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Lewis, A., Coates, L.N., Turner, A.D., Percy, L. and Lewis, J.

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A REVIEW OF THE GLOBAL DISTRIBUTION OF *ALEXANDRIUM MINUTUM*, DINOPHYCEAE WITH COMMENTS ON ECOLOGY AND ASSOCIATED PARALYTIC SHELLFISH TOXIN PROFILES, WITH A FOCUS ON NORTHERN EUROPE[1]

Adam Michael Lewis[2], Cefas, The Nothe, Barrack Road, Weymouth, Dorset, DT48UB, Faculty of Science and Technology, The University of Westminster, 115 New Cavendish Street, London, W1W6UW, adam.lewis@cefas.co.uk, 01305206769

Lewis Nicholas Coates, Cefas, The Nothe, Barrack Road, Weymouth, Dorset, DT48UB

Andrew D Turner, Cefas, The Nothe, Barrack Road, Weymouth, Dorset, DT48UB

Linda Percy, Faculty of Science and Technology, The University of Westminster, 115 New Cavendish Street, London, W1W6UW

Jane Lewis, Faculty of Science and Technology, The University of Westminster, 115 New Cavendish Street, London, W1W6UW

Running Title:

Global distribution, toxin profiles and ecology of *Alexandrium minutum*

Abstract

Alexandrium minutum is a globally distributed harmful algal bloom species with many strains that are known to produce paralytic shellfish toxins (PSTs) and consequently represent a concern to human and ecosystem health. This review highlights that *A. minutum* typically occurs in sheltered locations, with cell growth occurring during periods of stable water conditions. Sediment characteristics are important in the persistence of this species within a location, with fine sediments providing cyst deposits for ongoing inoculation to the water column. Toxic strains of *A. minutum* do not produce a consistent toxin profile, different populations produce a range of PSTs in differing quantities. Novel cluster analysis of published *A. minutum* toxin profiles indicates five PST profile clusters globally. Some clusters are grouped geographically (Northern Europe) whilst others are widely spread. Isolates from Taiwan have a range of toxin profile clusters and this area appears to have the most diverse set of PST producing *A. minutum* populations. These toxin profiles indicate that within the UK there are two populations of *A. minutum* grouping with strains from Northern France and Southern Ireland. There is a degree of interconnectivity in this region due to oceanic circulation and a high level of shipping and recreational boating. Further research into the interrelationships between the *A. minutum* populations in this global region would be of value.

Keywords

Alexandrium minutum, ecology, geographic distribution, Paralytic Shellfish Toxins, toxin profiling,

Abbreviations

C, N-sulfocarbamoyl; FISH, Fluorescent In-situ Hybridisation; GTX, Gonyautoxin; Neo, Neosaxitoxin; STX, Saxitoxin

Introduction

The marine phytoplankton species *Alexandrium minutum* Halim (Halim 1960), is an armoured dinoflagellate with a global distribution (Hansen et al. 2003, Lilly et al. 2005). It is a known producer of paralytic shellfish toxins (PSTs) the causative agents for the condition in humans known as paralytic shellfish poisoning (PSP). These toxins are a family of neurotoxic alkaloids the parent molecule of which is saxitoxin (STX). To date around 60 saxitoxin analogues have been described, those produced by *A. minutum* fall into the most common groups which are the hydrophilic toxins (Wiese et al. 2010). To date there are very few records of non-toxic *A. minutum*. As a consequence of this scarcity (Touzet et al. 2007a) the predominant impact of *A. minutum* is that caused by the toxic strains. As a result, *A. minutum* is recognised as one of the many harmful algal species which naturally occur around the globe and is known to have had impacts on humans, food security and other marine organisms (Costas and Lopez-Rodas 1996, Usup et al. 2002, Ranston et al. 2007, Erdner et al. 2010, Santos et al. 2014). With the continuing expansion of the global human population there is a concomitant rise in the need for safe food sources, the presence of PSTs in seafood poses a risk which is becoming increasingly important (McPartlin et al. 2017), the contribution of *A. minutum* to this risk is therefore an important factor to be assessed.

The first population of *A. minutum* was discovered and described by Halim in 1960 at the entrance to the port of Alexandria in Northern Egypt, from where the genus takes its name. Following many years of discussion around the correct taxonomy for this important group of microalgae the morphological characteristics which define the species were redescribed by Balech in 1989 to provide greater clarity. This identification guide from 1989 provides the basis of many of the identifications which appear within the literature, especially those predating the advent of affordable molecular techniques. In more recent studies a number of molecular techniques, such as PCR (Anna Godhe et al. 2001b, Galluzzi et al. 2004, Touzet et al. 2007a), FISH (Touzet et al. 2009) and microsatellite markers (McCauley et al. 2009a, Casabianca et al. 2012), have been utilised, either for the independent or confirmatory identification of *A. minutum*. Work in this field has resulted in a greater understanding of the species complex (Lilly et al. 2005, McCauley et al. 2009b) and the reclassification of related species - *A. lusitanicum* (McCauley et al. 2009a) and *A. angustibulatum* (Hansen et al. 2003) into *A. minutum* as recommended by Lilly et al. (2005). It has also highlighted variability within some of the morphological traits, such as the presence of a ventral pore, traditionally utilised for microscopic identification (Hansen et al. 2003, Lilly et al. 2005, Touzet et al. 2007a, Touzet et al. 2008, Penna et al. 2008). The occurrence of *A. minutum* has been confirmed by morphological taxonomy at many locations and is still used as a principle tool in species identification for this genus in many areas where molecular tools are either unavailable or have not been applied (Ranston et al. 2007, Satta et al. 2010, Baylón et al. 2015). Often this is because *A. minutum* has not been the primary focus of the research in question (D’Silva et al. 2013). Light microscopy also remains the key methodology in monitoring programmes where molecular tools may not be practicable for regular application (Godhe et al. 2001b).

Details of the distribution of this species have been assessed and reviewed previously (Hansen et al. 2003, Lilly et al. 2005) but this has not been carried out recently. Since the last comprehensive review (Hansen et al. 2003) which detailed global distribution, a number of new populations have been discovered globally, in regions such as central America, which makes the compiling of more recent data a valuable exercise. Further to this, toxin profiles of

A. minutum populations have been determined from a number of source populations but little work has been conducted to explore the relationship of these. Therefore, the purpose of this brief review is to provide updated details on the current known global distribution of *A. minutum,* as well as the toxin profiles associated with this species globally, alongside information pertaining to the ecology of this important harmful species. Finally, the situation within Northern Europe and the United Kingdom are evaluated in more detail. In this way it is intended that this review offers key information on the current known distribution and toxicity of the species as well as providing an overview of traits which typify areas currently experiencing detected levels of *A. minutum.*

Specific ecosystem characteristics associated with *A. minutum*

Geographically *A. minutum* has been detected at sites which are widely dispersed. Although populations may be spatially isolated from one another, the conditions of the local environments where populations are known to occur share similarities. Through a number of laboratory studies and observations of *A. minutum* events in the field it is possible to determine some common characteristics of the conditions which favour the growth and accumulation of *A. minutum* to detectable levels. Predominantly, *A. minutum* is found in sheltered harbours, lagoons, estuaries or embayments, where stratification occurs and hydrographic shear stresses are typically low (Delgado et al. 1990, Lassus et al. 2004, Ranston et al. 2007, Bravo et al. 2008, Touzet et al. 2010b, D’Silva et al. 2013, Le Bec et al. 2016). It is of note that these are normally areas of higher human activity and so also represent areas more likely to be monitored, it is therefore possible that further populations exist in different habitats which remain to be discovered (Alacid et al. 2017). Those recordings of *A. minutum* from open waters or exposed areas are limited in the literature with only a handful of papers documenting such findings (Godhe et al. 2001b, Yoshida 2002).

