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Diversity and regional distribution of harmful algal events along the Atlantic margin of Europe

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Abstract

The IOC-ICES-PICES Harmful Algal Event Database (HAEDAT) was used to describe the diversity and spatiotemporal distribution of harmful algal events along the Atlantic margin of Europe from 1987 - 2018. The majority of events recorded are caused by Diarrhetic Shellfish Toxins (DSTs). These events are recorded annually over a wide geographic area from southern Spain to northern Scotland and Iceland, and are responsible for annual closures of many shellfish harvesting areas. The dominant causative dinoflagellates, members of the morphospecies '*Dinophysis acuminata* complex' and *D. acuta* are common in the waters of the majority of countries affected. There are regional differences in the causative species associated with PST events; the coasts of Spain and Portugal with the dinoflagellates

Alexandrium minutum and *Gymnodinium catenatum*, north west France/south west England/south Ireland with *A. minutum*, and Scotland/Faroe Islands/Iceland with *A. catenella*. This can influence the duration and spatial scale of PST events as well as the toxicity of shellfish. The diatom *Pseudo-nitzschia australis* is the most widespread domoic acid producer, with records coming from Spain, Portugal, France, Ireland and the UK. Amnesic Shellfish Toxins (ASTs) have caused prolonged closures for the scallop fishing industry due to the slow depuration rate of DA. Amendments to EU shellfish hygiene regulations introduced between 2002 and 2005 facilitated end-product testing and sale of adductor muscle. This reduced the impact of ASTs on the scallop fishing industry and thus the number of recorded HAEDAT events associated with ASTs. Azaspiracids (AZAs) are the most recent toxin group responsible for events to be characterised in the ICES area. Events associated with AZAs have a discrete distribution with the majority recorded along the west coast of Ireland. Ciguatera Poisoning (CP) has been an emerging issue in the Canary Islands and Madeira since 2004. The majority of aquaculture and wild fish mortality events are associated with blooms of the dinoflagellate *Karenia mikimotoi* and raphidophyte *Heterosigma akashiwo*. Such fish killing events occur infrequently yet can cause significant mortalities. Interannual variability was observed in the annual number of HAEDAT areas with events associated with individual shellfish toxin groups. HABs represent a continued risk for the aquaculture industry along the Atlantic margin of Europe and should be accounted for when considering expansion of the industry or operational shifts to offshore areas.

Keywords. Paralytic Shellfish toxins, Diarrhetic Shellfish Toxins, Amnesic Shellfish Toxins, Azaspiracid Shellfish Toxins, Ciguatera Poisoning, Mortality events, IOC-ICES-PICES HAEDAT

1. Introduction

Marine ecosystems represent an important source of cultural identity, food security and economic activity (Willis et al., 2018). In 2018 the capture of aquatic animals (fish, crustaceans, molluscs, etc.) in marine waters worldwide amounted to 84.4 million tonnes and aquaculture production was 30.8 million tons (FAO, 2020). Within Europe, fisheries and the development of the aquaculture industry since the 1960s has brought and sustained employment to remote coastal areas as well as maintaining ecosystem services and a social connection with the sea (Krause et al., 2020). The value of shellfish production within the European Union was valued at EUR 1.2 billion (~ $1.46 billion US) in 2017 while production of Atlantic salmon was valued at EUR 1.34 billion (~$1.63 billion US) (European Union 2019). Aquaculture also provides an important source of seafood protein for the European market through the production of farmed fish and shellfish (Gutierréz et al., 2020). While the fish farming industry is expected to expand in the future in some countries in the EU (STECF 2018), a 20% decline has been observed in shellfish production in Europe between the late 1990s and 2016 with revenue remaining low due to the small size of enterprises involved, imports from out with the EU and access to space (Avdelas et al., 2020). Harmful algal blooms (HABs), as well as factors such as pollution, diseases/parasites, climate change and ocean acidification have been identified as environmental threats which can challenge the economic performance of the EU mussel industry (Avdelas et al., 2020). The sea also represents an important source of well-being, recreation and tourism (Kūle et al., 2013, Fleming et al., 2014, Elliot et al., 2018) particularly in coastal areas. For example, in 2010 the UK estimated a market turnover associated with recreation and leisure activities in the marine environment of £2.74 billion (~$3.74 billion USD) with £1.29 billion (~$ 1.76 billion USD) gross value added (UKMMAS 2010).

In Europe, the first recorded impacts from HABs date from over 100 years ago and are circumstantially associated with human consumption of shellfish contaminated with toxins of microalgal origin (phycotoxins). The earliest reports of human illness and fatalities associated with Paralytic Shellfish Toxins (PSTs) come from the UK in 1827 (Ayres 1975) and Norway in 1901 (Yndestad and Underdal, 1985). Increased consumption of shellfish from the 1960s led to further reporting of events of human illness associated with PSTs with incidents reported from Portugal in 1946 and 1955 (Correia 1946, Pinto and Silva 1956) and the UK in 1968 (Ayres and Cullum, 1978). A major outbreak of paralytic shellfish poisoning in 1976 intoxicating 176 people from several European countries was traced to mussels imported from the same cultivation site in Galicia (Lűthy 1979). These events led to the establishment of the first European monitoring programmes aimed to protect human health and safeguard the mussel industry (Campos et al., 1982) and to Directive 79/923/EEC. The subsequent increasing risk of phycotoxins to human health led to the development and implementation of the Shellfish Hygiene Directive in 1991 within the European Union (EU 91/492, later regulation 854/2004 and now EU regulation 2019/627), to ensure the production of safe seafood products and the protection of human health. This directive and regulations oblige member states to monitor shellfish for the presence of phycotoxins as well as shellfish growing waters for the presence of causative microalgae. The directive prohibits the harvest and sale of product if toxin levels exceed defined regulatory limits and led to the establishment of formal routine monitoring programmes in all shellfish growing EU countries, with non-members Norway and Iceland following the same criteria. The implementation of this directive greatly increased the volume of data on phycotoxins in shellfish and toxin producing microalgae collected in Europe. For some countries, fulfilling the monitoring requirements of the Shellfish Hygiene Directive generated the first comprehensive datasets on toxin producing microalgae and toxin levels in shellfish from their coastal areas.

The farmed fish sector is the most economically valuable part of the aquaculture industry in Europe (Davidson et al., 2020, Froelich et al., 2020). An example of the economic impact associated with HAB events on the farmed fish industry is provided by the massive bloom of *Chrysochromulina leadbeateri* Estep, Davis, Hargreaves & Sieburth 1984 in Norwegian waters in 2019, which was estimated to have killed 8 million salmon, with a direct value of over 850 million NOK (approx. $100 million US). The inclusion of future sales losses, clean-up costs, extra mitigation, loss of tax revenue and requirements to fund unemployment/social benefits led to direct and indirect gross economic impact of the bloom estimated between 2.3 and 2.8 billion NOK (approx. $0.27 – $0.33 billion USD) (Kontali, 2020).

Phycotoxins in fish are an emerging problem across the globe. Ciguatera Poisoning (CP), contracted through consumption of fish contaminated with ciguatoxins (CTX), is the most commonly reported seafood borne illness related to natural marine toxins globally (Friedman et al., 2017). Previously CP was thought to be restricted to tropical and subtropical areas of the world's oceans (Pérez-Arellano et al., 2005), with cases in Europe associated only with returning visitors after local consumption in the tropics, consumption of fish imported into Europe or caught recreationally while abroad (de Haro et al., 2003, Epelboin et al., 2014, Mattei et al., 2014). Since 2004 endemic cases have been detected in the Canary Islands and the Selvagens Islands, resulting in a multilateral approach to examine the diversity of causative organisms and toxins, and to develop criteria for definitions of CP and responsive management actions (Friedman et al., 2017, Rodriguez et al., 2017, Bravo et al., 2019, Estevez et al., 2019, Sanchez-Henao et al., 2019).

There is an increasing need to understand the impacts of HABs and their effects on economies. The FAO (2020) has formally recognized the contribution of the marine environment plays in the production of seafood via aquaculture on a global level. Within the North Atlantic and adjacent waters, the International Council for the Exploration of the Sea (ICES), the world's oldest intergovernmental science organisation, has provided impartial evidence on the state and sustainable use of the seas and oceans since its founding in 1902. In 1992 the ICES Working Group on Phytoplankton and Management of their Effects summarised experiences from different countries dealing with negative impacts from HABs and produced recommendations for cost effective monitoring programmes to provide an early warning of HAB species and toxins to protect human health (ICES 1992). For the past 30 years members of the ICES Working Group on Harmful Algal Bloom Dynamics, co-sponsored by the Intergovernmental Oceanographic Commission of UNESCO (IOC) (ICES-IOC WGHABD), have been presenting annual national reports of harmful algal events in their respective countries. This led to the development of the IOC-ICES-PICES Harmful Algal Event Database (HAEDAT) to archive summaries of these national reports. HAEDAT is an open access database that places these data in the public domain to facilitate access by scientists, managers and policy makers.

This study herein accesses data from HAEDAT along with a literature review to present for the first time a regional overview of the diversity and spatial distribution of harmful algal events that affect the Atlantic coast of Europe. HAEDAT data is also used to present temporal and spatial changes since the 1980s.

2. Methods

2.1 The IOC-ICES-PICES Harmful Algal Event Database (HAEDAT)

HAEDAT (http://haedat.iode.org/) was developed in the 1990s and contains more than 8,000 entries on harmful algal events from across the globe. Within Europe, data since the 1980s associated with monitoring programmes and *ad hoc* reports have been entered. Records prior to 1980 exist in the scientific literature, national monitoring data sets and public press .

Within HAEDAT a harmful algal event follows a strict definition and must be associated with a negative impact or management action resulting from; (i) a water discoloration, scum or foam causing a socio-economic impact due to the presence of toxic or non toxic microalgae; (ii) phycotoxin accumulation in seafood above levels considered safe for human consumption; (iii) any event where humans, animals or other organisms are negatively affected by microalgae; or (iv) precautionary closures of shellfish harvesting areas based on predefined thresholds of causative phytoplankton cells in the water.

HAEDAT is not a database of cell counts, records of the presence of harmful species, or toxin concentrations. Information about phycotoxin concentrations below regulatory criteria is not entered. It is important to note that HAEDAT does not record negative results. Where no records exist, it is not possible to distinguish between an absence of events or a lack of monitoring.

National editors collate data from regulatory monitoring bodies, industries and *ad hoc* reports into individual ‘events’. The information on individual events is recorded over two pages with required information on dates, type of impact, area code, point of contact. Optional information such as maximum microalgal cell densities and toxin levels, species impacted and geographic range covered, and environmental information such as temperature, salinity, and chlorophyll concentrations can also be entered. In many instances only the genus level cell densities are reported where species identifications are difficult e.g. for *Alexandrium* and *Pseudo-nitzschia* spp. The data are searchable by country, region, syndrome and year. Points to note when interpreting HAEDAT data from Europe are given in Suppl_ 1.

2.2 Assembling a HAEDAT record

A central tenet behind defining an ’event’ in HAEDAT is that it is associated with a specific bloom of harmful algae and that the impacts recorded and/or management actions taken are directly related to this bloom. This association requires a degree of expert judgement and as a result, recorded harmful algal events in HAEDAT can be subjective in terms of how many events are entered and their duration. In some instances, blooms and their impacts can be discrete, impacting a local area for only a few days or weeks and are easily categorised. In contrast, some species such as *Dinophysis acuminata* Claparède & Lachmann 1859 have long growing seasons and yield blooms that can be transported in and out of coastal areas with changing wind intensity and direction, and water masses. This can result in discrete periods when toxin concentrations exceed regulatory limits interspersed with periods when toxin concentrations decline and harvesting of shellfish beds is allowed to resume. This can make characterisation of these blooms into a single event for HAEDAT subjective. Some countries record each closure period as an individual event whereas others record the closures over the whole growing season as one event.

Some countries enforce precautionary closures in some areas based on putatively toxigenic phytoplankton cell counts exceeding defined trigger levels as an early warning until the results of toxin analysis are obtained. Precautionary closures based on phytoplankton trigger levels are frequently used in areas where wild shellfish banks are harvested as these are more difficult to sample than aquaculture farms.

2.3 Introduction of area codes

A major modification to the database took place in 2003 when the coastline of each country was divided into ‘areas’ approximately 200km in length and each assigned a unique area code. This allowed events extending over wide spatial areas to be reported as a single event, spanning multiple area codes. Prior to this, these widespread events had been entered as multiple individual records covering different areas. Figure 1A shows the central point of the area codes included in this study. Reports from the Canary Islands, the Azores archipelago, the Madeira archipelago and the Selvagens Islands are included in this study. Figure 1B presents the geography of Macaronesia. The Selvagens Islands are assigned to the HAEDAT area code that includes the Madeira archipelago (see Figure 1A/1B).

2.4 Distribution of data entry

Some countries have not been able to consistently contribute to HAEDAT and their datasets are therefore incomplete. The lack of HAEDAT entries from these countries does not mean that no harmful algal events have occurred, and the paucity of entries may represent an incomplete dataset. For example in the area studied herein, the low number of reported events from Iceland and Faroe Islands indicate incomplete datasets.

2.5 Phycotoxins in Shellfish

Within the European North Atlantic area included in this study there are 2,345 HAEDAT records until the end of 2018. Of these ~80% relate to management actions associated with levels of phycotoxins in shellfish flesh (referred to here as shellfish toxins) exceeding regulatory limits. This means monitoring effort and thus the potential to detect events can be influenced by the scale and intensity of aquaculture and wild shellfish harvesting. Figure 2 shows the current distribution of sites for routine monitoring of shellfish toxins within the study area.

Monitoring for phycotoxins associated with different toxin syndromes began at different times in different countries. For example routine monitoring for PSTs began between 1986 and 1995 with some countries monitoring local areas since the 1960s, DSTs between 1986 and 1992, ASTs between 1995 and 1999 (Table 1). All analytical and assay methods currently employed for the quantitative and qualitative monitoring of phycotoxins in shellfish detection have undergone progressive refinement, standardization and/or replacement within the past three decades. In some instances regulatory limits have changed e.g. yessotoxins (YTXs) changed from 1 mg kg^{-1} in 2002 to 3.75 mg kg^{-1} in 2013. Furthermore, individual countries have adopted new methodologies and protocols within their respective regulatory framework at different rates. This is problematic for the direct comparison of toxin data recorded in national databases. Within Europe, general adoption of standardized reference methods certified by AOAC and/or European regulatory authorities, and greater availability of certified analytical toxin standards and reference materials, has alleviated many of these discrepancies. For example, standardised liquid chromatography -ultra violet (LC-UV) methods are now used for analysis of amnesic shellfish toxins (ASTs), whereas almost complete replacement of mouse (MBA) or rat (RBA) bioassays for paralytic shellfish toxins (PSTs) and lipophilic toxins (LTs) have taken place. LTs are now routinely monitored by LC coupled to tandem mass spectrometry (LC-MS/MS) whilst PSTs are analysed using an approved LC- fluorescence detection (LC-FD) method. Metadata on the monitoring methods from countries involved in this study are presented in Table 1A. Current regulatory limits for shellfish toxins are presented in Table 1B.