The temperature range of *A. minutum* occurrence, as determined from field observations, is broad globally, with cell proliferations occurring from 12°C (Vila et al. 2005; Touzet et al. 2010a) to 30°C or more (Glibert et al. 2001, D'Silva et al. 2013). Local populations seem to have a smaller temperature range at which optimal growth occurs, this has been evidenced by variable optimum growth conditions having been determined for different source populations (Grzebyk et al. 2003b, Lim et al. 2006, Bravo et al. 2008). The salinity tolerance of *A. minutum* is also broad, ranging from growth in natural samples at 11 (Ranston et al. 2007) to 46 (Abdenadher et al. 2012). Studies on cultures have demonstrated growth at salinities as low as 5, *A. minutum* is therefore considered euryhaline (Lim and Ogata 2005) and concentrations can develop near to freshwater inputs as well as in lagoons, pools or harbours without such inputs. The optimal levels for both temperature and salinity appear to vary by the geographic location of the source population and potentially represent an acclimation to prevailing conditions (Lim and Ogata 2005, Van Lenning et al. 2007). This would suggest that *A. minutum* exhibits a degree of local adaptation, presumably altering certain physiological parameters to enable optimum growth conditions to meet those of the local environment. If this is the case *A. minutum* would be able to become competitive in a range of environments if initial introductions are able to survive whilst adaptation takes place.

Nutritionally *A. minutum* is photosynthetic but mixotrophy has been observed (Fagerberg et al. 2009, Anderson et al. 2012). *A. minutum* displays a high affinity for nitrogenous material, displaying relatively low half-saturation constants for both nitrate and ammonium, which is unusual for a dinoflagellate (Maguer et al. 2007). Furthermore *A. minutum* is capable of sequestering both N and P intracellularly to support growth during conditions when nutrients may otherwise be limiting (Maguer et al. 2007, Touzet et al. 2007b, Labry et al. 2008)

Globally *A. minutum* can occur at high abundances, Smayda (1997) suggested that bloom status is species and locality specific, considering a bloom as a rapid increase in cell numbers to levels considerably in excess of background population concentrations allows for a decoupling of bloom status to specific cell densities. Certainly, many authors refer to the proliferation of *A. minutum* within their study areas as a bloom without defining their criteria for what a bloom is, this results in considerable differences in maximum *A. minutum* concentrations within a 'bloom'. Levels of *A. minutum* have been frequently recorded above 10^4 cells per litre (Chang et al. 1995, Ranston et al. 2007, Bravo et al. 2010a, Touzet et al. 2010b, Anglès et al. 2012, Baylón et al. 2015) and maximum densities exceeding 10^7 cells per litre (Hwang et al. 1999, Pitcher et al. 2007, Chapelle et al. 2015) have been reported. A concentration of *A. minutum* above 10^3 cells per litre was used as the criteria to determine a bloom event by Anglès et al. (2012) and above 10^5 in Le Bec et al. (2016). For the purposes of this review we have considered blooms to occur when stated as such in the literature, this follows the criteria applied by Smayda (1997) of bloom status being determined by the conditions considered normal for each individual location.

In many regions experiencing blooms these accumulations tend to occur from spring, into the summer months, March to August in the Northern Hemisphere (Lassus et al. 2004, Blanco et al. 2009, Le Bec et al. 2016, Guallar et al. 2017) and September to February in the Southern Hemisphere (Chang et al. 1997a, Hwang et al. 1999, Baylón et al. 2015). There are some cases of winter blooms of this species, with most of these reported in the Northern Mediterranean (Bravo et al. 2008, Alacid et al. 2017). In all cases early onset of blooms coincides with differing conditions depending on the location. Table 1 outlines some such bloom initiators where they have been determined.

In several cases the presence of a cyst deposit, alongside a parameter such as those above, has been strongly implied as a primary contributor for a successful population of *A. minutum* developing (Bravo et al. 2010b, Anglès et al. 2012, Cosgrove et al. 2014). The presence of cysts is not enough to guarantee the formation of a bloom, environmental conditions need to be suitable to support cell division following an inoculation from a cyst deposit, the cysts therefore provide for a ready source of vegetative cells which can bloom under favourable conditions. Initiation of a bloom may well be dependent on the specific locality, in all locations there will be several thresholds which need to be met to initiate a bloom. Which of these factors is limiting to bloom formation is likely to vary from site to site based upon prevailing local conditions and the requirements of the local population of *A. minutum.* Consequently, the final trigger condition for bloom formation will vary between sites, this makes it difficult to specify a key parameter which needs to be monitored, instead a range of measurements need to be considered.

Bloom termination is similarly a complex process and is likely to vary between locations and also between years. Many factors have been identified for bloom decline, examples of which are detailed in Table 2.

Bloom decline is an aspect which needs to be assessed on a case by case basis, long term data on the frequency and duration of occurrence becomes a very useful tool in this regard but is normally limited to areas which are well studied or where routine monitoring exists.

Blooms of *A. minutum* are rarely monospecific, *A. minutum* will sometimes form the majority component of the assemblage (Delgado et al. 1990, Maguer et al. 2004) but at other times reaches high densities whilst still being a relatively small proportion of the total algal assemblage (Chang et al. 1997b). The proportion of the assemblage represented by *A. minutum* is often not reported as quantifying all species from different phyla can be an extensive task. Consequently, reports are often limited to abundance in relation to other dinoflagellates or simply stating the number of cells present alongside whether it was dominant or not. Where *A. minutum* co-occurs with other potential PST producers or species producing other biotoxins this can increase the complexity of the management strategy required to safeguard human health as well as having a more pronounced impact on the fisheries affected.

Life History

The life history of *A. minutum,* which is presented graphically in figure 1, contains both a haploid motile vegetative stage as well as a diploid planozygote formed following sexual fusion, both of these stages are able to encyst leading to a loss of motility and a change in morphology. Two types of cysts are known to feature within the life cycle of *A. minutum*. Of these, one is an asexually produced pellicle cysts which has been found in two forms, the most common of which possesses a thin wall with yellow/brown inclusion bodies, the other retains the theca of the vegetative cell and has rarely been reported. In addition to these asexual cysts, a more resilient resting cyst, possessing a thick double wall can be formed following sexual reproduction. The presence of the thin walled pellicle cyst and the double walled resting cyst has been commonly observed but the thecate cyst has been encountered rarely. One study where all three cyst morphologies were observed in natural samples was conducted by Bravo et al. (2010a), working with sediment traps from the Bay of Baiona, Spain. The different cyst types have also been demonstrated as mechanisms to survive short and long periods of inclement conditions with cyst formation observed in conjunction with deteriorating conditions, such as a reduction in available nutrients (Figueroa et al. 2007). Sexual hypnocysts are also known to form from sexual encounters between cells during normal vegetative cell growth (Figueroa et al. 2015). This has been shown to result in a low percentage of background encystment (Anglès et al. 2012) whenever a population is present in the water column. If these cysts remain in the surface layers of sediments following their mandatory dormancy period, shown to be between 1 and 1.5 months (Figueroa et al. 2007, Bravo et al. 2010b), then they represent a potential rapid inoculum for future *A. minutum* proliferations. Recurrence of *A. minutum* within a location has been shown to result from the formation of ‘seed’ banks (Cosgrove et al. 2014) composed of resting cysts in surface sediments. Excystment has been demonstrated over a broad range of physical characteristics which affect the vegetative growth of the species and it has been concluded that cyst germination occurs under all conditions where both light and oxygen are available (Blanco et al. 2009, Ní Rathaille and Raine 2011). Indeed a study by Anglès et al., (2012) found a constant low level of background excystment which increased dramatically in periods immediately prior to bloom formation. Consequently, vegetative cells of *A. minutum* are potentially present at very low densities at any sites where cysts have formed but will only escalate to densities likely to cause issue or be detected when conditions within the water column become favourable.

Following their formation, *A. minutum* hypnocysts lose their motility and subsequently behave as passive particles with a lower density than clays (Anglès et al. 2010) these then sediment out of the water column and are deposited in the surface layers of the benthos. Where the benthos of a location is dominated by larger particles or the sediment is scoured by strong water movements *A. minutum* cysts are lost from the upper sediment or dispersed (Narale and Anil 2017) and so are unable to form a future inoculum. Therefore, waterbodies which accumulate and retain fine sediments are required for the successful establishment of long term cyst deposits (Narale and Anil 2017). A degree of sediment circulation is required also, to ensure that cysts are not buried too deeply. Bays and harbours often represent suitable habitats as they typically provide reduced water movement except in proximity to entrances. This allows for the accumulation of the fine sediments required for cyst deposit formation as well as encouraging the development of stable stratified water when temperatures increase towards summer months. The introduction of harmful algal bloom species to new locations through cyst or motile cell transport is an area of concern for food safety reasons as new introductions may bring previously unseen issues with relation to human and ecosystem health. Although the frequency of anthropogenically mediated transfers is debatable (Smayda 2007) many authors have cited ballast water as a potential vector, although this has only been conclusively demonstrated in relatively few cases (Burkholder et al. 2007), this would suggest that ports and harbours could represent a higher risk environment not only due to favourable conditions for *A. minutum* to persist within them but also potentially as points of introduction where shipping occurs between regions where *A. minutum* is known to occur and new sites.

Current global distribution

Since its first discovery at Alexandria port, *A. minutum* has subsequently been isolated from a range of further locations and has now been found to inhabit coastal regions globally, it has now been discovered from the coasts of all continents with the exception of Antarctica. In some areas, including some of those where it has recently been discovered, it is known in only a relatively small range or even a single specific location (Pitcher et al. 2007, Baylón et al. 2015). Several of these areas where occurrence is limited in range represent understudied regions, in some cases comprehensive plankton surveys are being carried out for the first time. In other parts of the world it has been identified at several locations, such as multiple sites around northern Europe (Elbrachter 1998, Nehring 1998, Godhe et al. 2001a, Hansen et al. 2003, Touzet et al. 2007a, Brown et al. 2010, Guallar et al. 2017), which represents a well studied area both geographically and temporally. The limited known distribution in some areas may be as a result of a lack of suitable conditions or it could simply be that surrounding areas have not received sufficient attention from research or monitoring groups to determine the presence or absence of *A. minutum*. Figure 2 displays the locations of known occurrences of *A. minutum*. No weighting is given to those sites where multiple studies have occurred and in some cases a marker may cover more than one site of occurrence if it is in close proximity to others.

The study of Lilly et al., (2005) subdivides *A. minutum* into four clades, based upon differences in the D1-D2 regions of the large ribosomal sub-unit DNA. Of these the smallest two clades were comprised of only three strains, with one clade of one and the other containing two, all of which were originally isolated from Japan. The remaining two clades contained the majority of those strains studied. These two major clades are the Global clade, which, of the strains assessed, includes those populations isolated from areas around Europe, South Africa and Australia and the smaller Pacific clade, which encompasses those populations originating from Asia and New Zealand. Since the work carried out by Lilly et al., (2005) further populations of *A. minutum* have been identified from Cape Town in South of Africa (Pitcher et al. 2007), the Azores (Santos et al. 2014) East Asia (Baula et al. 2011) South America (Menezes et al. 2007, Baylón et al. 2015) and Central America (Ranston et al. 2007, Lozano-Duque et al. 2011).With the exception of the population in South Africa these newly discovered populations have only been identified via the use of morphological traits and as such detailed information on their phylogeny is currently lacking. It is therefore not possible to classify these into either the Global or Pacific clade at this time. This is also the case for less well studied areas or those where culturing of detected populations has not been actively undertaken. As well as lacking phylogenetic information, the toxicity of the populations from Jamaica, Peru and India has yet to be determined. It was noted by Ranston et al., (2007) that there have been no recorded cases of PSP in Jamaica, implying that the *A. minutum* population present there is either not toxic, does not reach abundances sufficient to cause issues in areas where harvested shellfish are also present, or existing management programmes are sufficient to prevent human exposure. As with phylogenetic relationships, where *A. minutum* occurs but cultures have yet to be established it is uncommon for toxicity of a population to have been elucidated. Given the potential impact, on humans, of PSTs from *A. minutum* it should be of paramount importance to determine what if any toxicity is exhibited by a population following discovery.

The more recent identifications of *A. minutum* now increase its known spread to encompass South America, where it was previously unrecorded but also increase its spread in the Southern Hemisphere where it was previously known only from Eastern areas. It is possible that with further studies carried out along the coasts of both Africa and South America the number of detections would increase. From Figure 2 it is apparent that under the current taxonomic designation, *A. minutum* is truly a global species. This raises the possibility for human health concerns related to *A. minutum* across a wide range of geographies. As a species *A. minutum* should be considered by monitoring agencies worldwide, as the detection of further populations seems likely given the ability of *A. minutum* to survive across such a large range of latitudes and longitudes.

Current global toxin profile information

Other than morphological and genetic markers toxigenic strains of *A. minutum* also produce determinable PST profiles. The toxin profiles for *A. minutum* populations are presented in Table 3 alongside their geographic origin. Where these toxin profiles have been determined in conjunction with complete phylogenies, either within the same study or via separate studies utilising cell cultures sourced from the same location, it is noted as to which of the two clades they belong. The most common method for determination of toxin profiles within the literature has been the use of high performance liquid chromatography with fluorescence detection and post column oxidation of PSTs. For determination of toxins in algal cultures many studies have utilised high performance liquid chromatography with post column oxidation (HPLC-PCOX) such as the AOAC method (Anon, 2011) or have utilised similar methods developed or refined within their own research group.