2.6 Fish kills

The mechanism for reporting fish mortalities is less formal than that for shellfish toxins, being reliant on industry reporting local events at sites of aquaculture operation and members of scientific agencies or the public reporting mortalities of wild fish. Whilst most major mortality events *are reported* to national agencies (sometimes with a time lag), some kills of wild or farmed fish occur where the association with phytoplankton blooms may be ambiguous or happen in remote areas, thus such events may be underreported.

2.7 High biomass blooms, benthic mortalities, scums and foams

Impacts from high biomass blooms are poorly recorded in HAEDAT as there is no category to specifically record these events. The classification ‘high phytoplankton count’ has been used and in some instances these blooms are simultaneously recorded in the ‘water discolouration’ and ‘mass mortality’ categories. Also included in the ‘high phytoplankton count’ are records of when phytoplankton trigger levels have been exceeded and have been associated with shellfish toxin events. Some records of high phytoplankton counts have been entered in HAEDAT which are not associated with any known harmful event. Thus the ‘high phytoplankton count’ category needs to be screened to remove these records when investigating high biomass bloom events that impact the ecosystem. France in the only country in the study area with a formal monitoring programme (*Phenomer* https://www.phenomer.org/) for reporting water discolourations (Siano et al., 2020) and these data have been included in HAEDAT. In other countries reports of water discolourations, scums, foams and benthic mortalities are reliant on members of the public providing information to national editors and may be underrepresented in HAEDAT.

2.8 Ciguatera poisoning events

Only ciguatera poisoning (CP) events arising from consumption of endemic fish are currently recorded in HAEDAT from Europe. Two records of fishery closures to protect human health from CP have been entered from Portugal (Selvagens Islands) in 2013 and Spain (Canary Islands in 2016). HAEDAT records from Europe have been associated with human poisoning outbreaks associated with individual contaminated fish species such as *Balistes capriscus*, *Canthidermis sufflamen, Epinephelus* sp., *Mycteroperca fusca*, *Pagrus pagrus*, *Pomatomus saltatrix*, *Seriola dumerili, Seriola fasciata* and *Seriola rivoliana*.

2.9 HAEDAT metrics

Due to way that HAEDAT has evolved since its inception, the number of HAEDAT events is not used as a metric within the ICES-IOC WGHABD dataset. Instead within the North Atlantic area, the metrics used are the number of years with events per area code, or the number of area codes with events. For a more robust analysis, it is recommended that the national editors be contacted for access to the raw phycotoxin/microalgal monitoring datasets and comprehensive metadata. It is important to avoid double counting of events due to inconsistent event assembly, e.g. entering multiple records from one area, when they should be combined as one. HAEDAT data from 1998 onwards should be used when examining changes over time from the countries in this study.

2.10 Production of maps, bar charts and tables

The maps in Figures 4 - 10 show the number of years with events per area code for each toxin syndrome. HAEDAT data from Iceland, the Faroe Islands, UK, Ireland, France (area codes 1 – 11), Spain (area codes 1-11, 28 -29) and Portugal have been used (Figure 1A). The total number of years with HAEDAT events per area code up until 2018 has been assembled and plotted using R. Maps of HAEDAT data from 1999 until the end of 2018 is presented in five year blocks to show regional changes over time. Barcharts showing the total number of area codes with events per year since monitoring began are plotted in Figure 3. These barcharts are plotted per country to avoid differences between countries in interpreting and assembling a harmful algal event biasing trends over time.

The incidence of human illness in the study area is summarised in Table 2 gathered from medical and epidemiological reports, and the scientific literature. The maximum toxin levels recorded in shellfish from the study area has been gathered from the raw monitoring data is presented in Table 3. Table 1A highlights the differences between countries in reporting units for the different toxin groups and so the units in Table 3 have been standardised to the reporting units described in regulation 853/2004 to provide consistency between countries. Harmful algal species from the IOC-UNESCO Taxonomic Reference List of Harmful MicroAlgae (http://www.marinespecies.org/hab/) as well as species associated with mortality events recorded in the study area are listed in Table 4.

3. Results

3.1 HAEDAT areas with events

Figure 3 shows the number of HAEDAT area codes per year where harmful algal events have been recorded in this study from 1987 to 2018. The data in Figure 3 is sensitive to monitoring, reporting effort and efficiency. In some countries how events have been assembled and entered in HAEDAT have changed over time. For example, in the HAEDAT dataset from Ireland the criteria for assignment of events changed from 2002 (see Suppl. Information 1) which resulted in a decrease in the number of HAEDAT area with events, e.g. for DSTs (Figure 3E) which can give the appearance of a declining trend. In the UK a reduction in monitoring effort during 2005 means that DST events may be underrepresented during that year (Figure 3D).

Figure 3 shows the interannual variability in the number of HAEDAT areas with events associated with different toxin types. Events associated with DSTs have been recorded in multiple HAEDAT areas during most years whereas fewer areas have experienced events associated with PSTs and they are more regionally and temporally variable. Ireland exhibits the highest number of HAEDAT areas with AZT events (Figure 3E), while Spain and

Portugal are the only countries where events associated with endemic CP have been recorded (Figures 3 A and B).

In some countries the introduction of toxin monitoring for new syndromes e.g. introduction of monitoring for ASTs, or detection of new syndromes such as CP resulted in an increase in the total number of areas with events per year. The start dates of routine monitoring for the different toxin syndromes is given in Table 1A and this is reflected in the individual bar charts of the countries from when events are first recorded, e.g. 1995 and 1998 for AST events in Spain and the UK, respectively (Figure 3). Ireland and the UK show a reduction in the number of areas with AST events since monitoring started.

3.2 Harmful Algal Events

In this section the spatial and temporal distribution of harmful algal events using data from HAEDAT are described as well as their incidence prior to HAEDAT, causative organisms and their ecology.

3.21 Paralytic Shellfish Toxins (PST) Events

3.211 Event description

Consumption of shellfish contaminated with Paralytic Shellfish Toxins (PSTs) can result in the human illness, Paralytic Shellfish Poisoning (PSP). The earliest reports of human illness and fatalities putatively associated with PSTs in northern Europe were from the UK between 1827 and 1909, (Ayres 1975), Ireland in 1872 and 1890 (Ayres 1975), Portugal in 1946 and 1955 (Correia 1946, Pinto and Silva 1956) and the UK in 1959 (Ayres 1975). In 1968 78 people in the UK were admitted to hospital with symptoms of PSP (Ayres and Cullum, 1978) and in Galicia, Spain in 1976, a major PSP outbreak poisoned 176 people from several European countries (Lűthy 1979, Gestal et al., 1980). These events led to the establishment of the first European monitoring programmes aimed to protect human health and safeguard the shellfish industry (Campos et al., 1982). In 1984 four people became ill in the Faroe Islands in 1984 after eating mussels containing high levels of PST toxins (Mortensen 1985).

The incidence of human illnesses associated with PSTs within the study areas are summarised in Table 2.

HAEDAT records of PST events from the region in this study start from the mid-1980s – 1990s (see Table 1). The majority of records are high concentrations of PSTs exceeding regulatory limits and have resulted in closures of shellfish harvesting areas. Figures 3 and 4 show that concentrations of PSTs regularly exceed regulatory limits, although these events are not reported annually in all areas. The maximum toxin levels recorded for different shellfish species are presented in Table 3. Maximum levels have been recorded in mussel species (*Mytilus edulis, M. galloprovincialis)* while PST levels in oyster species (e.g., *Ostrea edulis*) are relatively low. Figure 4 (A – E) shows that PST events are not continuously distributed in areas with routine monitoring, but have been recorded along the Atlantic coast of Spain and Portugal, northwest France, (southern Ireland, south west of the UK, and the east and west coast of Scotland and Orkney and Shetland islands. There are some HAEDAT areas where PSTs have not been detected above regulatory limits since routine monitoring began, such as along the east and west coasts of Ireland and the eastern part of the English Channel. Records of events are sparse where monitoring effort is low (Figure 2), such as the southern part of the Bay of Biscay and the south east coast of England.

Records from the Faroe Islands are incomplete; however, a closure of a shellfish harvesting area was enforced in 1989 due to high levels of PSTs. In Iceland, there was a precautionary closure of shellfish harvesting areas in 1994 due to high cell densities of the dinoflagellates *Alexandrium catenella* (Whedon & Kofoid) Balech, 1985 (formerly identified as *Alexandrium tamarense* (Lebour) Balech 1995) and *A. ostenfeldii* (Paulsen) Balech & Tangen 1985. Events associated with PSTs have been recorded in the HAEDAT data from Iceland entered since 2016.

Since monitoring of shellfish for the presence of PSTs became routine across Europe, there have been six incidents of human illness associated with PSTs from the Atlantic coast (Table 2), from Spain in 1993 and 1994 (DXSP, 1996), and mainland Portugal in 1994, 2007, 2018 due to ingestion of PST-contaminated wild-harvested shellfish (cockles, clams). These Iberian mainland PST events coincided with blooms of the dinoflagellate *Gymnodinium catenatum* H.W.Graham 1943 (de Carvalho et al., 1998, Rodrigues et al., 2012, de Carvalho et al., 2019). In São Jorge Island in the Azores archipelago, a bloom of *A. minutum* Halim 1960, in 2013 caused a multifaceted PST-event including human illness (Santos et al., 2014). PSTs have also been associated with a few mortality events impacting both wild and farmed fish and domestic animals such as dogs (section 3.2713).

3.212 Causative organisms

The diversity of PST producing organisms in this study area are listed in Table 4. Regional diversity exists, with three dinoflagellate species responsible for the PST events along the Atlantic coast of Europe although additional PST toxin producers have been recorded. Along the west coast of the Iberian Peninsula the dominant species associated with PST events is the dinoflagellate *G. catenatum*. Within Europe, high cell abundances of *G. catenatum* are restricted to the Atlantic coasts of Spain and Portugal, with further occurrences in the Mediterranean Sea (Jaén et al., 2008). The toxin profile of *G. catenatum* in Spain and Portugal is distinct from that produced by species of *Alexandrium* and is dominated by the N-sulfocarbamoyl toxins (C1&C2; C3 & C4), the gonyautoxins GTX5&6 (Anderson et al., 1989, Oshima et al 1993) and the hydroxybenzoate toxins GC 1&2, 3 and 6. Other PSTs from these same sub-classes, or the decarbamoyl group, may also be present in *G. catenatum* from Iberia, but made up only a low proportion of the total toxin composition (Costa et al., 2015).

Alexandrium minutum has a wide geographic range in Europe and has also been responsible for distinct PST events in the Iberian Peninsula (Vale et al., 2008), where it has bloomed frequently in the northern and southern Rias of the Galician coast as well as in Óbidos lagoon in Portugal. *Alexandrium minutum* is a key cause of PST events on the Atlantic coast of France, and along the English Channel, where it affects the southwest UK, and the south of Ireland (Nascimento et al., 2005, Percy 2006, Touzet et al., 2010, Lewis et al., 2018). Geographic variability is apparent in the toxin profile of *A. minutum*, with strains from north west Spain producing GTX1 and GTX4 as the principal toxins (Franco et al., 1995), whereas GTX2 and/or GTX3 are more dominant in strains from Ireland, southwest UK and France (Lewis et al., 2018). In Ireland and the UK both toxigenic and non-toxigenic strains of *A. minutum* have been recorded (Touzet et al., 2007, Brown et al., 2010). There is a clear relationship between the presence of toxigenic *A. minutum* and PST events in the south of Ireland and south west of the UK (Touzet et al., 2007, Lewis et al., 2018).

Further north, PST events on the west and east coasts of Scotland, and including the Orkney and Shetland Islands, have been associated with *A. catenella* (Whedon & Kofoid) Balech 1985*.* This toxin-producing species has previously been designated as *Gonyaulax excavata* (Braarud) Balech 1971, *A. fundyense* Balech 1985, *A. tamarense* North American ribotype and/or *A. tamarense* Group I ribotype before taxonomic studies reassigned this taxon to *A. catenella* (Higman et al., 2001, Collins et al., 2010, John et al., 2014, Fraga et al., 2015). The PST profile of *A. catenella* (reported as *A. tamarense* Group I) from Scotland contains a range of congeners, being saxitoxin (STX), neosaxitoxin (Neo), gonyautoxins GTX1&4 and C1&2; other detected toxins included gonyautoxins GTX2&3 as well as GTX5 (=B1) (Brown et al., 2010). PST-producing *A. catenella* (identified as *A. tamarense*) has also been reported from the Faroe Islands (Mortensen 1985, Moestrup and Hansen 1988) and Iceland (Burrell et al., 2013).

3.213 *Ecology*

Along the western Iberian Peninsula the timing of *G. catenatum* blooms has been associated with relaxation of upwelling events at the end of summer and subsequent advection into coastal areas (Fraga et al., 1988, Bravo et al., 2010, Hallegraeff et al., 2012). *Gymnodinium catenatum* populations growing offshore on the Lisbon shelf can be carried poleward as upwelling relaxes and surface flow carries vegetative cells northward. As these cells are transported onshore they proliferate in coastal waters and have been detected along the northwest Portuguese coast (Pitcher et al 2010, Botelho et al., 2019). If these advected populations reach the Galician coast of Spain, *G. catenatum* blooms are enhanced within the complex rias system where the bays and inlets act to concentrate advected *G. catenatum* populations originating in shelf waters (Tilstone et al., 1994). In addition, there is evidence for locally driven populations in lagoons in Portugal (Brito et al., 2012), as well as within other embayments where they can proliferate alongside upwelling boundaries, being retained and concentrated by local water circulation. The first record of *G. catenatum* cysts in the sediment in this area comes from the late 1800s in southern Portugal. The mode of entry for this species into Portuguese waters is not clear, with advection from the African coast or introduction by ships ballast both suggested as mechanisms (Ribeiro et al., 2012).

The majority of *A. minutum* blooms are observed in coastal embayments e.g. within the Galician Rias and along the Portuguese coastl (Bravo et al., 2010, Franco et al., 1992) . The Bay of Brest in northwest France has been subject to closures of shellfish harvesting areas almost annually since the occurrence of a bloom of *A. minutum* in 2012 when PSTs in shellfish exceeded regulatory limits (Chapelle et al., 2015, Belin et al., this issue). Molecular methods have shown cysts *A. minutum* have been present in the area since the late 1800s becoming more abundant during the last 20-30 years and are currently widespread (Klouch et al., 2016a, 2016b). Cysts beds of *A. minutum* are also present in the Fal Estuary in the south west of England (Blanco et al., 2009) and Cork Harbour in the south of Ireland (Touzet et al., 2007, Ní Rathaille et al., 2008) where blooms of vegetative cells are responsible for closures of shellfish harvesting areas.

The role of cyst beds in seeding *A. catenella* blooms in the UK remains to be fully investigated. Studies in the 1990s revealed high cyst abundances in northeast England and the Firth of Forth on the west coast of Scotland (Lewis et al., 1995, Brown et al., 2010), but there has not been a subsequent investigation to determine if cyst abundances have declined or if this influenced vegetative cell densities of *Alexandrium* and hence PST levels in shellfish. A cyst study from the northwest coast of Iceland found moderate quantities (maximum ca. 400 cysts cm^3 of surface sediments) of *A. catenella* (reported as *A. fundyense*) cysts (Richlen et al 2016) in association with PSTs in bivalve shellfish (e.g., the Arctic clam *Arctica islandica*) (Cembella et al., 2016). Laboratory studies on the germination and growth kinetics of cultures from cysts from Icelandic fjords (Richlen et al., 2016) support the hypothesis that the conditions in shallow Icelandic fjords would be suitable for proliferations of *A. catenella* from indigenous, self-seeding blooms in these areas.

3.214 *Changes over time*

The first published report of *G. catenatum* as the suspected agent of the 1976 PSP event in Galicia was based on the taxonomic identification of this chain-forming dinoflagellate (Estrada et al.,1984) that was newly observed in European waters. This species was not reported again in Iberia until 1985 (Fraga et al.,1988), then occurred annually until 1995 but was subsequently not observed until 2005 (Vale et al., 2008). Interannual variability in the number of area codes with PST events from the HAEDAT areas in Portugal and Spain reflect this pattern of *G. catenatum* even though PST events associated with *A. minutum* are included (Figure 3A - B) and also in the dearth of areas with PST events along the Portuguese coast between 1999 and 2004 (Figure 4B).

Further north, *A. minutum* is associated with PST events along the Atlantic coast of France. Figures 4(B– E) shows that PST events are not recorded every year in that area with a six-year period (2004 – 2009) within which no PST events were reported (Figure 3C).

A. minutum is also responsible for PST events in southwest England, the Channel (Nascimento et al., 2005, Percy 2006, Lewis et al., 2018), and Cork Harbour in southwest

Ireland (Touzet et al., 2010). Interannual variability has been observed in the occurrence of PST events in these areas (Figure 3, Figure 4 B-E). Although *A. minutum* has been associated with management actions involving shellfish harvesting closures in southwest England the majority of PST events within the UK have been recorded from Scottish waters, including the North Sea and east and west coasts, and the Orkney and Shetland islands. High PST reports in shellfish in Scottish water are invariably (when confirmed) associated with *A. catenella.* Very high PST levels spatiotemporally linked with a bloom of *A. catenella* were recorded on both the east and west coasts of Scotland in 1990 (maximum >10,000 µg STXeq kg^{-1}). The number of areas with recorded PST events increased from 1990 as monitoring expanded to deal with the apparent enhanced risk from PSTs. The number of areas with events then declined until 2004 due to a decline in the recorded levels of PSTs in shellfish from Scotland (Bresnan et al., 2008) when there were no management actions for PSTs enforced for Scottish waters, but subsequently increased after 2011 (Turner et al., 2014). This pattern of events is captured in Figure 3D. The number of sites monitored on the Orkney Islands declined from 2000 with shellfish from only two sites being received in recent years (Marine Scotland/Food Standards Scotland unpubl data).

3.22 *Diarrhetic Shellfish Toxins (DST) Events*

3.221 Event description

The first records of human illness apparently associated with what were later defined as Diarrhetic Shellfish Toxins (DSTs) (Yasumoto et al.,1978) came from the Netherlands in 1961, with a further four events recorded from the Netherlands (Kat, 1983), and also from Spain by 1981(Campos et al., 1982) (Table 2). Among all phycotoxin-related harmful algal events, those associated with DSTs have had the biggest impact along the Atlantic coast of Europe since routine monitoring began, both in terms of geographical distribution and frequency. Figure 3 shows that DST events were recorded in the most HAEDAT areas in this study. The majority of events are associated with closures of shellfish harvesting areas, historically due to DST-positive mouse or rat bioassays results, and since the introduction of analytical chemical methods into the European regulatory framework to the presence of DSTs above regulatory limits (see Table 1A). The maximum levels of DSTs in bivalve shellfish species from the study area can be seen in Table 3.

The DSTs are considered within a wider group of phycotoxins, termed Lipophilic Toxins (LTs), because of their high extractability in relatively non-polar organic solvents for application in lipophilic MBA and chemical analytical methods. In addition to DSTs the general classification LT comprises structurally diverse groups of phycotoxins, e.g. yessotoxins (YTX), pectenotoxins (PTX), cyclic imines, including spirolides (SPX) and pinnatoxins, and azaspiracids (AZA). Members of these various LT groups vary widely in specific potency in the lipophilic MBA, mode of action, and respective toxicity syndromes elicited in human consumers of contaminated seafood. Interpretation of toxicity risk of LTs from the MBA is further complicated by the fact that multiple groups they may co-occur within seafood and may be derived from different toxigenic source organisms.

Strictly structurally defined, DSTs are restricted to okadaic acid (OA) and related dinophysistoxin (DTX) analogues, but early work based on DSP MBA and RBA toxicity included PTXs and often also YTXs in the “DSP toxin group”. Confusingly, not all structurally defined DSTs are diarrheagenic (or the property is unknown), and many LTs cause “DSP-like” symptoms (e.g., abdominal pain, cramps and diarrhoea) but are defined within other syndromes.

True DSTs known to cause DSP in humans and found in shellfish with the northeastern Atlantic margin region are most commonly OA, DTX1 and DTX2, together with their esterified counterparts, e.g. members of the DTX3 sub-group. The presence of AZAs in shellfish has also been linked to “DSP-type” events in Europe (Alexander et al., 2008; Twiner et al., 2008) given the diarrheic symptomology associated with Azaspiracid Shellfish Poisoning (AZP) (Blanco et al., 2017). Pectenotoxins (PTXs) and yessotoxins (YTXs) are also toxic to mice following intraperitoneal administration in the lipophilic MBA, but there is no evidence for any oral toxicity in humans (Munday, 2014; Miles et al., 2004; Torgersen et al., 2008).PTXs are currently included in the OA-group toxins during the calculation of total OA equivalents per kg of shellfish tissue but are in the process of being deregulated. Levels of YTXs are low in bivalve shellfish from this study area (Table 3). There have only been two incidents of YTXs (2011 and 2012 on the west coast of Scotland) above the regulatory limit (1mg kg^{-1}) (Dhanji- Rapkova et al., 2019).

Until 2011, all DSP regulatory controls in Europe were performed with the DSP MBA, which provided only a qualitative indication of OA/DTX toxins plus a range of neurological clinical signs indicating the possible presence of other LTs, such as AZAs and YTXs (Ito et al., 2000) and YTXs (Dominguez et al., 2010). Subsequently, quantitative LC-MS/MS has been adopted as the EU reference method for monitoring all regulated LTs (McNabb et al., 2005), enabling the accurate assessment of LT levels in shellfish marketed in Europe, including the OA/DTX group specifically associated with the DSP syndrome (EURLMB, 2015).

Figure 5 A-E shows that the distribution of DST events is widespread along the Atlantic coast of Europe for the last 20 years. HAEDAT areas with less Atlantic water influence and are more tidally mixed, e.g. the southeast coast of England and the Irish Sea coast have had fewer DST events recorded. Areas with few or no shellfish farms do not have many recorded HAEDAT events. For example, commercial aquaculture has only recently been implemented in open marine waters of the Basque region of northern Spain and thus reported data for this area are still scarce. This does not mean that harmful species/shellfish toxins have not been present in these regions, but rather that shellfish have not been commercially harvested at a large scale, and thus the sites have not been monitored frequently or required to implement management actions. No management actions associated with DSTs have been recorded from the Azores or Canary Islands, although okadaic acid (OA) and other DSTs have been recorded in several bivalve shellfish species and other vectors in the Azores archipelago (Silva et al., 2015).

Human illness associated with DSTs (Diarrhetic Shellfish Poisoning (DSP)) in this study area have been reported in most countries in this study area, and is the toxin syndrome responsible for the most incidents. Three events before monitoring of shellfish for DSTs impacted more than 1,000 individuals (Spain 1981, France 1983 & 1984). Since monitoring became routine, the number of individuals afflicted with DSP has been considerably less. These incidents are summarised in Table 2.

3.222 Causative organisms

Diarrhetic shellfish toxins are produced by certain species within the genera *Dinophysis* Ehrenberg, 1839 and *Prorocentrum* Ehrenberg, 1834*.* Table 4 shows that a diverse range of potentially toxigenic *Dinophysis* species are found along the Atlantic coast of Europe. *Phalacroma rotundatum* (Claparéde & Lachmann) Kofoid & J.R.Michener, 1911 (formerly *D. rotundata* Levander, 1894) has been included in the table as it is on the IOC-UNESCO Taxonomic Reference List of Harmful MicroAlgae (http://www.marinespecies.org/hab/) although it has not been associated with harmful events in this region and evidence for toxigenicity of this heterotrophic species is highly doubtful (Cembella et al., 1989; Pleasance et al., 1990; González-Gil et al., 2011). The dominant species responsible for DST accumulation in bivalve shellfish along the Atlantic coast of Europe is *Dinophysis acuminata*, with *D. acuta* Ehrenberg, 1839 also an important contributor of DSTs. Two additional related species, *D. sacculus* F.Stein, 1883 and *D. ovum* (F.Schütt) T.H.Abé, with morphology similar to *D. acuminata* are also recorded in the region. Detailed studies of *D. acuminata* and *D. sacculus* have shown that these species can be distinguished by the shape of their hypothecal plates (Zingone et al., 1998), but intermediate morphological forms have been observed in samples from Galicia, Spain (Bravo et al., 1995). Raho et al., 2008 used morphological characteristics and molecular analysis to distinguish between *D. acuminata* and *D. ovum*, finding that the mitochondrial cytochrome oxidase 1 (*cox1*) gene could distinguish between them. Studies on Korean strains of *D.*

acuminata and *D. ovum*, however, could not categorically discriminate between the two species by morphological criteria and the *cox1* gene (Park et al., 2019). This complicates the definitive attribution of DST events to particular taxa within the *Dinophysis* genus. Such morphological variability has led to these species for many years being referred to as the "*Dinophysis acuminata* complex" (Lassus and Bardouil, 1991, Reguera et al., 2012). Nevertheless, some monitoring programmes separately record individual species in routine monitoring programmes based on their morphology, as notated in Table 4.

Dinophysis species occurring within the North Atlantic margin region may produce a diverse array of DSTs and other LT analogues. Both *D. acuminata* and *D. acuta* produce OA, with *D. acuminata* also producing dinophysistoxin-1 (DTX 1) and PTXs; *D. acuta* more often associated with production of DTX 2, but in some instances along the Iberian Peninsula this species was also found to produce PTXs (Fernandez et al., 2006). *Dinophysis caudata* Saville-Kent, 1881 and *D. sacculus*, both known producers of OA and DTXs, along with *D. tripos* Gourret, 1883, a PTX producer, exhibit a more southerly distribution in Europe, along the Iberian and French Atlantic coasts (Table 4). Recently, *D. tripos* has been found in increasing cell abundance and frequency in Irish waters (Salas and Clarke, 2019), and occasionally cells are recorded in waters in the west of Scotland (S. Swan pers. comm.).

Dinophysis norvegica Claparède & Lachmann, 1859 is typically recorded in more northerly waters of the northeastern Altantic, e.g., Iceland and the Faroe Islands, although there are a small number of records from further south (Table 4). *Dinophysis norvegica* can produce OA and DTX1 (Lee et al., 1989), as well as PTX (Miles et al., 2004). Recently *D. norvegica* from Maine in the USA has been found to produce dihydro-DTX1 (Deeds et al., 2020). OA and DTX 1 are particularly associated with blooms of the species and DST events recorded in HAEDAT from the Norwegian Sea and western coast of Sweden (see Karlson et al., this volume and references cited therein).

Benthic species of the dinoflagellate genus *Prorocentrum* Ehrenberg are globally known as diverse sources of DSTs (Durán-Riveroll et al., 2019) but unlike certain *Dinophysis* spp., do not apparently produce PTXs. *Prorocentrum lima* (Ehrenberg) F.Stein, 1878 is a confirmed producer of DSTs in the coastal waters of the UK (Foden et al., 2005), Galicia, Spain (Bravo et al., 2001) and Portugal (Vale et al., 2009), and is also recorded in the study area (Table 4). *Prorocentrum hoffmannianum* M.A.Faust, 1990 has also been identified as an OA-producer in the Canary Islands (Rodríguez et al., 2018) and *P. cf rhaythmum* A.R.Loeblich III, Sherley & Schmidt, 1979 has been recorded in Portugal (Table 4). There are no confirmed DST events associated with these species in the Atlantic waters of Europe. Phytoplankton monitoring focuses on pelagic species and thus incidents associated with benthic or epiphytic species may be underreported.

3.223 Ecology

Members of the *Dinophysis acuminata* complex are coastal species and tolerant of a wide range of environmental conditions. They are present over a long growing season, in some areas occurring from spring to late summer/early autumn (Reguera 1993, Reguera et al., 2012, Whyte et al., 2014, Swan et al., 2018, Fernandez et al., 2019, Salas and Clarke 2019). The occurrence of *D. acuta* is more regionally and temporarily variable, often blooming later in the summer/early autumn in Spain, Portugal, France, Ireland and the UK (Reguera et al., 1993, Moita et al., 2006, Vale et al., 2008, Reguera et al., 2012, Diaz et al., 2016, Dhanji-Rapkova et al., 2018, Swan et al., 2018, Fernandez et al., 2019 Salas and Clarke 2019, Belin et al., in press). The occurrence of *D. acuta* blooms in late summer and early autumn can mean that DSTs persist in shellfish over winter, and as a result shellfish harvesting areas can remain closed for lengthy periods, e.g., up to nine months.

Hydrographic conditions and transport play an important role in the development *Dinophysis* blooms. Both *D. acuminata* and *D. acuta* can form thin-layers at the thermocline or pycnocline (Moita et al., 2006). In the Galician Rias, the onset of *D. acuminata* blooms is associated with the beginning of upwelling, but blooms of this species can occur from spring to autumn (Reguera et al., 1993, Moita et al., 2016). In contrast, *D. acuta* blooms are associated with the relaxation of upwelling at the end of summer and northward longshore transport from an epicentre near Aveiro, Portugal into the Galician Rias, a transport journey of 100s of kms (Moita et al., 2006, Escalera et al., 2010, Diaz et al., 2019). Sudden outbreaks of *D acuta* in the Galician Rías can be explained by this advection demonstrating that advective effects are equally important as the local conditions for bloom development (Moita et al., 2016).