From Table 3 it is possible to see that the most common toxins produced by *A. minutum* are GTX 1&4 with GTX4 being the most dominant toxin in a range of strains which are widely dispersed geographically. Additionally, both non-toxic and toxic strains belong within the same clade. The exception to this toxic profile appears to occur around Northern Europe with strains identified from Ireland, England and Northern France being dominated by GTX2&3 rather than GTX1&4 or the populations from Denmark and some areas of Northern France containing C1&2 alongside either GTX2&3 or dcGTX2&3. Whether there is an underlying genetic separation between these populations and other members of the global clade which has yet to be determined or if the toxin production in this region is driven by a specific set of environmental parameters or stressors is currently unknown. For the most part other STX analogues are scarce in *A. minutum* strains, appearing in low or trace levels within most strains analysed. Both STX and Neo occur in higher proportions but only in a limited number of strains analysed, predominantly from New Zealand. This may indicate that these toxins are comparatively scarce or that populations producing these toxins are less well studied.

To determine if any global patterns existed within toxin profile further analysis was undertaken. Where toxin profile was available within the available literature it was collated and normalised to allow for comparison. Additionally, owing to a desire to maximise the data pool but also to reduce variability, allowing data to be classified into a meaningful number of clusters, the epimeric toxin pairs were summed and considered as a single value for further analysis. K-means clustering analysis was performed, in MS Excel, on this toxin profile data for a range of algal strains from different geographic locations. The analysis generated 5 clusters of PST toxin profiles via iterative generation of minimum distances to each of 5 centres taken from within the source data. Figure 3 graphically represents the geographic spread of the different clusters. The first cluster was predominated by GTX1&4 and covered a broad range of source populations from sites in Europe as well as Australia, Taiwan and Malaysia. The second cluster included those strains producing high levels of GTX2&3 and included most of the strains analysed from Northern Europe as well as some from South Taiwan. The remaining three clusters were smaller in terms of representation in the literature. Cluster 3 contains 2 strains from New Zealand and 1 from Brazil, all of which contained a high proportion of Neosaxitoxin. Cluster 4 contains exclusively strains from Northern Europe including those from Denmark and some from Northern France. The defining feature of cluster 4 is the significant amount of C1&2 within the profile, this appears to be unique to this geographic region as of those profiles analysed the C toxins only featured at low levels outside of Northern Europe, in one strain, AL2V, from the Mediterranean. Finally, Cluster 5 grouped some strains from the South of England with some from New Zealand, the characteristics of this profile were the presence of GTX2&3 alongside a considerable contribution to overall toxicity by STX, those New Zealand strains in cluster 5 were also found to produce GTX1&4 and in some cases Neo. Data for one strain from Taiwan was also found to fall into cluster 5.

Cluster 1 is the most widely dispersed of the reported profiles encompassing a broad range of different populations globally. Excluding the Taiwanese strains, the remaining clusters are either closely grouped together or represent only a handful of sites. Cluster 2 represents a small distribution in NW Europe, cluster 3 is confined to two locations, cluster 4 like cluster 2 is limited to Northern Europe and cluster 5, whilst occurring at very distant locations is only representative of populations from four locations, two of which are in New Zealand. The data from Taiwan is interesting as cultures from this region were found to exhibit toxicity profiles indicative of three of the five clusters generated. Strains from this area occupied clusters 1,2 and 5. The example of a Taiwanese occurrence in cluster 5 is, additionally, profile information generated within a study where the profile during exponential growth of *A. minutum* fell into cluster 5 whereas the PST profile during the stationary phase was grouped with cluster 1. Work by (Lim et al. 2007a) arrived at a similar conclusion, albeit using a smaller range of toxin profiles during analysis, where a dendrogram was used to display the divergence of profile between strains. It is therefore difficult to generalise regarding the toxin production of *A. minutum* on a global basis, the Global clade contains representatives from 3 of the 4 clusters and strains from the Pacific clade fall within 2 clusters, leading to a number of genetically differentiated strains existing within the same toxin profile cluster. This would imply that the toxicity of a strain is not linked to its clade but more likely to the geographic region from which it is isolated, with specific regions appearing to have characteristic toxin profiles which may be driven by the local environment. Although in both Taiwan and Northern Europe there are areas where two or more profiles exist within a very small geographic range, this may give credence to the notion that toxin profile is genetically driven as these populations will be exposed to very similar environmental conditions but still produce distinct toxin profiles. The similarity in profile between the New

Zealand strains included within the analysis and the single strain tested from Brazil is an interesting development. Work to compare these two populations using molecular techniques would be of value in determining what, if any, connectivity exists between these geographically disparate populations as they represent an otherwise uncommon PST profile within the *A. minutum* species.

With the Exception of Taiwan, where the same region or strain has been assessed repeatedly it is possible to see that the toxin profile remains relatively stable (Flynn et al. 1994, Yang et al. 2011), one strain which has been extensively used within research, AL1V, for example was always found to appear in cluster 1 during our analysis. Some studies have found the PST profile within *A. minutum* and other toxic dinoflagellates to be variable (Hwang and Lu 2000, Maas et al. 2007) but these have often been studies conducted using cultures exposed to artificial conditions, such as the removal of associated bacteria, and so extrapolating their findings directly to natural populations is not easy (Hansen et al. 2003). It has been demonstrated by other studies that the cellular toxin quota is also modified by environmental conditions, examples of these are detailed in Table 4.

From the analysis of multiple toxin profiles generated globally for *A. minutum* it is possible to determine patterns of toxin profile distribution which could indicate related populations or those which may have diverged. This provides clear regions globally to compare genetically and an indication of where either variance or similarity could be expected. Consequently, the assessment of PST profiles within *A. minutum* populations constitutes another mechanism for assessing global variation within the species.

Studies where the toxicity of *A. minutum* has been determined are quite common but it is unfortunately also common for comprehensive toxin profile data to be omitted in many publications. Instead the principle toxins and their proportions are listed or only total toxicity given. It is of value, for further research efforts, for the full toxin profile to be released if it is determined to allow for further exploration of links between genetic structure and toxin production. This would be useful as the toxicity of harmful species is of key importance with regard to the impact which they have on society and health. Holistic studies which have determined morphology, toxicity and phylogeny are more rare still but are also incredibly useful (Hansen et al. 2003; Pitcher et al. 2007; Brown et al. 2010; Touzet et al. 2010b), wherever possible studies of harmful algal species should provide data on all of these aspects and in the case of well-studied populations it is of value to compile this data over time even if it is not possible to determine all of it within a single study.

North European distribution and toxin information

There are a number of sites spread across North Europe which are currently known to experience growth of *A. minutum*, for several of these sites incidents of growth are recurrent, happening either annually or sporadically but within the same locations. Figure 4 presents a map of Northern Europe highlighting areas where *A. minutum* has been detected within this region as well as denoting whether a specific population was found to be toxic or non-toxic.

Of the populations of *A. minutum* in North Europe, some have been extensively researched. For example, sites from the North of Brittany have been well studied, especially the Penzé Estuary. This area has been well characterised with studies detailing *A. minutum* toxicity (Lassus et al. 2004), parasitism (Erard-Le Denn et al. 2000, Chambouvet et al. 2008) and nutrition (Maguer et al. 2004). As a result, a comprehensive knowledge set exists for *A. minutum* in the region and consequently further studies in this area can be well designed using the knowledge already present as a suitable basis. Similarly, the *A. minutum* population originating in Cork Harbour in Ireland has been extensively assessed leading to knowledge of toxicity (Touzet et al. 2007a) bloom dynamics (Touzet et al. 2010a), factors affecting excystment (Ní Rathaille and Raine 2011) and phylogeny (Touzet et al. 2009). As with the Brittany coast this presents Cork Harbour as a model population to carry out further research with many characteristics being well documented allowing for the exploration of more specific questions. Cultured cell lines from these populations would make an excellent choice if investigating the effect of environment on toxin production, as their PST profile is both well characterised and uncommon globally. This rare toxin profile also makes these populations an important inclusion in any study wishing to investigate genetic divergence between strains globally, the high abundance of GTX2&3 indicating that there may be an underlying genetic distinction between these Northern European populations and others globally. However, conclusions drawn from work conducted on these specific populations may be limited in scope to the local region as *A. mintum* populations exhibit a broad range of tolerance for certain parameters depending upon their origin so that work conducted with one population may not be directly applicable to populations occurring in disparate regions. Work by Hansen et al., (2003) in Denmark was also comprehensive as were the studies in the South of England by Percy (2006) and Nascimento et al., (2005). Although these populations were thoroughly evaluated within these works further work at these sites has either not been conducted or published. Other sites within Northern Europe where *A. minutum* has been identified have been less comprehensively studied. As a result, information is patchy especially relating to longer temporal scales or broader geographic areas than the direct area of study. It is therefore possible that as yet undiscovered populations exist within this region, without comprehensive surveys the full extent of the species distribution cannot be conclusively stated.

One common feature of *A. minutum* from this region is the production of GTX2&3 or C1&2 in much larger quantities than exhibited elsewhere in the world. Interestingly the production of GTX1&4 which is a common feature of many other *A. minutum* populations is entirely absent from these North European strains. The exact reason for this difference has not yet been determined to our knowledge and further studies assessing this particular aspect would be of interest to improve the understanding of the *A. minutum* species as a whole. It is recognised that work with the genomes of dinoflagellates is complicated by the large size of the total genome, the dynamic nature of the genome evolutionarily and the variability in chromosome number (Casabianca et al. 2017). As a dinoflagellate *A. minutum* exhibits a large genome, where multiple strains of *A. minutum* were assessed they were found to contain a genome size ranging from 22.5-29.6 pgDNA cell (Stüken et al. 2015). Consequently, it may be some time before it is possible to resolve any genetic basis for these global differences or define intraspecific markers which allow for the separation of *A. minutum* populations where differences are known to exist. Recent work by (Casabianca et al. 2012) has successfully resolved genetic differences in multiple strains isolated from within the Mediterranean via the use of microsatellite markers. This is therefore an area warranting further research especially in and between those areas for which an extensive amount of background work has been conducted, such as the populations of Brittany.

Current understanding of the *A. minutum* species group in the UK

A. minutum populations are known from both the Northern and Southern extent of the UK. The Scottish algal assemblages have been thoroughly analysed on several occasions and where *A. minutum* has been detected it has been shown to be non-toxic and belonging to the global clade (Brown et al. 2010), shown as a black X in the North East of Scotland in figure 4. Both populations identified from the south of England, denoted by the two black circles in Southern England in figure 4, have been determined to be toxic with both producing primarily GTX3 alongside GTX2 which appears to be typical of Northern Europe. The population of *A. minutum* from the Fleet Lagoon was also found to be producing STX (Nascimento et al. 2005) in sufficient quantity to cluster it with strains from New Zealand rather than the others in Northern Europe. These two sites in Southern England where toxic *A. minutum* has been identified do not represent the only occurrences of PSP toxicity from southern areas of the UK. Data supplied in Turner et al., (2014) clearly shows that PSP toxicity occurs in shellfish at a number of sites around the South coast with further incidents in Devon, Cornwall and South Wales. The analysis performed within that paper suggested that the toxin profile determined in shellfish via pre-column oxidation and high performance liquid chromatography in these regions most closely aligned with the profile of *A. minutum* from the Fal, except in the Fowey estuary which showed a profile clustering more closely to that from Scotland possibly due to its STX content. The Fowey profile could be representative of the profile exhibited by *A. minutum* from the Fleet Estuary population, which also contains significant STX. Within our own cluster analysis performed here the populations from the Fal and the Fleet fell into different clusters, the factor affecting this was again the presence of STX in the Fleet population which is absent from the Fal population. Considering the dominance of GTX2&3 in both the Fleet and Fal populations of *A. minutum* it would appear that the PST events in shellfish from the South Western regions of the United Kingdom where the algal producer has not been identified are most likely caused by undetermined populations of toxic *A. minutum*. Shellfish toxicity associated with *A. minutum* within the UK has only been demonstrated conclusively in one location with intoxications in the Fal directly linked to the presence of *A. minutum* (Percy 2006). The only other demonstrably toxic population from the Fleet Lagoon has no known associated shellfish intoxications, although the area does contain shellfish production and is monitored on a regular basis. Therefore, in other UK shellfish harvesting areas which have been affected by

PST contamination it can only be inferred from the evidence supplied by toxin profile that *A. minutum* is the most likely causative algal species and further research within this area could shed more light on the current situation and bring this part of Northern Europe in line with other, comparatively better studied sites within this global region.

Further to this information from within the UK it is worth considering the relationship between the South coasts of England and Wales with other sites containing populations of toxic *A. minutum,* such as the South East of Ireland and Northern France. Although dispersal of viable vegetative cells via ocean currents is unlikely across broad geographic scales there is a weak current which runs up the Brittany coast and crosses the mouth of the channel passing Cornwall, this flow then joins the Celtic sea circulation, which runs anti-clockwise to the South coast of Wales and either into the Irish sea or crosses the St George's Channel before flowing South West along the Irish coast (Bailly Du Bois et al. 2002, Brown et al. 2003). The other prevailing currents in the area flow from West to East along the English Channel until joining the North Sea circulation (Bailly Du Bois et al. 2002, Brown et al. 2003). Further to this water from the Celtic Sea flows into and out of the Bristol Channel, along the South coast of Wales due to tidal forcing (Pearce et al. 2012). This has been well described and is shown visually in figure 5, from Bailly Du Bois et al. (2002).

This large-scale water exchange connects the known toxic populations of *A. minutum* in Southern England, Southern Wales, Southern Ireland and Northern France providing the possibility for cells to move between the regions via oceanic transport. These areas are also connected via anthropogenic means. Recreational boating is popular in the area which is noted as being well connected (Pearce et al. 2012) with direct yachting and recreational cruising between Northern France and Southern England as well as Southern Ireland, although this is less common (Tidbury et al. 2016). The South of Cornwall and the region around Cork harbour both scored highly for intensity of pathway activity with regard to the introduction of non-native invasive species in the paper by Tidbury et al. (2016) Earlier work assessing non-native species introductions by Pearce et al. (2012) rated several regions of the South West of England, the South of Ireland and the West of Wales as a medium risk of introduction of planktonic species when considering pathways such as commercial shipping, in ballast water or biofouling, and the movement of aquaculture organisms. Tidbury et al. (2016) noted also that whilst a lower risk than recreational shipping, commercial shipping posed a risk with live animal movements representing a lower threat to this South-Western region. Nationally, both France and Ireland fall within the top 10 in terms of shipping volumes to and from the UK, scoring second and sixth respectively (GOV.UK 2016). Therefore, whilst shipping into the South-Western region of the UK is relatively light it may still originate from other areas with established populations of *A. minutum.*

Whilst some sites of PST toxicity in shellfish within the South West of the UK have been linked to *A. minutum* the remaining areas remain undetermined. In other parts of the world and even within the UK the algal assemblages have been thoroughly analysed and the results published in such a way as to allow more definitive conclusions to be drawn regarding the causative organisms of intoxications both within studied and nearby locations. To provide a greater depth of understanding it would be of benefit for further field surveys to be conducted in the South West of the UK to elucidate the distribution of *A. minutum* within the region as well as the potential presence of other noxious algal species. This would allow for a greater understanding of the true extent of *A. minutum* in this region of Europe as well as providing an opportunity to assess relatedness between the populations from this region. In turn this information can be fed into management strategies and decisions to allow for more effective monitoring as well as better planning for any aquaculture expansions in the South of the UK.