Local hydrodynamics and weather events can also influence DST events in coastal areas. Wind driven advections coupled to tidal forcing have been responsible for the transport and the dispersal of *D. acuminata* blooms to the coast of Normandy, France (Soudant et al., 1997). In the Bay of Biscay, coastal *D. acuminata* events have been related to the onshore transport from offshore eddies (Xie et al., 2007) or transport through river plumes (Velo-Suarez et al., 2010, Batifoulier, et al., 2013), both resulting in physical water structures trapping phytoplankton populations. Strong westerly winds inducing northwards currents have been observed prior to *Dinophysis* events suggesting winds as potential mechanisms of cross-shelf transport of off shore populations towards shellfish farms zones (e.g. into Arcachon Bay) (Batifoulier, et al., 2013). Winds have been also indicated as potential causes of collapse of *Dynophysis* blooms (Velo Suarez et al., 2010).

Offshore *Dinophysis* populations have been observed at the tidal front in the Celtic Sea off the south east coast of Ireland (Raine et al., 2017). These populations can be transported into bays where shellfish are harvested, with the bloom driven by prevailing winds and transported in coastal jets along the coast (Farrell et al., 2012, Salas and Clarke 2019). In Scotland prevailing winds resulted in a *Dinophysis* population being trapped in Loch Fyne in Scotland during the summer of 2009 causing sustained closures due to DST levels above the regulatory limit for many weeks (Morris et al., 2010). A sudden change in wind direction resulted in onshore advection of an offshore *Dinophysis* population, leading to a rapid rise in DST levels in shellfish in the Shetland Islands in 2013, and causing several cases of human illnesses with DSP symptoms (Whyte et al., 2014). A study in the Clyde Sea in on the west coast of Scotland has shown that the formation of hydrographic fronts can prevent the entry of *Dinophysis* populations into sea lochs, protecting the shellfish harvesting areas within (Paterson et al., 2017).

3.224 Changes over time

The criteria used to judge the presence of DSTs over regulatory limits were not consistent among European countries until 2004. For example, in Galicia a positive DST MBA result requiring regulatory action was considered when 2 out of the 3 test mice died in less than 12 h, while in the rest of Spain 24 h was used, and in the UK, France and Portugal a 5 h observation period was adopted (Gestal-Otero, 2003). European regulatory legislation until then required only that the customary biological methods had to give a negative result (91/492/EEC). This death time observational period was standardized to 24 h in 2002 (2002/225/EC), as well as the allowable levels for each of the toxins involved in the DSP syndrome. In spite of these changes, and along with the move to chemical methodologies (see Table 1) in some countries the annual number of HAEDAT areas with DST events is relatively consistent, as in France and the UK over the last 20 years (Figure 3). In Spain the number of areas with DST events is also consistent, aside from increase in reporting from Asturias to HAEDAT and the increase in monitoring effort on the Atlantic coast of Andalusia since 2013. In contrast, some countries exhibit more interannual variability. In 2001 a low number of areas with DST events were recorded in Portugal. In Ireland no closures of shellfish harvesting areas were enforced in 2007 despite consistent monitoring effort (Figure 3).

Previous studies reported in the scientific and monitoring literature do not reveal significant trends. Analysis of a 30-year time-series of *D. acuta* occurrence in the Galician Rias (1985 – 2014) showed no evidence of a trend towards increasing frequency or intensity of *D. acuta* blooms (Diaz et al., 2016), although the time window of *Dinophysis* outbreaks off NW Iberia, was slightly wider during the decade 2004-2013 in comparison to previous decades (Moita et al., 2016). The maximum number of days that shellfisheries were closed to harvesting in the Atlantic coast of Andalucia due to DSTs was variable between 2007 and 2017 but without apparent pattern (Fernandez et al., 2019). Similarly, Belin et al., (this issue) did not find a trend in DST events along the Atlantic coast of France or within the English Channel. Salas and Clarke (2019) did not observe an increase in the periodicity or intensity of DSTs in Irish shellfish from 2005 – 2017. Finally, Swan et al., (2018) described substantial interannual variability in the presence of *D. acuta* in Scottish waters but without evident decadal trend.

Species level records of HAB phytoplankton distribution before the 1990s are scarce. Nevertheless, changes in the distribution of *Dinophysis* (recorded to genus level) from Continuous Plankton Recorder (CPR) data in the North Sea have been identified, shifting in abundance from the western North Sea to the south east coast of Norway from the 1960s to the early 2000s (Edwards et al., 2006). There are reports of a widespread distribution of *D. norvegica* along the east coast of the UK from Scotland to the English Channel before the early 1980s (Dodge 1981), as well as reports of very high cell densities of this species in the north east of England in 1971 (Dodge 1977). These reports do suggest changes in the abundance and distribution of this species in eastern UK waters as *D. norvegica* is currently infrequently recorded.

3.23 *Amnesic Shellfish Toxins (ASTs) Events*

3.231 *Event description*

The first reported outbreak of AST poisoning in humans occurred in Canada during 1987 caused by consumption of mussels (*Mytilus edulis*) originating from Prince Edward Island in Atlantic Canada (Bates et al., 1989). At least 153 people were affected with symptoms including both diarrheic and neurological, including confusion and memory loss and there were three mortalities (Quilliam et al., 1988, 1989; Quilliam and Wright, 1989; Perl et al., 1990). This event led to the determination of domoic acid (DA) as the causative toxin and the subsequent rapid development of monitoring methods using liquid chromatography (LC) with UV or diode-array detection (DAD), for shellfish tissues, and shortly thereafter a more sensitive fluorescence-based method (LC-FD) for detection in plankton and seawater matrices (Pocklington et al., 1990, Devez and Delmas 2013). These chemical analytical methods were implemented into an ongoing regulatory management programme from the early 1990s for prevention of subsequent outbreaks affecting human health of shellfish consumers (Quilliam et al., 1991).

Although some countries have shifted mostly to LC-MS/MS, at least for confirmatory analysis of DA in various matrices, LC-UV remains the standard method for routine DA analysis in shellfish for regulatory purposes in Europe. In addition to DA, there exist other AST analogues, primarily iso-DA, which can be produced by the causative diatom *Pseudo-nitzschia* spp. and found in AST-contaminated shellfish, but they are less abundant (usually <10% total AST) and also less potent as glutamate agonists – the toxic mode of action of ASTs. These AST analogues are, therefore, usually ignored in regulatory monitoring schemes, even those based on LC-MS/MS analyses.

The first management actions in response to ASTs above regulatory levels were implemented in the late 1990s following the introduction of routine testing in Europe (Figure 3). No records of reported events associated with ASTs exist in this area of Europe prior to routine monitoring commencing. Events recorded to date are primarily management actions associated with shellfish harvesting or offshore fishing areas resulting from DA levels exceeding regulatory limits. There is one record of human illness associated with ASTs from Spain after a family ate mussels collected from an area where a harvesting ban had already been implemented (Pazos et al., 2016) (Table 2). Figure 6 shows that the majority of management actions have been enforced along the Iberian Peninsula, northwest France, southwest Ireland and the west and north coasts of Scotland. Events along the southern Bay of Biscay, the English Channel, Irish Sea and western side of the North Sea are rare.

The maximum level of DA recorded in shellfish species shows variability between species and between countries (Table 3). In some areas of the Atlantic coastline, e.g., Galicia, Ireland and Scotland, extended closures of harvesting or fishing grounds for king scallop *Pecten maximus* and the possibly conspecific Mediterranean scallop *Pecten jacobaeus* have been enforced as a result of the very slow depuration rate of DA from the digestive- and gonadal tissues (Blanco et al., 2002, Bogan et al., 2006, Bresnan et al., 2017). These closures have lasted, at times, for up to or even over a year. This contrasts with the duration of closures for high DA levels in other bivalve shellfish species such as the mussels *Mytilus edulis* and *M. galloprovincialis* where closures last a few weeks in the majority of cases (Blanco et al., 2002, Bresnan et al., 2017).

In 1999 an area of ~37,000 km^2 in Scottish waters was closed for scallop harvesting with an approximate economic impact at the time of £17 million (~ $23 million US) (Gallacher et al., 2001). These closures were driven by the uptake and slow depuration of DA from the gonadal tissue of king scallops (*Pecten maximus*) (Campbell et al., 2001, Blanco et al., 2002, Bresnan et al., 2017). Research into the compartmentalisation of DA within *Pecten maximus* tissues revealed that the digestive glands contained the highest levels of DA while the lowest were found in the adductor muscle (Campbell et al., 2001, Blanco et al., 2002). The EU Shellfish Directive was amended in 2002 and again in 2004 to facilitate tiered testing and shucking (for removal of the gonad and/or digestive tissue) and sale of the *Pecten maximus* adductor muscle only when DA concentrations in the whole tissue was <250 µg g^{-1} (now 250 mg kg^{-1}) and in the adductor muscle were <4.6 µg g^{-1} (now 4.6 mg kg^{-1}) with a move towards end product testing (2002/226/EC, EU854/2004). Since 2005, there has been no legal requirement for offshore monitoring of *Pecten maximus* fishing grounds in Scotland by the responsible authority and this time-series of data recording within HAEDAT has stopped. As a result, the number of HAEDAT areas associated with AST events in Scottish waters has reduced due to this change in monitoring effort, as can be seen in Figure 6 B - E.

In addition, the maximum concentration of DA associated with UK AST event data has also declined as prior to 2005 the majority of shellfish tested for DA were *Pecten maximus*. Figure 7 A – B shows how maximum toxin concentration in the HAEDAT UK dataset has changed as a result of this monitoring change in Scottish waters since 2005.

The move to shucking and end-product testing was not applied in all countries. In contrast to the case in Scotland, France began monitoring ASTs in *Pecten maximus* in 2004 and continues to close *Pecten maximus* harvesting areas when DA levels exceed regulatory limits, resulting in continued reporting of HAEDAT records associated with ASTs.

No marine mortality events have been associated with ASTs in Europe, although concern has been expressed about the detection of DA in the urine and faeces of seals in Scottish waters (Hall and Frame 2010, Jensen et al., 2015). ASTs have also been detected in crabs, fish and octopus (Hess et al., 2001, Vale and Sampayo, 2001, Costa and Garrido, 2004, Lopes et al., 2018), but without reports of related human illnesses.

3.232 Causative organism

Species belonging to the diatom genus *Pseudo-nitzschia* H. Peragallo, 1900 are confirmed to be the primary DA producers and hence the main causative agent for amnesic shellfish poisoning. There is confirmation of closely related *Nitzschia* species also capable of DA production but these are likely not a major factor in AST events in Europe. The diatom *Halamphora coffeaeformis* (C.Agardh) Levkov is also listed on the IOC-UNESCO Taxonomic Reference List of Harmful MicroAlgae (http://www.marinespecies.org/hab/) as a DA producer although there have been some doubts its toxicity (Bates et al., 2000). On a global basis, the taxonomy and nomenclature of *Pseudo-nitzschia*, as well as known biogeographical distributions, have undergone substantial and ongoing revisions over the past few decades (comprehensively reviewed by Trainer et al., 2012; Bates et al., 2018). *Pseudo-nitzschia* (as

Nitzschia) has been observed in Atlantic waters of Europe for over 100 years (Herdman and Riddell 1911, Churro et al., 2009).

Reliable routine identification of *Pseudo-nitzschia* species is problematic because of the high degree of infraspecific variation and details of the morphotaxonomic descriptors which lie close to the limit of resolution by light microscopy. The difficulty in implementing phytoplankton monitoring for DA toxin risk assessment is further compounded by the fact that cell DA content can vary among and within morphospecies, on a strain- or population-specific basis, and even in response to environmental triggers or chemical cues. In routine monitoring programmes, therefore, *Pseudo-nitzschia* is often only identified to genus level or may be split into two different size categories, e.g. *P. delicatissima* group (diameter < 5µm) and *P. seriata* group (diameter >5 µm), e.g. as in Fehling et al., (2006). Species level identifications are made by electron microscopy or molecular methods on selected samples (Cusack et al., 2004, Fehling et al, 2006, Churro et al., 2009, Orive et al., 2010, 2013, Fehling et al., 2012, Bresnan et al., 2015, Bresnan et al., 2017, Delegrange et al., 2018, Clark et al., 2020). These have shown *Pseudo-nitzschia* populations along the Atlantic margin of Europe are seasonally variable and diverse (Table 4). *Pseudo-nitzschia australis* Frenguelli 1939 is the species most associated with AST events along the Atlantic coast, being a confirmed DA producer and associated with closures of shellfish harvesting areas in Spain (Miguez et al., 1996), Portugal (Vale and Sampayo 2001), Scotland, (Campbell et al., 2001, Fehling et al., 2004), France (Klein et al., 2010) and Ireland (Cusack et al., 2004, Lefran et al., 2020). Additional confirmed DA toxin producers along the Atlantic region are *P. multiseries* (Hasle) Hasle, 1995 in the southwest UK, France and Portugal (Percy et al., 2006, Amzil et al., 2001, Godinho et al., 2018); *P. plurisecta* Orive & Pérez-Aicua, 2013*, P. pungens* (Grunow ex Cleve) G.R.Hasle, 1993, and *P. pseudodelicatissima* (Hasle) Hasle, 1993 in France (Amzil et al., 2001, Caruana et al., 2019); and *P. seriata* (Cleve) H.Peragallo, 1899 in Scotland and Iceland (Fehling et al., 2004, Lundholm et al., 2018).

3.233 Ecology

During blooms highest concentrations of DA in *Pseudo-nitzschia* cells and released into the water column were recorded from old cells (Trainer et al., 2012) which are a source of DA for the benthos when aggregates of marine snow comprised of degraded *P. australis* cells sink to the sea bed (Schnetzer et al., 2017). In coastal areas increased light availability, salinity, freshwater runoff and shifting nutrient ratios are also important in driving development of *Pseudo-nitzschia* blooms (Fehling et al., 2006, Thorel et al., 2010, 2017).

Hydrographic conditions influence the development of *Pseudo-nitzschia* blooms. Upwelling conditions along the Iberian Peninsula bring nutrient-rich waters from the deep and have the potential to seed populations with vegetative cells or resting stages (Smayda and Trainer 2010). In Portugal, diatom assemblages in the water column during upwelling are composed primarily of *Chaetoceros* and *Pseudo-nitzschia* species (Abrantes and Moita 1999) and increases in *Pseudo-nitzschia* cell densities have been observed 4 –6 days after an upwelling pulse (Palma et al., 2010). In Galicia, Spain, upwelling events can promote the formation of thin layers of *Pseudo-nitzschia* cells when the pycnocline shoals, providing an environment with appropriate nutrient concentrations and light intensity for cell growth (Velo-Suarez et al., 2008). These thin layers can be also be displaced downwards during downwelling introducing these toxigenic cells to the benthos (Velo-Suarez et al., 2008).

Nutrient enrichment from freshwater inflow and upwelling contribute to the spatial differences in the occurrence of AST events in embayments in France along the channel and Atlantic coast (Husson et al., 2016). An anomalous *Pseudo-nitzschia* bloom and linked AST event was observed in France in 2010 following storm ‘Xynthia’ which was thought to have triggered a *Pseudo-nitzschia* bloom which impacted *Pecten maximus* fisheries (Husson et al., 2016).

In Irish waters, upwelling conditions can also drive the development of *Pseudo-nitzschia* blooms (Cusack et al., 2015). Transport from offshore populations also influences abundances in coastal waters. *Pseudo-nitzschia* populations can also be present in thin layers in the Celtic Sea off southwest Ireland (Farrell et al., 2012) and can be transported to the coastal embayments in the coastal current (Raine 2014). *Pseudo-nitzschia* has also been observed in the shelf waters and off the shelf to the west of Scotland, with the diversity of the *Pseudo-nitzschia* differing between the shelf and off shelf populations (Fehling et al., 2012).

3.234 Changes over time

The annual number of HAEDAT areas with AST events shows interannual variability, varying with a change in monitoring effort and intensity, e.g. in countries moving to shucking and end product testing of scallops influencing the number of events recorded, whereas in other countries the number of HAEDAT areas with AST events remains variable despite a consistent monitoring regime. Hasle et al., (1996) reported declines in *P. multiseries* in the Skagerrak in the 1980s and early 1990s, hypothesised as a result of milder winters. Examination of sediment cores in Mariager Fjord in Denmark have also identified a shift from *P. multiseries* to *P. pungens* dominance after 1947, most likely due to increasing temperatures and nutrient loading (Lundholm et al., 2010). Data from the Continuous Plankton Recorder (CPR) has revealed an increase in the abundance of *P. seriata* 'type' larger cells since the mid-1990s in the North East Atlantic (Hinder et al., 2012). Unfortunately, the CPR does not identify *Pseudo-nitzschia* cells to species level and thus it is not known if the emergence of ASTs in this region is linked to an increase in potentially toxigenic *Pseudo-nitzschia* species, such as *P. australis*.

3.24 *Azaspiracid Shellfish Toxin (AZT) Events*

3.241 Event description

Azaspiracids (AZAs) are the most recently characterized major phycotoxin group that cause closures of classified aquaculture producing areas along the Atlantic coast of Europe, particularly in Irish coastal waters (Clarke, 2020). These were initially called Killary Toxins (KTs) when first identified, due to human illness recorded in the Netherlands after consumption of mussels *Mytilus edulis* harvested from Killary Harbour, on the west coast of Ireland, in 1995. The first lipophilic marine toxin of this group was isolated from Irish shellfish, structurally elucidated, characterised as azaspiracid (AZA) (Satake et al., 1998, Ofuji et al., 1999); since then, more than two dozen naturally occurring AZA analogues have now been described from contaminated shellfish and from isolated strains of the causative dinoflagellate species within the family Amphidomatacea. To date, management actions associated with AZA levels exceeding regulatory limits (as prescribed in EU legislation 853/2004 Table 1B) have only been enforced in Europe. Figure 8 A-E shows events associated with AZA have had a high occurrence and incidence in Ireland since their first detection in 1995. Ireland was the first country to introduce LC-MS-MS analysis for the determination and quantification of AZA-1, -2 & -33, reported as µg AZA-1 eq. g^{-1} in shellfish species, Annual closures of shellfish harvesting areas have been enforced for varying periods each year, with the exception of 2004, predominantly along the southwest, west and northwest coasts of Ireland. Similar to DSTs, AZAs can be retained in shellfish tissues above regulatory limits during winter periods, as observed in Ireland during 2005 – 2007, and 2013-2014 (Clarke 2020). Table 3 shows the highest AZA levels in this study area since chemical analytical monitoring by LC-MS/MS commenced were found in *M. edulis* from Irish waters (8970 µg kg^{-1}).

There have been sporadic reports of human illness resulting from consumption of Irish shellfish associated with AZAs recorded in the Netherlands 1995, Ireland 1997, Italy 1998, France, 1998, UK 2000, Belgium via the Netherlands 2006, France and the USA in 2008 (Twiner et al., 2008), in Switzerland 2013, and during the same year, a major outbreak of human illness was reported in France due to AZA poisoning. (Table 2).

A low number of incidents of AZA above regulatory limit in shellfish has also been recorded in Scotland in 2011, 2012 and 2013, and also in southwest England in 2015 (Figure 8) . Recently, AZA levels exceeding the closure limit were recorded near Huelva in Spain during a period of high DSTs in shellfish (Tillmann et al., 2017). Elsewhere, low concentrations of AZAs have been recorded from bivalve shellfish in France, Spain and Portugal (Braña Magdalena et al., 2003, Vale et al., 2008) but have not resulted in any management actions. Table 3 shows the maximum levels AZA toxin levels in shellfish from this study area. Within Europe a small number of harvesting closures have been enforced in Norway in 2003, 2005, 2015-2016 and also in Sweden during 2018 (Karlson et al., this issue).

3.242 Causative organism

In the early 2000s, initial field studies screening individual phytoplankton cells identified the dinoflagellate *Protoperidinium crassipes* (Kofoid, 1907) Balech, 1974 as a producer of AZAs (James et al., 2003); however, there was doubt about this attribution due to lack of detection of AZA production in this species and the heterotrophic nature of this dinoflagellate (Gribble et al., 2007). Later investigations discovered that AZAs were produced by *Azadinium spinosum* Elbrächter & Tillmann, 2009*,* a newly described dinoflagellate species, and requiring the erection of a new genus belonging to the Amphidomataceae family Sournia, 1984 (Tillmann et al., 2009). *Azadinium spinosum* was found to produce the EU regulated isomers AZA-1, -2 & -33 in Irish and Norwegian coastal waters; in Norwegian waters, a ribotype of *Az. spinosum* has recently been identified to produce the non EU regulated isomers AZA-11, -51 (Wietkamp et al., 2020). Further investigations discovered additional AZA producers, including *Az. poporum* (Tillmann et al., 2011, Krock et al., 2012, Luo et al., 2017), from which Norwegian strains were found to produce AZA-37. A Mediterranean strain of *Az. dexteroporum* (Percopo et al., 2013) was found to produce various AZAs, but to date no strains isolated from the North Atlantic have produced known AZAs (Tillmann et al., 2015, 2020). *Amphidoma languida* Tillmann, Salas & Elbrachter, 2012 has been found to produce AZA-38,-39 as the dominant toxins (Krock et al., 2012, Wietkamp et al., 2019); however, different toxin profiles (primarily AZA-2 and -43) have been determined in strains from the Andalusian coast, Spain (Tillmann et al., 2017) and AZA-52 and -53 have been detected in Norwegian strains of *Am. languida* (Tillmann et al., 2018).

All of these species have been recorded in the North Atlantic area and are shown in Table 4, where *Az. spinosum* is the main causative organism which results in closures of shellfish production areas routinely monitored by EU countries by LC-MS/MS (Table 1A) for the EU regulated toxins AZA-1, -2, -33 and reported as µg AZA-1 equivalents kg^{-1}.

3.243 Ecology

Until recently very little was known about the spatial and temporal distribution of Amphidomataceae. In 2018 a survey of the North Sea, Celtic Sea, and Irish and UK coastal waters using on-board microscopy, qPCR and LC-MS/MS revealed that 3 out of the 4 currently known primary toxigenic species (*Az. spinosum, Az. poporum* & *Am. languida*) were present and with measurable AZA toxin composition in selected isolates.

Both *Az. spinosum* & *Am. languida* were present at the majority of stations on the Irish coast, but in low cell numbers, whereas *Am. languida* was observed in the North Sea at cell densities 1.2 X 10^5 cells L^{-1}. *Az. poporum* was detected with a less regular occurrence at stations, mainly in the North Sea and along the south coast of Ireland (Wietkamp et al., 2020). It should be noted that this distributional pattern reflects only short-term discrete sampling from a field expedition and not seasonal or annual variation.

3.244 Changes over time

The causative toxigenic species of AZAs have only been recently described. *Azadinium* species are difficult to monitor in routine monitoring samples by light microscopy due to their small cell size. Hence *Azadinium* cells tend to pass through standard phytoplankton nets (20 µm) and can be frequently misidentified under microscopic observation even at genus level (e.g., as *Heterocapsa*). Since the first identification of the dinoflagellate *Az. spinosum* in 2009, and confirmation of its AZT toxigenicity, knowledge about Amphidomataceae and their global biogeography is continually increasing, with both toxigenic and non-toxigenic novel species being described (Tillman et al., 2018, 2019). Previously identified toxigenic species are now recorded as having variable toxin profiles, and non-toxigenic strains/ribotypes within "toxic" species have also been recently identified (Tillman et al., 2018, 2019). Reliable molecular probes and primers i.e. based on PCR methods, are available and currently in use in the Irish monitoring programme to distinguish between three *Azadinium* species*, Az. spinosum, Az.* poporum *and Az. obesum* (Toebe et al., 2013) and the PCR confirmation *of Am. languida* (Wietkamp et al., 2019), and also for Amphidomatacea, using a genus assay for the detection for *Azadinium* & *Amphidoma* (Smith et al., 2016)

3.25 *Ciguatera Poisoning Events*

3.251 Event description

Within Europe, endemic CP events are restricted to the Canary Islands, Spain, the Selvagens Islands, and Madeira, Portugal (Figure 3, 9), with HAEDAT records containing reports of human illness associated with fish contaminated with ciguatoxins (CTXs). Two CP events within HAEDAT are records of a ban on fishing in the Selvagens Islands in 2013 and fishing and shellfish harvesting to contain an outbreak of CP in the Canary Islands in 2016. The first endemic case of CP in Europe was recorded in the Canary Islands in 2004, when five people became ill after eating amberjack fish (*Seriola* spp.) caught in the locality (Perez-Arellano et al., 2005). Additional cases were reported in 2008 from fish caught around Selvagens Islands (Costa et al., 2018) and from the Canary Islands in 2008

Difficulties in identifying authentic cases of CP illness means that cases may have been underreported in earlier years. More than half of the CP records in the HAEDAT database from Macaronesia come from Tenerife, with additional records from Gran Canaria, Lanzarote and La Palma. Approximately half of the cases there have occurred from November to January.

Since 2008 there has been at least one case of CP reported annually in the Canary Islands, with the exception of 2014. The incidents of human illness associated with CP are presented in Table 2.

The Caribbean form of ciguatoxin (C-CTX) has been identified as the main toxin responsible for CP in the Canary and Selvagens Islands, Madeira archipelago (Costa et al., 2018, Estevez et al., 2019). The causative vector for CP incidents in this region was identified as the large predatory fish, such as amberjack (*Seriola* spp.). However, CP toxins have also been found in sedentary grazers such as grouper (*Epinephelus* sp*., Mycteroperca* sp.) and eels, indicating accumulation through the food web (Rodriguez et al., 2017, Sanchez-Henao et al., 2020). Fish species included in HAEDAT CP records from Macaronesia include *Balistes capriscus*, *Canthidermis sufflamen, Epinephelus* sp., *Mycteroperca fusca*, *Pagrus pagrus*, *Pomatomus saltatrix*, *Seriola dumerili, Seriola fasciata* and *Seriola rivoliana*.

3.252 Causative organisms

Along the eastern North Atlantic edge, a number of species belonging to the dinoflagellate genus *Gambierdiscus* have been identified in the Canary Islands; *G. australes* Chinian & M.A.Faust, 1999*, G. belizeanus* M.A.Faust, 1995*, G. caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester, 2009*, G. carolinianus* Litaker, Vandersea, M.A.Faust, Kibler, W.C.Holland & P.A.Tester, 2009 (Fraga et al., 2011, Tudo et al., 2020) with two new species described from the area *G. excentricus* S. Fraga, 2011 (Fraga et al., 2011) and *G. silvae* S.Fraga & F.Rodríguez, 2014, and an additional putative species *Gambierdiscus* ribotype 3 (Bravo et al., 2019). Of the five species described, screening with neuroblastoma cell-based assays showed all species to produce CTX and maitotoxin (MTX) like compounds, with the highest CTX activity detected from *G. excentricus* and in selected strains of *G. australes* (Fraga et al., 2011, Rossignoli et al., 2020, Tudo et al., 2020). Maitotoxin activity was also highest in *G. excentricus* and *G. australes* (Rossignoli et al., 2020). Highest cell abundances of *G. australes* and *G. excentricus* from the Canary Islands have been observed in Lanzarote and Fuerteventura, the two islands closest to the African mainland (Rodriguez et al., 2017). A CTX-risk assessment of fish from the islands also found higher concentrations of CTX in amberjack from the area (Sanchez-Henao et al., 2019).

3.253 Ecology

The high diversity of *Gambierdiscus* in the Canary Islands suggests that these species were not introduced but are endemic. Water temperatures in the Canary Islands are within the growth tolerance for *Gambierdiscus* species and thus an increase in water temperature due to climate change may extend favourable conditions for growth into the winter months (Rodriguez et al., 2017).

3.254 Management actions

A review global status and trends of CP is presented in Chinain et al., this issue. The emergence of CP as a threat to human health has led to the development of coordinated management actions to address the issue. Within the north eastern Atlantic marginal area, the regional government of the Canary Islands established a monitoring program for CTXs in 2011. Under this framework. a list of fish species and respective weights is issued and reviewed every year. All fish specimens caught that correspond to the species listed and are over the threshold weight cannot be sold before obtaining a negative result in the *in vitro* cell based assay (N2A) for ciguatera-like toxicity (Sánchez-Henao et al., 2019). At present fish species considered to present a CP risk in the region include all species from the genus *Seriola*, *Epinephelus marginatus*, *Acanthocybium solandri* and *Pomatomus saltatrix*. In 2009 the Regional Health Directorate of the Canary Islands established an epidemiological programme for ciguatera and since 2015, CP has been declared a notifiable disease in the Canary Islands. Recent efforts have been made to better identify cases of CP and manage the risk of outbreaks, with a formal description of criteria used to define CP illness in humans that will aid the recording of incidents (Friedman et al., 2017) and predictive CP risk scores developed for fish for human consumption (Sanchez-Henao et al., 2019).