Conclusion

Globally *A. minutum* has been well studied, with a number of investigations evaluating different features of its biology and ecology. Due to the occurrence of predominantly toxic strains of *A. minutum* it exists as a concern for human health in locations where it occurs alongside the harvest of filter feeding organisms, usually bivalve molluscs. Recent studies highlight that *A. minutum* may well be more widely distributed than is currently realised and given the range of localities it has now been detected from it should form a species of interest for anyone undertaking a comprehensive phytoplankton survey. Due to the variations in different strains of the species globally, data from areas where limited research has been carried out must be treated cautiously as extrapolation to the *A. minutum* species as whole may not be appropriate. Further to this it is not possible to assume with confidence that these comparatively under studied populations will behave in the same way as those where the literature is more abundant leading to a need to determine certain characteristics for these strains. Conversely well studied areas such as the Ria de Vigo and Penzé Estuary are very useful as they allow for ever more detailed and specific studies to be carried out as ground work in many areas, such as optimal growth characteristics, has already been completed. In this way studies can be planned more precisely and outcomes can be interpreted with confidence that the results obtained do not arise from the effects of undetermined variables. Due to the potentially harmful nature of *A. minutum* and the expanding knowledge of its distribution it is important that research continues to further our knowledge of the species. The toxin profiles of populations are a key area, especially for any identifications where toxicity has not been tested. Novel statistical analysis of global *A. minutum* toxin profiles has shown that, at present, 5 distinctive profiles exist globally. Of these profiles, 2 encompass the majority of strains analysed and both major molecular clades as outlined by Lilly et al. (2005) include representatives of these 2 most common toxin profiles. Also, further characterisation of populations under the existing clades as well as further work to find genetic markers capable of differentiating between populations would certainly be of value in the comparison of *A. minutum* from different sites and could allow for an understanding of source populations and population divergence within this important global species. Further examination of microsatellite markers may be a promising area for future work in this regard.

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Table 1: A sample of factors attributed to *A.minutum* bloom initiation with the location of occurrence.

Factor	Specifics	Location	Reference
Competitive advantage	Abnormal conditions suppressed expected bloom of competitor: *Lingulodinium sp.*	Kastela Bay, Croatia	(Marasović *et al.* 1995)
Nutrient loading	Decreased N:P ratios	Ganzirri Lagoon, Italy	(Giacobbe *et al.* 1996)
Nutrient loading	Increasing levels of Phosphates	Gulf of Gabes, Tunisia	(Abdenadher *et al.* 2012)
Salinity & Temperature	Excystment triggered with decreasing salinity and increasing temperature	Sungai Getting, Malaysia	(Lau *et al.* 2017)
Stratification	Thermal stratification following increasing temperatures	Ganzirri Lagoon, Italy, Baiona Bay, Spain	(Giacobbe *et al.* 1996, Bravo *et al.* 2010)
Temperature	Apparent threshold temperature required for effective cell growth	Bay of Brest, France	(Chapelle *et al.* 2015)
Tidal event	Rapid increase following spring tide	Cork Harbour, Ireland	(McCoy *et al.* 2014)

Tidal event	Bloom maxima attained during times of low tide coefficient, (neap tides)	Bay of Brest, France	(Chapelle *et al.* 2015)

Table 2: A sample of factors attributed to *A. minutum* bloom decline with the location of occurrence.

Factor	Specifics	Location	Reference
Dispersal	Increased wind speed and offshore direction dispersed the bloom	Arenys de Mar, Spain	(Van Lenning *et al.* 2007)
Encystment	Encystment was the dominant contributor to bloom decline	Penzé estuary, France	(Erard-Le Denn *et al.* 2000)
Grazing	Microzooplankton are capable of causing substantial reductions in algal numbers	Arenys de Mar, Spain (laboratory study)	(Calbet *et al.* 2003)
Meteorology	Elevated winds, decreased temperature and irradiance	Cork Harbour, Ireland	(Touzet *et al.* 2010)
Nutrient loading	Decreased availability of phosphates	Ganzirri Lagoon, Italy	(Giacobbe *et al.* 1996)
Parasitism			(Blanquart *et al.* 2016, Alacid *et al.* 2017)

	High levels of parasitic infection can cause high levels of mortality	Arenys de Mar, Spain; Penzé and Rance Estuaries, France	
Stratification	Decreased water column stability and increasing salinity	Ganzirri Lagoon, Italy	(Giacobbe *et al.* 1996)

Table 3: Table detailing the toxins present where determined within the literature alongside classification by the clades proposed in Lilly *et al.*, (2005) where this information is available. Toxin abbreviations are as follows: STX – Saxitoxin, GTX – Gonyautoxin, NeoSTX- NeoSaxitoxin C1 – N-sulfocarbamoyl Gonyautoxin 2, C2 - N-sulfocarbamoyl Gonyautoxin 3.

			PST Profile		**Phylogeny**		
Location		**Strain Identifier**	**Principle Toxins**	**Secondary Toxins**	**Pacific Clade**	**Global Clade**	**Reference**
Africa	Cape Town Harbour, South Africa	CTCC22	GTX1,4	GTX2,3		X	Pitcher *et al.* 2007
Asia	Do Son, Vietnam		GTX4	GTX1-3, NeoSTX, dcSTX	X		Lim *et al.* 2007
Australasia	Australia	AMAD01, AMAD06, AMAD21	GTX1,4			X	Franco *et al.* 1995

	New Zealand, Bay of Plenty	AMBOPO006	NeoSTX	STX, GTX1-4	X		Chang *et al.* 1997
	New Zealand, Marlborough Sounds	CAWD11, CAWD12, CAWD13	GTX1,2,4, NeoSTX, STX	GTX3	X		MacKenzie and Berkett 1997
Europe	Denmark, Kosor Nor	GHmin04	C1,2	GTX2,3 STX		X	Hansen *et al.* 2003
	England, Fleet Estuary	3.9h	GTX3	GTX2, STX		X	Nascimento *et al.* 2005
	France, Bay of Morlaix	AM89BM	GTX2,3	dcGTX2,3		X	Grzebyk *et al.* 2003
	Ireland, Cork Harbour	CK.A02, CK.A14, CK.A17, CK.A20, CK.A23, CK.D04	GTX3	GTX2, dcGTX2/3		X	Touzet *et al.* 2007, 2007b, 2008
	Ireland, Killary Harbour	Kill.A12, Kill.C6, Kill.E4, Kill.G3	Non-toxic	Non-toxic		X	Touzet *et al.* 2007,