3.26 *Emerging toxins*

Certain phycotoxins can be described as “emerging” either because the nature of their specific toxicity remains unclear or because the toxin syndromes associated with their presence have not yet been manifest. They are not subject to regulatory control within the region and no events from this study area associated with these emerging toxins have been entered in HAEDAT to date. National laboratories in Europe continue to test for emerging toxins such as the cyclic imines, including pinnatoxins and spirolides, that often exhibit “fast-acting toxin” (FAT) responses in the intraperitoneal mouse bioassay (MBA), but have not been confirmed to pose a toxin risk for humans via shellfish consumption. The cyclic imine toxins are frequently detected in shellfish and phytoplankton from European waters but have yet to cause known problems for seafood consumers and the respective compounds are not current subject to EU regulation. For example pinnatoxins produced by the benthic dinoflagellate *Vulcanodinium* E.Nézan & N.Chomérat, 2011 have been detected in shellfish from France, Ireland and Spain (McCarthy et al., 2015, Lamas et al., 2019, Otero et al., 2019, Arnich et al., 2020), and spirolides have been detected in plankton or shellfish from all Atlantic edge countries (Ruhl et al., 2001, Gonzalez et al., 2006, Amzil et al., 2007, Touzet et al., 2011, Silva et al., 2013, Davidson et al., 2015). The potentially toxigenic benthic dinoflagellates *Ostreopsis* cf. *ovata* Fukuyo, 1981 and *Ostreopsis* cf. *siamensis* Johs.Schmidt, 1901 may produce potent lipophilic polyether toxins, such as palytoxins (PLTXs) and ovatoxins (OVTXs), causing respiratory distress in humans via aerosol formation from blooms in coastal areas, particularly in the Mediterranean and in the tropics. For the north Atlantic margin, both species have been found along the Atlantic coast of Portugal, Spain and France, with *O.* cf. *siamensis* appearing sometimes at high cell densities in the Basque country along the southwest Bay of Biscay (David et al., 2013; Seoane and Siano, 2018, Santos et al., 2019, Drouet et al., submitted). *Ostreopsis heptagona* D.R.Norris, J.W.Bomber & Balech, 1985 has also been recorded in the Azores archipelago (Silva et al., 2010). Both OVTXs and PLTX remain in the emerging toxin category because they have caused no apparent HAB events for the region.

The potent guanidinium neurotoxin tetrodotoxin (TTX) has also been detected in shellfish in Europe (Turner et al., 2015), most notably in the Wadden Sea of the Netherlands (Gerssen et al., 2019). While this emerging toxin in European shellfish is most likely of bacterial origin and hence not technically a phycotoxin or recorded as a HAB event, the presence of TTX can enhance the potential risk of shellfish consumption when it co-occurs with other biotoxins, particularly PSTs (STXs) (Boente-Juncal et al., 2020). TTX shares a similar paralyzing mode of action with PSTs as a sodium-ion channel blocker (Durán-Riveroll and Cembella, 2017) and synergy would be expected.

3.27 Mortality events

HAEDAT records of mortalities represents a rather loose categorization of often over-lapping events, including mortality of natural and aquaculture finfish and shellfish, benthic and pelagic invertebrates, plankton, marine mammals and seabirds, as well as marine macroalgae and seagrasses. Although most HAEDAT mortality events refer to those directly (if only circumstantially) linked to specific HABs of known harmful species, certain HAB mortality events, particularly of marine mammals and seabirds, may be inferred from high toxin body burden of accumulated phycotoxins characteristic of HAB species, but not attributable to specific blooms. HAEDAT mortality entries may or may not include details of the individual species affected, number or mortalities, or confirmed identification of the putative causative HAB species and/or cell densities during the event. In many instances the mechanism of mortality is unclear, i.e. in cases of fish and other faunal mortalities it is most often not possible to attribute the effect to defined ichthyotoxins or indirectly to anoxia.

These events also differ from those associated with “shellfish toxins” or ichthyotoxins because there are no formal monitoring programmes for recording mortalities in the

HAEDAT areas presented, with the exception of France, where a citizen science project '*Phenomer'* to monitor water discolourations has been in operation since 2013 (Siano et al., 2020).

With these above caveats, Figure 10 (A-D) presents the number of years where mortality events in natural fish, aquaculture fish, benthic fauna and dogs/seabirds have been recorded by accessing all relevant HAEDAT entries. This is the first time these HAEDAT events have been assembled and presented in the same forum. The majority of mortality events entered in HAEDAT have been recorded from Ireland, the west coast of Scotland, the Orkney and Shetland Islands and northern France. While information about major mortality events will reach national editors, minor mortality events (e.g. small numbers ofwild fish, benthic fauna washed onshore) are almost certainly under-reported

3.271 Event description and causative organisms

3.2711 Karenia mikimotoi

The dinoflagellate *Karenia mikimotoi* (Miyake & Kominami ex Oda) Gert Hansen & Moestrup, 2000, formerly often recorded as *Gyrodinium aureolum* Hulbert in northern Europe, is responsible for most of the mortality events recorded in HAEDAT. In France the first observation of *K. mikimotoi* in Brittany (Douarnenez Bay) was in 1976. Since then several blooms of this species with observed harmful effects on benthic fauna have been reported along the Atlantic coast of France, especially from the bays of Brest and Douarnenez (Erard-Le Denn et al., 1990). In July-August 1985 a massive bloom (maximum 0.8 x 10^6 cells L^{-1}) caused disturbances to breeding stages of the king scallop *Pecten maximus* in the Bay of Brest. Mass mortalities of post-larval stage (0.25-3 mm) individuals in nursery and grow-out trays and retardation of development of the juvenile stages (5-30 mm) were observed (Erard-Le Denn et al., 1990, Chauvaud et al 1998). In 1987, water discoloration caused by a *K. mikimotoi* bloom in southern Brittany (Bay of Vilaine) was associated with lugworm mortalities (Sournia et al., 1991). Between May and August, 1995, a massive bloom (maximum 4.8 x 10^7 cells L^{-1}) extending along the entire Atlantic coast of France caused mortalities of both benthic invertebrates (sea urchins, gastropods, lugworms, shellfish) and pelagic fish (including trout and conger eels). Significant losses were reported by shellfish producers in scallop hatcheries, and from shellfish and finfish nurseries (Arzul et al., 1995).

Fish mortality events have also been reported in the Gulf of Biscay and adjacent waters. In September 2003 fish mortalities were recorded in northern Brittany from Saint Malo Bay during a bloom of *K. mikimotoi* (maximum 5.6 x 10^5 cells L^{-1}); a direct link, however, between the specific bloom and the fish kill was not established (http://envlit.ifremer.fr/layout/set/print/content/view/full/17479).

Mass mortalities of shellfish and finfish in the west of Ireland have also been caused by high density blooms of *K. mikimotoi*. Cells of this species are a regular, annual presence in Irish coastal and inshore waters from May to September but have formed massive blooms (recorded at up to 9 x 10^6 cells L^{-1}) only during a few years. The earliest recorded event of *K. mikimotoi* causing faunal mortalities in Irish waters occurred in the southwest in 1971 with the observation of lugworm mortalities (Helm et al., 1974). In the mid- to late 1970s, three separate events were linked to *K. mikimotoi* blooms: one in 1976 along the south coast with recorded mortalities of fish and invertebrates (Ottway 1979), and two in the southwest, associated with mortalities of fish and invertebrates in 1978 (Pybus 1980), and in 1979 with widespread mortalities of gastropods, echinoderms and rainbow trout (Cross & Southgate 1980, Roden et al.,1981, Southgate *et al.,* 1984). Throughout the 1980s and 1990s there were a few records of high cell density blooms of *K. mikimotoi* along the west, southwest and south coasts of Ireland, but no associated faunal mortalities were observed or recorded (Raine et al., 1993, 2001; Silke et al., 2005). The highest number of marine faunal mortalities in Irish coastal waters were recorded in 2005 when a protracted high cell density bloom of *K. mikimotoi* was observed over a wide area from the end of May to the beginning of August (Silke et al., 2005). Maximal cell densities (3 x 10^6 cells L^{-1}) were recorded along the northwest and west coasts of Ireland. A second bloom became established from July to

August in the southwest at cell densities that reached 3.7 x 10^6 cells L^{-1}. Observations of the benthic communities and habitats of the affected areas revealed significant impacts on the benthos with high faunal mortalities and lethargy of affected species.

In 2012, persistent blooms of *K. mikimotoi* were similar in impact and duration to the 2005 events, but were confined to two successive blooms during June and July in coastal waters of northwest Ireland. These dense blooms (maximal record of 4.5 x 10^6 cells L^{-1}) resulted in mass mortalities of invertebrates, including shellfish and finfish. In August 2015 a bloom of *K. mikimotoi,* with the highest cell densities ever observed (up to 9 x 10^6 cells L^{-1}) in southwest Ireland caused mortalities of several invertebrates along the Celtic Sea shoreline. In this case, there was obvious water discolouration but high cell densities of *K. mikimotoi* are not always associated with observations of water discolouration and coincident faunal mortalities. For example, neither were observed during bloom events in 2016 at cell densities of 1.5 x 10^6 cells L^{-1} nor in 2019 with cell densities of 1.5 x 10^6 cells L^{-1}.

In the UK most fish-killing events are also linked to massive blooms of the dinoflagellate *K. mikimotoi.* Although this species is frequently present in summer phytoplankton community, fish mortalities are infrequent. Observations from routine monitoring phytoplankton programs suggests that *K. mikimotoi* is a regular member of the phytoplankton in Scottish waters at cell densities typically reaching 5 x 10^3 cells L^{-1} in summer. At these cell densities few environmental consequences or mortalities are recorded. Prior to 1999, reports of impacts from *K. mikimotoi* in Scottish waters were relatively rare, or at least unrecorded, with only one event in the Firth of Clyde in 1980 when, a massive bloom (2 x 10^7 cells L^{-1}) was associated with mortalities of farmed fish (Jones et al., 1982; Roberts et al., 1983; Potts and Edwards, 1987). Since 1999, dense blooms of *K. mikimotoi* have become more common although still far from annual events in UK waters. Blooms associated with benthic mortalities were recorded in 1999 in Orkney and in 2003 in the Orkney and Shetland Islands, with the 2003 bloom resulting in the death of 53,000 farmed fish (Atlantic salmon). In 2006, a major *K. mikimotoi* bloom, both in terms of spatial magnitude and temporal duration, affected the west, north and east coasts of Scotland (Davidson et al., 2009). Fortunately, this bloom resulted in relatively few losses at aquaculture sites, but did cause extensive benthic faunal mortalities at a wide range of locations, including commercial areas of wild scallop harvesting. Modelling-based analysis of the bloom (Gillibrand et al., 2016) demonstrated the role of advection in governing bloom transport round the Scottish coast over a number of months.

More recently, a massive *K. mikimotoi* bloom occurred in the Firth of Clyde in 2016 (Baptie and Swan 2017). Cell densities were sufficient to bring about hypoxic conditions and mass mortalities of marine fauna, but the bloom was not in a fish farming area and hence the impact on aquaculture was low. *Karenia mikimotoi* has also been associated with faunal mortalities in the benthos in Wales in 1990 and 1994, and fish kills and benthic mortalities in the southwest of England in 2007. There is also a report of a fish kill associated with *Gymnodinium* sp. in 1990 in the Faroe Islands. Whether this was synonymous with *K. mikimotoi* is unclear. France and Ireland have experienced a reduced number of impacts from *K. mikimotoi* since the 1970s and 1980s, and indeed this is true for the entire eastern Atlantic margin of Europe, as well as for adjacent waters of the North Sea, Norwegian Sea and Kattegat-Skagerrak (Karlson et al., this issue) where this species has typically posed less of a threat to fish populations,

The mechanism(s) whereby *K. mikimotoi* causes faunal mortalities remains unclear – numerous alternative but not mutually exclusive modalities have been proposed. For *K. mikimotoi* these hypothesized mechanisms range from production of known ichthyotoxins (e.g gymnocin –A and B) to membrane-disruptive fatty acids and sterols, and/or involving oxidative stress and induction of reactive oxygen species (ROS), mucus production causing gill clogging and/or simple anoxia due to oxygen depletion from the decaying bloom (see Li et al., 2019 and references therein). There is no consensus for the blooms that have caused mortalities in the north Atlantic margin.

3.2712 Harmful Raphidophytes and Prymnesiophytes

Phytoflagellates not belonging to the dinoflagellates have also caused faunal mortalities in the eastern north Atlantic region. In Portugal HAEDAT records contain information about mortalities in Obidos lagoon due to *Prymnesium parvum* N.Carter, 1937 in 1988, *Heterosigma* akashiwo (Y.Hada) Y.Hada ex Y.Hara & M.Chihara, 1987 in 1989 and in 1996 in the Formosa lagoon in the Algarve due to *Heterosigma inlandica*, (now a synonym of *H. akashiwo)* (total cell density 17.5 x10^6 cells L^{-1}). In Galicia, Spain one record in HAEDAT reports mortalities of farmed fish associated with *H. akashiwo (*18.0 x10^6 cells L^{-1}) in 1987. In France in late September 1994; a high density bloom (maximum 1.3 x 10^8 cells L^{-1}) of *H. akashiwo* was recorded from coastal waters at Camaret, Brittany corresponding with yellow-brownish discolouration of the water. Two salmonid farms were affected by the bloom event: one registered 100% mortality (= estimated loss of 50K $US) of salmon and rainbow trout and the latter 10%. In mid-August 2013, the citizen program *Phenomer* (www.phenomer.org), reported dark-brown water discolourations from separate shallow locations in the Bay of Vilaine. Prompt phytoplankton sampling revealed co-occurring blooms of *H. akashiwo* (maximum 3.0 x 10^6 cells L^{-1}) and the dictyochophyte *Pseudochattonella verruculosa* (Y.Hara & M.Chihara) S.Tanabe-Hosoi, D.Honda, S.Fukaya, Y.Inagaki & Y.Sako, 2007 (maximum 1.9 x 10^6 cells L^{-1}). At one site massive mortality of bivalve shellfish and small crustaceans was observed (Siano et al., 2020), corresponding to very low (4.9 and 5.5 mg L^{-1}) dissolved oxygen concentrations at the sea floor in both locations. *Heterosigma akashiwo* is reported to have resulted in mortalities of farmed fish on the Isle of Mull in the West Coast of Scotland in 1990. There is one record of mortalities of farmed fish in Iceland in HAEDAT from 1987 associated with a bloom of *Heterosigma akashiwo* (570,000 cells L^{-1}) believed to be an introduced species in the area (Thorarinsdottir et al., 2014).

On the west coast of Scotland prominent incidents related to an unidentified phytoflagellate (designated as “Flagellate X”) that bloomed in Loch Striven and Loch Fyne between 1972 and 1982. Flagellate X has never been definitively identified due to lack of archived specimens but appeared “*Chattonella*-like” and might in fact be affiliated with the dictyochophyte *Pseudochattonella* (Y. Hara et Chihara). This species was implicated in three major kills of farmed salmon (Ayres et al., 1982, Gowen 1984, Gowen 1987, Tett, 1980) at cell densities ranging from 8 to 34 x 10^6 cells L^{-1}. Flagellate X also bloomed (3.5 x 10^3 cells L^{-1} recorded) during 1982 on the western Scottish coast at Ullapool accompanied by some salmon mortalities (Ayres et al., 1982), and in nearby Loch Kinaird and Loch Broom but without recorded fish mortalities (Gowen, 1987). Flagellate X also caused mortalities in fish farms in the west of Ireland in the mid 1980s (Silke and Jackson 1990).