Ireland, Shannon Estuary	SHA.A12, SHA.B11, SHA.B12	Non-toxic	Non-toxic	X	Touzet *et al.* 2007,
Italy, Gulf of Trieste	AL3T, AL9T	GTX4	GTX3, STX	X	Yang *et al.* 2010
Italy, Gulf of Trieste	AL1T	Non-toxic	Non-toxic	X	Yang *et al.* 2010
Scotland, Orkney Islands	W07/001/01, W07/025/01	Non-toxic	Non-toxic	X	Brown *et al.* 2010
Spain, Ria de Vigo	AL1V, AL2V, AL3V	GTX1,4		X	Franco *et al.* 1995
Spain, Ria de Vigo	AL1V	GTX1,4	GTX2,3	X	Bricelj & Cembella 1995, Yang *et al.* 2011

Table 4: Examples of biotic and abiotic factors and their associated impact on PST quotas in *A. minutum.*

Factor	Impact on toxicity	Reference
Associated bacteria	Antibiotic treatment of cultures reduced PST levels by up to 87%	(Maas *et al.* 2007)
Grazer cues	Cellular toxin quota significantly elevated in the presence of grazer cues	(Selander *et al.* 2006, Yang *et al.* 2011)
Growth stage	3 times higher cellular toxicity encountered in exponential than stationary phase	(Mascarenhas *et al.* 1995)
Nutrient limitation	10 times higher toxicity when exposed to grazer cue and limited phosphates	(Selander *et al.* 2008)
Nutrient source	4 times higher cellular toxicity when fed with ammonia rather than nitrate	(Lim *et al.* 2010)
Temperature	Increased temperature resulted in increased toxin production	(Lim *et al.* 2006)

Figure 1: Diagram representing the different life cycle stages within *A. minutum* modified after Berdalet *et al.* (2017)

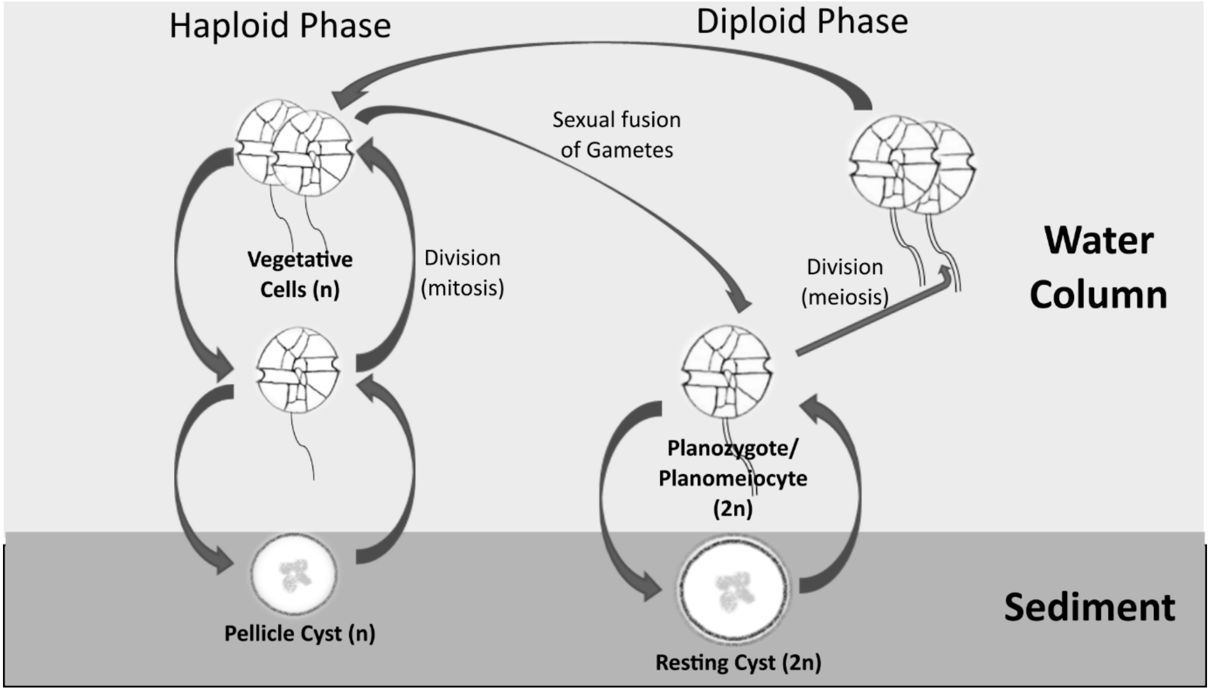


Figure 2: Map of the world showing locations from which *A. minutum* has been positively identified. In some areas with higher numbers of identification of occurrence points may overlap. Circles denote confirmed locations. Stars represent regions of occurrence listed in literature or reports but where original literature and definitive location is uncertain. Compiled from multiple sources, for full details of the literature used please see Appendix S1.

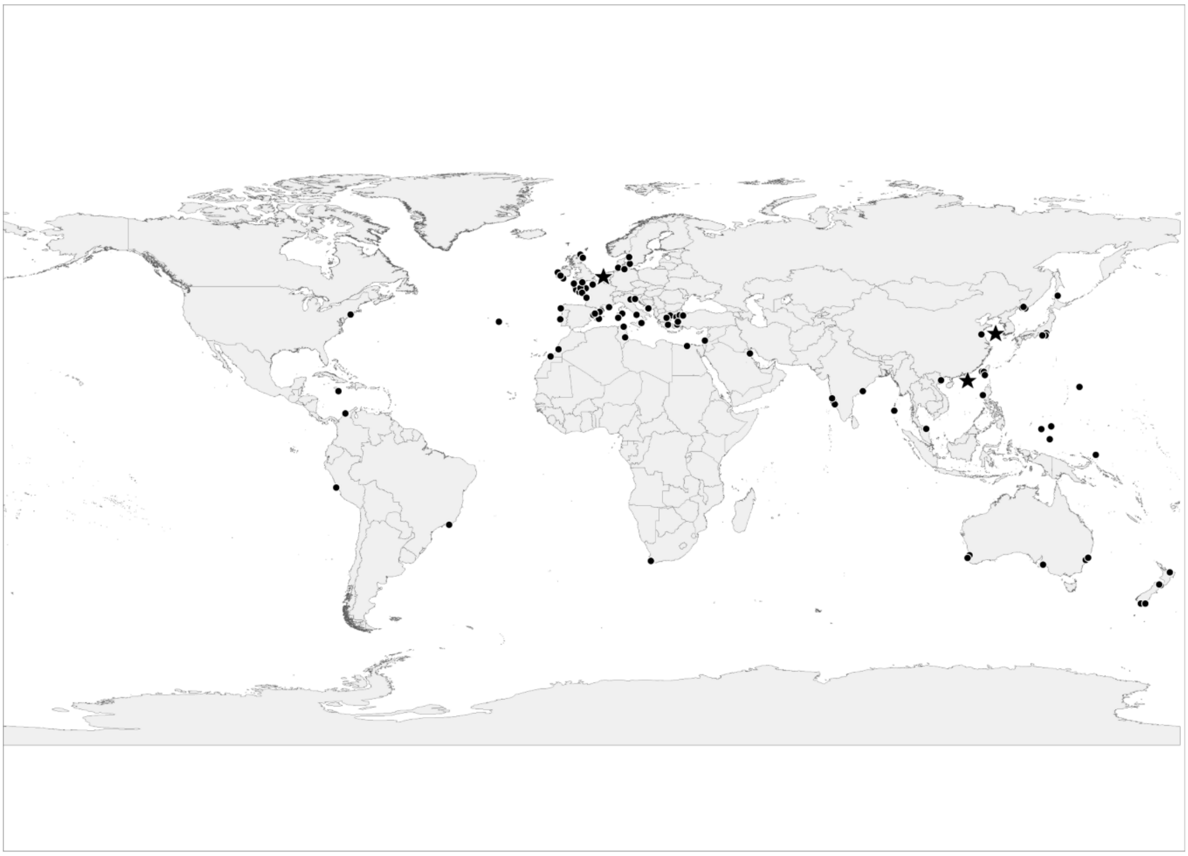
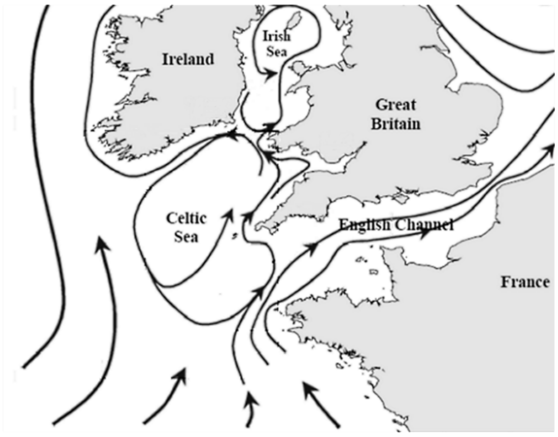