3.2713 Alexandrium catenella and *Alexandrium minutum*

Blooms of *Alexandrium* have also caused mortalities of seabirds, fish and dogs in this study area. The 1968 *Alexandrium catenella* bloom of the north east coast of England was attributed as the cause of mortalities of seabirds (Coulson et al., 1968) and sandeels (Adams et al., 1968) in the area. Mortalities of rainbow trout and salmon in the Faroe Islands have been associated with *A. catenella* in 1984 and 1987 (Mortensen 1985, Moestrup and Hansen 1988). During the 1984 bloom 27 metric tons of fish were killed and the event was laterally associated with PST intoxication of four people (Mortensen 1985). In September 2013, a bloom of *A. minutum* in a coastal lagoon in São Jorge Island within the Azores archipelago resulted in an orange/brown water discolouration and mortalities of pelagic fish (*Chelon labrosus*). This event was also associated with PST accumulation in shellfish and the reported intoxication of four people.

The incidence of PSTs on the east and south east coast of England is low, although there was a case of nine canine intoxications and two canine fatalities in Dec 2017/Jan 2018 in the East of England along the Norfolk and Suffolk coastlines after consumption of fish, crustacean and starfish contaminated with PSTs which had washed ashore (Turner et al., 2018). A subsequent study found high concentrations of PSTs in a number of benthic invertebrates in the North Sea, several toxic sample locations in the Southern Bight (Dean et al., 2020).

3.2714 Other species

Other phytoplankton species have also caused mortalities. Shellfish mortalities were reported in 1996 in the Salgada lagoon in the Algarve due to high cell densities of the non-toxigenic diatom *Chaetoceros calcitrans* (Paulsen) Takano, 1968 (total phytoplankton concentration 87.0 $x10^6$ cells L^{-1}) and in 1996 in the Albufeira lagoon due to high concentrations of diatoms and *Gymnodinium* sp. F. Stein, 1878 resulting in anoxia. In 2013 mortalities of wild fish were reported in the same area associated with the “red tide ciliate” *Mesodinium rubrum* (Lohmann, 1908). In 1998 mortalities of farmed fish were reported from Galicia due to a bloom of the dictyochophyte *Octactis speculum* (Ehrenberg) F.H.Chang, J.M.Grieve & J.E.Sutherland 2017 (Prego et al., 1998).

Green seawater discolorations are regularly reported in southern Brittany due to high biomass blooms of the naked dinoflagellate *Lepidodinium chlorophorum* M.Elbrächter & E.Schnepf) Gert Hansen, Botes & Salas, 2007. In 2014 the citizen science project *Phenomer* (www.phenomer.org), reported mortalities of wild fish (sole, sand eels, weevers), jellyfish, molluscs and crabs both in the water and washed up on the beach in association with high biomass of this dinoflagellate species. Those mortalities are more likely due to the anoxic conditions caused by the blooms, rather than by production of ichthyotoxins (Siano et al., 2020).

There is one report of mortalities in the north east of England in 1998 where DSP toxins were recorded in the livers of dead seabirds and this has been assigned as a DST event even though a correlation with *Dinophysis* blooms was not made at the time.

High cell densities of the dinoflagellate *Heterocapsa triquetra* (Ehrenberg) F.Stein, 1883 (now *Kryptoperidinium triquetrum* (Ehrenberg) U.Tillmann, M. Gottschling, M.Elbrächter, W.-

H.Kusber & M.Hoppenrath, 2019) (1 X10^6 cells L^{-1}) were also associated with mortalities of farmed fish in the Shetland Islands in 2000.

Two incidents of mortalities associated with the prymnesiophyte *Phaeocystis* sp. (Lohmann, 1908) have been recorded in the UK, in Wales in 1992 and in Vidlin Voe in the Shetland Islands in 2005.

Anecdotal evidence suggests that diatoms present a more regular threat to finfish aquaculture in Scottish waters, because the region is subject to frequent diatoms blooms (Fehling et al., 2006, Siemering et al., 2016). Unfortunately, barring two early studies (Bruno et al, 1989, Treasurer et al, 2003), these impacts have received relatively little scientific investigation.

4. Discussion

HAEDAT data captures the harmful algal events along the Atlantic margin of Europe. High levels of phycotoxins in shellfish, colloquially dubbed “shellfish toxins”, are the harmful algal events that most frequently impact the aquaculture industry and shellfisheries, both in terms of the number of HAEDAT areas affected and the number of years where closures of shellfish harvesting areas have been enforced. The spatial incidence of shellfish toxin events can be influenced by monitoring effort with areas with little or no routine monitoring recording a low number of events in HAEDAT.

High interannual variability can be observed in the number of HAEDAT areas with events per country for individual shellfish toxin syndromes but evidence for systematic trends in is generally inconclusive or absent. Human poisoning events linked to phycotoxins in consumed fish, almost exclusively CTXs associated with CP, has emerged as an established threat to human health from both imported and endemic fish species in Europe since 2004.

Since monitoring for phycotoxins and potentially toxigenic phytoplankton became routine there have been no fatalities associated with the consumption of contaminated shellfish in Europe since the 1950s. DSTs belong to the phycotoxins group that has caused the most incidents of seafood-borne human illness; however, epidemiological studies are scarce (Young et al., 2020) and many “DSP-like” events remain unconfirmed, particularly prior to the implementation of LC-MS/MS for confirmatory qualitative analysis.

For some countries, particularly France and Spain, the introduction of new monitoring requirements, e.g. for ASTs, led to an increase in the total number HAEDAT areas where events have been recorded. In addition to the European regulatory mandates for seafood safety, in some instances the scientific, regulatory and policy communities have come together on a national or multilateral basis to address the impacts from these harmful algal events e.g., the effects of ASTs on scallop (*Pecten maximus*) fisheries. Some countries, notably the UK and Ireland, have subsequently adopted new monitoring regulations to reduce the impacts on their shellfish industries.

The dinoflagellate *Karenia mikimotoi* and the raphidophyte *Heterosigma akashiwo* are the two species most associated with HAEDAT events of mortalities of benthos, natural and farmed fish from along the northeastern Atlantic coastal margin. This contrasts with fish-killing events in the Norwegian Sea, eastern North Sea and Kattegat-Skagerrak, where most mortality events, especially of fish, have been associated with prymnesiophytes (e.g., *Chrysochromulina* spp. and *Prymnesium parvum*) and the dictyochophyte *Pseudochattonella* spp. over the last few decades (Karlson et al, this volume). *K. mikimotoi* events are sporadic, but are declining in intensity and frequency in the greater North Sea region and adjacent waters. Some countries (e.g., Ireland, France) experienced frequent *K. mikimotoi* bloom events from the 1970s – 1990s and this species continues to cause problems in Ireland and the UK although not every year. Mortality events attributable to *H. akashiwo* also appear to be fading in importance within the last decade. With the exception of 2013, impacts from *H. akashiwo* have not been experienced since the 1990s. Unlike the situation in the eastern

Pacific and the Pacific northwestern coast of North America where *H. akashiwo* blooms continue to be a prominent cause of fish mortalities and bloom dynamics and causal mechanism of mortalities have been extensively researched, there is little information on the ecology of this species along the northeastern Atlantic margin. This is largely due to the consistent lack of major blooms in recent years, providing little opportunity for application of advanced research techniques.

Although promoted as a low carbon, healthful seafood, shellfish production is declining within the EU. Many shellfish aquaculture businesses are microenterprises (Gutierez et al., 2020) and are highly sensitive to impacts from HABs (Trainer et al., 2020). Economic studies on the impacts from HABs in Europe are scarce. Hoagland and Scatasta (2006) estimated the average annual economic effect of HABs in Europe to be 813 million $US based on impacts on public health, commercial fisheries, recreation and tourism, and monitoring and management, with approximately two thirds of the costs associated with noxious but non-toxic effects of macroalgal blooms affecting the human uses of the coastal zone. Studies have shown that the financial impacts from HABs can be significant on a national scale. The economic impact of HABs on mussel aquaculture in Europe was reviewed by Ferreira et al., 2014. They estimated that the average annual losses for the period 2000-2009 were 9.2, 6, 2.7, 2.2, 1.9 million $US for France, Spain, UK, Ireland and Iceland respectively. Recently Martino et al., (2020), using a Cobb-Douglas production function estimated annual losses from *Dinophysis*-generated toxins (DSTs) to be 15% (£1.37 million year^{-1} ~$1.87 million US) of the total Scottish shellfish production, representing a non-trivial financial loss. Accumulation of phycotoxins in shellfish has also been shown to have a negative impact on the Portuguese dredge-fishing fleet, potentially influencing its sustainability (Oliviera et al., 2015). Harmful algal events affecting the farmed fish industry have a dramatic financial impact, at least at the local level but this has not been investigated in depth in this study area. The direct and indirect gross costs of the recent 2019 *Chrysochromulina leadbeateri* bloom in northern Norway was estimated to be between 2.3 and 2.8 billion NOK (~0.27 to 0.33 billion $US) (Kontali, 2020).

Early warning of the timing, location and magnitude of HABs and their associated biotoxins is potentially of great value to users of the coastal zone, and in particular to the aquaculture industry, allowing informed business planning and ensuring human and fish health (Davidson et al., 2016, Anderson et al., 2019). A "traffic-light"-coloured warning system is in operation in some countries, as a tool for harvesters to manage the risk from HABs in shellfish harvesting (Food Standards Agency Scotland 2014, McLeod and McLeod 2016). Within Europe there have been a number of initiatives to develop HAB early-warning frameworks. Perhaps most prominent amongst these is the EU FP7 Asimuth project that sought to develop mathematical model based systems for HAB early warning on the Western European Seaboard (Maguire et al., 2016). Two outputs from this project that have proved enduring are the Irish and Scottish HAB and biotoxin early warning "bulletins" (e.g. www.HABreports.org), both of which employ oceanographic modelling approaches to predict the HAB risk to aquaculture (Aleynik et al 2016; Cusack et al., 2016). The ongoing Interreg Atlantic Arc project PRIMROSE (www.shellfish-safety.eu) is attempting to integrate these parallel approaches with initiatives in Portugal, Spain, France and England to develop a common web-based gateway for HAB risk assessment.

Due to the observed increase of CP outbreaks in Europe, the European Food Safety Agency (EFSA) launched the research project EUROCIGUA to characterise the risk of CP in Europe. The project established the criteria for case definition and a surveillance epidemiological protocol for Europe, which also includes cases associated with imported fish (Friedemann, 2019). Another output has been the toxicity evaluation of the different *Gambierdiscus* strains isolated in the Canary Islands (Rossignoli et al., 2020), Selvagens and Madeira, in fish caught in the same locations and the characterization of the CTXs present (Estévez et al., 2019a,b, Estévez et al., 2020a,b, Sánchez-Henao et al., 2020).

In 1995, Galicia established in its own legislation a list of criteria to manage shellfish sampling frequency; the criteria include not only the cell abundance of toxin producing species and the content of phycotoxins in shellfish, but also meteorological and remote sensing information (DOG, 1995). This methodology has been improved through different research projects as well as through the ongoing analysis of the time-series.

Phytoplankton blooms are natural phenomena and, therefore, HABs can be affected by natural hydroclimatic variability, as well as by global climate change and/or eutrophication and shifts in nutrient ratios. The interaction of multiple environmental factors makes very difficult to predict how global change will affect the distribution and frequency of HABs (Hallegraeff, 2010). Broad scale changes in phytoplankton life-forms have been recorded across the north west European shelf (Bedford et al., 2020). The carbonate chemistry of the oceans is also changing (Ostle et al., 2015) and the HAB community is at an early stage in understanding the effects of these changes on HAB species dynamics and biogeography and their impacts (Fu et al., 2012, Riesebell et al., 2018, Raven et al., 2020).

Wells et al., (2020) discuss the increasing concern that human-mediated environmental parameters may alter the patterns, distribution and intensity of HABs. Data from the continuous plankton recorder (CPR) has already revealed the redistribution of certain HAB taxa in the North Sea, including *Dinophysis* spp*.* over the last five decades (Edwards et al., 2006). In the southern North Sea, Nohe et al., (2020) have also observed a long-term increase in the occurrence of HAB genera, such as *Pseudo-nitzschia*, *Alexandrium* and *Prorocentrum,* during a period of de-eutrophication and ocean warming. In a model-based analysis parameterised with laboratory data, Gobler et al., (2017) propose that ocean warming has expanded the niche for harmful blooms of *D. acuminata* and *A. fundyense* (now *A. catenella*) in northwest European waters since 1982. In their analysis of changes in *Dinophysis* abundances identified to genus level in CPR data from offshore waters Dees et al., (2017) did not find any statistically significant increases in annual mean cell abundance, number of *Dinophysis* blooms, or bloom duration. Increases in the cell abundance of

Pseudo-nitzschia species in the northeast Atlantic as a result of changing wind speed and direction have been observed since the mid-1990s (Hinder et al., 2012). Analysis of DA levels in UK shellfish from 2008 - 2017 showed an increase in the frequency of both DA occurrence and toxic events in shellfish not including *Pecten maximus*, although there was considerable annual variability in intensity and geographical location of toxic episodes (Rowland-Pilgrim et al., 2019).

Species previously only recorded from the warmer Mediterranean Sea, such as *Alexandrium mediterraneum* U. John 2014, have recently been observed in the Atlantic coast in the Bay of Biscay (Laza-Martínez et al., 2017). During the last few decades, the warm water period has been prolonged by approximately a month in the Bay of Biscay and sea surface temperature has increased at 0.26 ± 0.03 °C per decade (Costoya et al., 2015), giving rise to concern that in a future warmer ocean scenario this area could become suitable for the settlement of other species currently present in the western Mediterranean Sea, such as the PST-producer *A. pacificum* R.W. Litaker 2014. Despite ocean warming and associated species distribution shifts being one of the most obvious consequences of climate change, shifts in hydrodynamic forces are also expected to impact phytoplankton dynamics. Shifts in winds affecting the upwelling off the Iberian Peninsula (Cabanas et al., 2003), cause changes in upwelling/downwelling dynamics influencing *Gymnodinium catenatum* and *Dinophysis* spp. occurrences.

In the decades to come an increased focus will be placed on the oceans and coastal and shelf sea areas to provide a source of seafood, as well as financial income to support society under changing climatic conditions. HAEDAT provides a data source on harmful algal events that need to be factored into future human adaption and management strategies.

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Figure headings

Figure 1. A, Map showing the central location (white dot) of each HAEDAT area code included in this study; PT – Portugal, ES – Spain, FR – France, GB – United Kingdom, IE – Ireland, FO – Faroe Islands, IS – Iceland. The African and Mediterranean coastlines are not included in this study. B, Map showing detail of Macaronesia.

Figure 2. Map showing locations (●) where routine monitoring for shellfish toxins takes place along the coastal areas of Spain, Portugal, France, Ireland and the UK included in the study area.