Figure 3: Map of the world displaying the geographic distribution of 5 individual toxin profiles as determined by K means clustering analysis. Inlay of NW Europe allows visualisation of the spread of multiple toxin profiles present within this region. Compiled from multiple sources, for full details of the literature used please see Appendix S1.

Figure 4: Map of Northern Europe displaying sites where *A. minutum* has been positively identified. Compiled from multiple sources, for full details of the literature used please see Appendix S1.

Figure 5: Map displaying prevailing currents around Great Britain, Northern France and Ireland, modified from Bailly Du Bois *et al.* (2002)



Appendix S1 – complete reference details for complex figures

Figure 2: Map of the world showing locations from which *A. minutum* has been positively identified. In some areas with higher numbers of identification of occurrence points may overlap. Circles denote confirmed locations. Stars represent regions of occurrence listed in literature or reports but where original literature and definitive location is uncertain. Compiled from: (Delgado *et al.* 1990, Hallegraeff *et al.* 1991, An *et al.* 1992, Yuki 1994, Chang *et al.* 1995, 1997b, Balech 1995, Franca *et al.* 1995, Franco *et al.* 1995, Marasović *et al.* 1995, Giacobbe *et al.* 1996, MacKenzie and Berkett 1997, Elbrachter 1998, Nehring 1998, Hwang and Lu 2000, Yoshida *et al.* 2000, Chen and Chou 2001, Godhe *et al.* 2001a&b, Yahia Kefi *et al.* 2001, Glibert *et al.* 2001, Guillou *et al.* 2002, Tillmann and John 2002, Usup *et al.* 2002, Yoshida 2002, Hansen *et al.* 2003, Garcés *et al.* 2004, Lassus *et al.* 2004, Montresor *et al.* 2004, Orlova *et al.* 2004, Lim and Ogata 2005, Nascimento *et al.* 2005, Vila *et al.* 2005, Bravo *et al.* 2006, 2008, 2010a, 2010b, López-Flores *et al.* 2006, Percy 2006, Harlow *et al.* 2007, Lim *et al.* 2007b, 2011, Maas *et al.* 2007, Menezes *et al.* 2007, Pitcher *et al.* 2007, Ranston *et al.* 2007, Touzet and Raine 2007, Touzet *et al.* 2007a, Abouabdellah *et al.* 2008, Touzet *et al.* 2008, Orlova and Morozova 2009, Brown *et al.* 2010, Chapelle *et al.* 2010, Alkawri and Ramaiah 2010, Satta *et al.* 2010, Uzar and Aydin 2010, Yang *et al.* 2010, Lozano-Duque *et al.* 2011, Silva *et al.* 2011, Baula *et al.* 2011, Abdenadher *et al.* 2012, Smida *et al.* 2012, Ignatiades 2012, Tang *et al.* 2012, Zina *et al.* 2012, D'Silva *et al.* 2013, Dhib *et al.* 2013, Farrell *et al.* 2013, Aydin and Uzar 2014, Santos *et al.* 2014, Dias *et al.* 2015, Aydin *et al.* 2015, Baylón *et al.* 2015, Balkis *et al.* 2016, Başdemir 2016, Bastianini *et al.* 2016, Bazzoni *et al.* 2016, Hii *et al.* 2016, Klouch *et al.* 2016a, Klouch *et al.* 2016b, Le Gac *et al.* 2016, Guallar *et al.* 2017, Narale and Anil 2017, Rubino *et al.* 2017)

Figure 3: Map of the world displaying the geographic distribution of 5 individual toxin profiles as determined by K means clustering analysis. Inlay of NW Europe allows visualisation of the spread of multiple toxin profiles present within this region. Compiled from: (Mascarenhas *et al.* 1995, Flynn *et al.* 1995, MacKenzie and Berkett 1997, Chang *et al.* 1997a, Béchemin *et al.* 1999, Hwang *et al.* 1999, 2003, Hwang and Lu 2000, Carreto *et al.* 2001, Moroño *et al.* 2001, Chen and Chou 2001, Guisande *et al.* 2002b, Parker *et al.* 2002, Guisande *et al.* 2002a, Hansen *et al.* 2003, Negri *et al.* 2003, Grzebyk *et al.* 2003a, Montresor *et al.* 2004, Chou *et al.* 2005, Nascimento *et al.* 2005, Wang *et al.* 2005, Lim *et al.* 2006, Percy 2006, Selander *et al.* 2006, 2008, Lim *et al.* 2007b, 2011, Menezes *et al.* 2007, Pitcher *et al.* 2007, Touzet *et al.* 2007a, Bergkvist *et al.* 2008, Touzet *et al.* 2008, Maas and Brooks 2010, Yang *et al.* 2010, 2011, Frangópulos *et al.* 2011, Fabioux *et al.* 2015; Stüken *et al.* 2015)

Figure 4: Map of Northern Europe displaying sites where *A. minutum* has been positively identified. Compiled from: (Elbrachter 1998, Nehring 1998, Arzul *et al.* 1999, Guillou *et al.* 2002, Hansen *et al.* 2003, Lassus *et al.* 2004, Nascimento *et al.* 2005, Percy 2006, Touzet *et al.* 2007b, Brown *et al.* 2010, Klouch *et al.* 2016, Guallar *et al.* 2017)