Figure 3 A- G. Barcharts of number of HAEDAT areas with events per year. The number of area codes per country are after the country name. The category 'Total Mortalities' includes events of benthic mortalities as well as dogs, birds, aquaculture and natural fish. Figures 3 F and G are highlighted in grey to flag that the datasets for Iceland and Faroe Islands are incomplete.

Figure 4. Distribution of HAEDAT events associated with PSTs along the European Atlantic margin; (A) total number of years with events in HAEDAT until 2018, number of years with events between; (B) 1999 – 2003, (C) 2004 – 2008, (D) 2009 – 2013, (E) 2014 - 2018. Symbol size represents the number of years with events per area code. Data from 1992 -1994 and 2016 - 2018 have been entered for Iceland; no events from the Faroe Islands have been entered since 1990. Data from the Mediterranean and mainland African coastlines are not included.

Figure 5. Distribution of HAEDAT events associated with DSTs along the European Atlantic margin; (A) total number of years with events in HAEDAT until 2018, number of years with events between; (B) 1999 – 2003, (C) 2004 – 2008, (D) 2009 – 2013, (E) 2014 - 2018. Symbol size represents the number of years with events per area code. Data from 1992 -1994 and 2016 - 2018 have been entered for Iceland; no events from the Faroe Islands have been entered since 1990. Data from the Mediterranean and mainland African coastlines are not included.

Figure 6. Distribution of HAEDAT events associated with ASTs along the European Atlantic margin; (A) total number of years with events in HAEDAT until 2018, number of years with events between; (B) 1999 – 2003, (C) 2004 – 2008, (D) 2009 – 2013, (E) 2014 - 2018. Symbol size represents the number of years with events per area code. Data from 1992 -1994 and 2016 - 2018 have been entered for Iceland; no events from the Faroe Islands have been entered since 1990. Data from the Mediterranean and mainland African coastlines are not included.

Figure 7. Plot showing the (A) maximum DA levels in *Pecten maximus* (circles) and other bivalve shellfish species (triangles) other shellfish (triangles) in the UK HAEDAT data (B) DA levels in *Pecten maximus* (circles) and other bivalve shellfish species (triangles) from associated monitoring data. The grey line on the x axis represents the

period when the requirement to monitor offshore *Pecten maximus* fishing areas had stopped due to move to end product testing and shucking.

Figure 8. Distribution of HAEDAT events associated with AZTs along the European Atlantic margin; (A) total number of years with events in HAEDAT until 2018, number of years with events between; (B) 1999 – 2003, (C) 2004 – 2008, (D) 2009 – 2013, (E) 2014 - 2018. Symbol size represents the number of years with events per area code. Data from 1992 -1994 and 2016 - 2018 have been entered for Iceland; no events from the Faroe Islands have been entered since 1990. Data from the Mediterranean and mainland African coastlines are not included.

Figure 9. Distribution of HAEDAT events associated with Ciguatera Poisoning along the European Atlantic margin. Symbol size represents the number of years with events per area code. The first record of CP comes from the Canary Islands, Spain in 2004 Routine monitoring began in the Canary Islands in 2011. There is no routine monitoring for CP toxins elsewhere along the Atlantic European coastline.

Figure 10: Distribution of HAEDAT events showing mortalities of (A) aquaculture fish, (B) natural fish, (C) benthic animals and (D) birds and dogs. Records start in the 1980s.

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Table 3. Maximum toxin levels measured in different shellfish species from the study area and date of occurrence

Table 4: Harmful microalgal species from the IOC taxonomic list and species associated with fish and benthic mortality events from the study area

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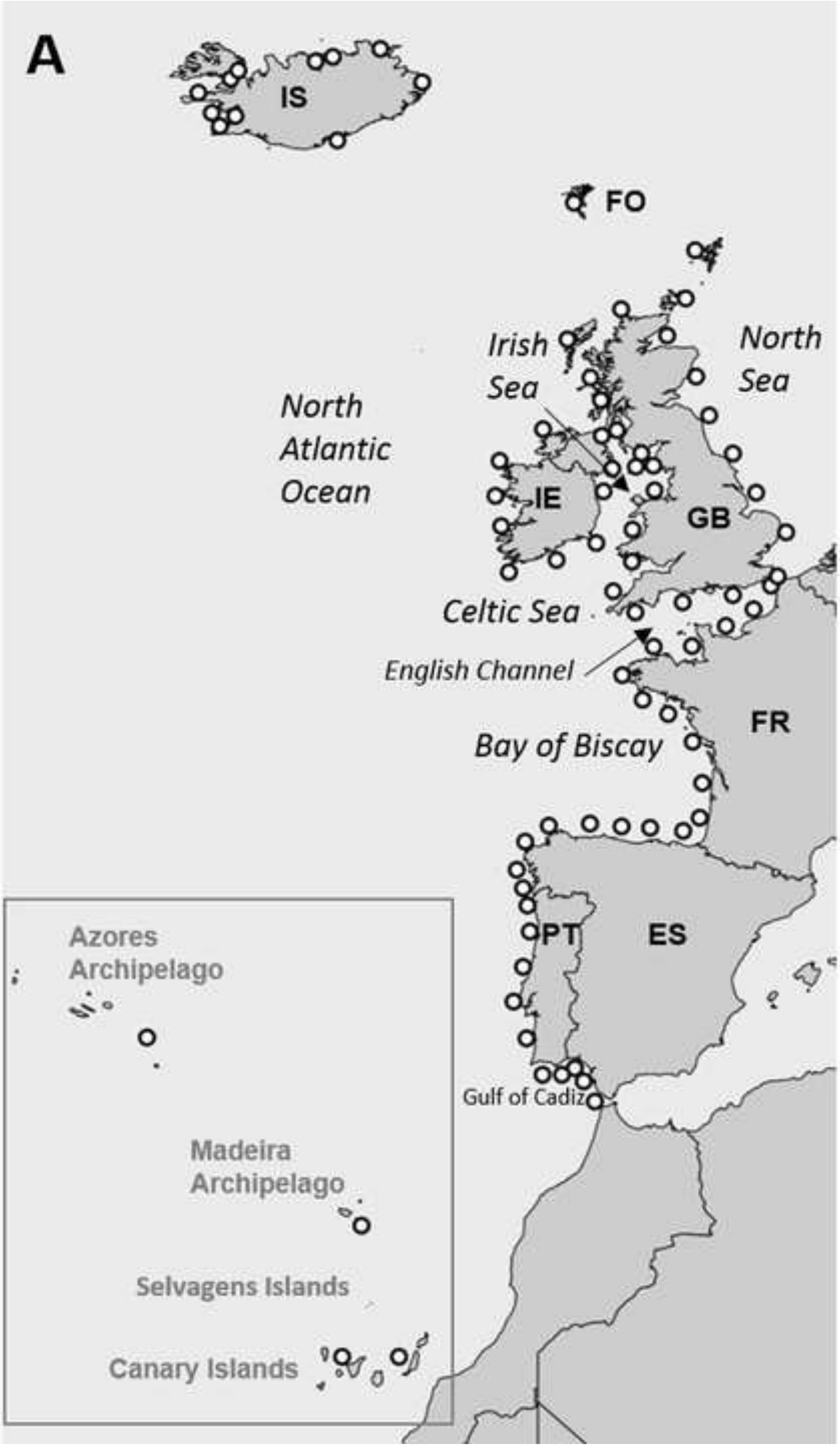

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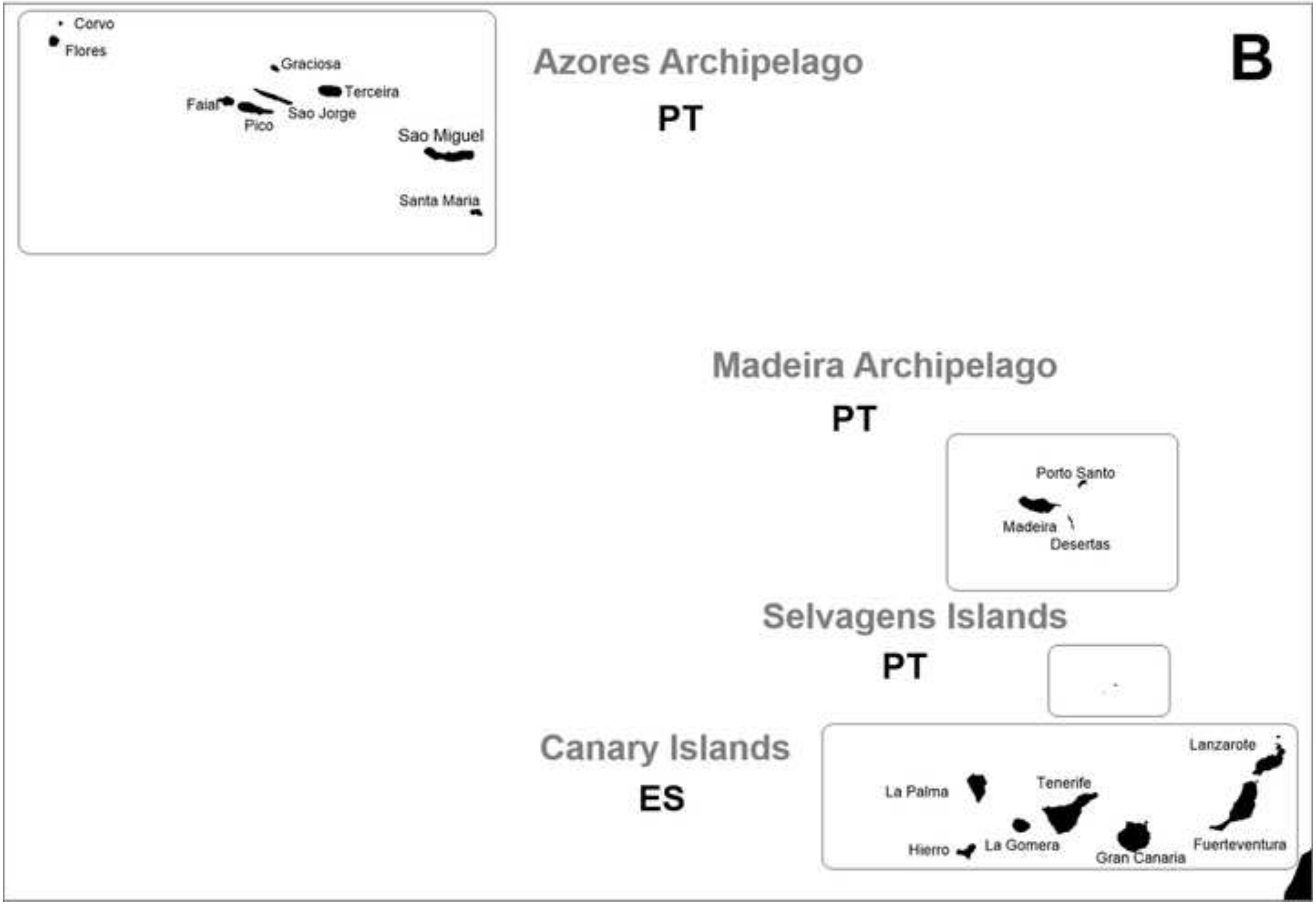

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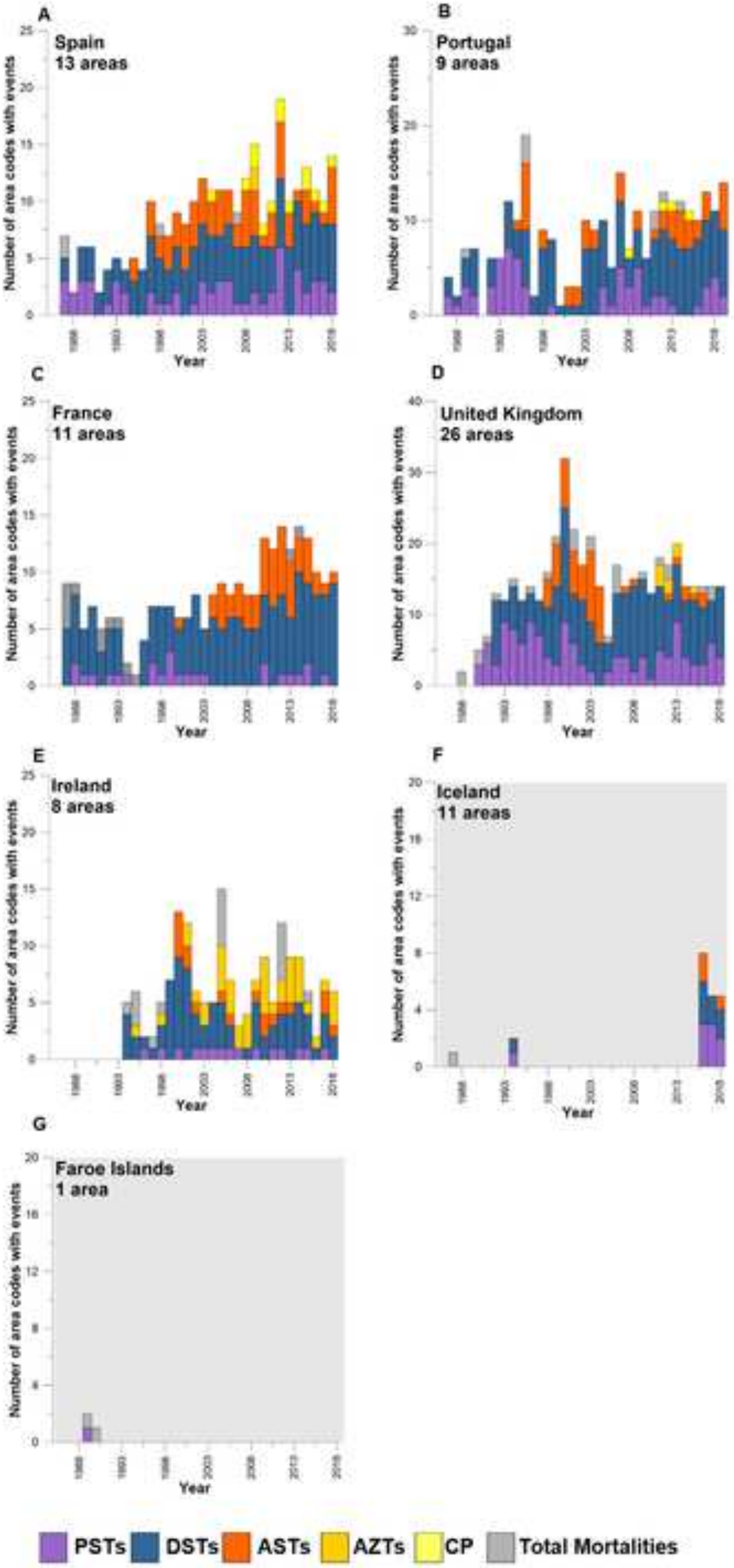

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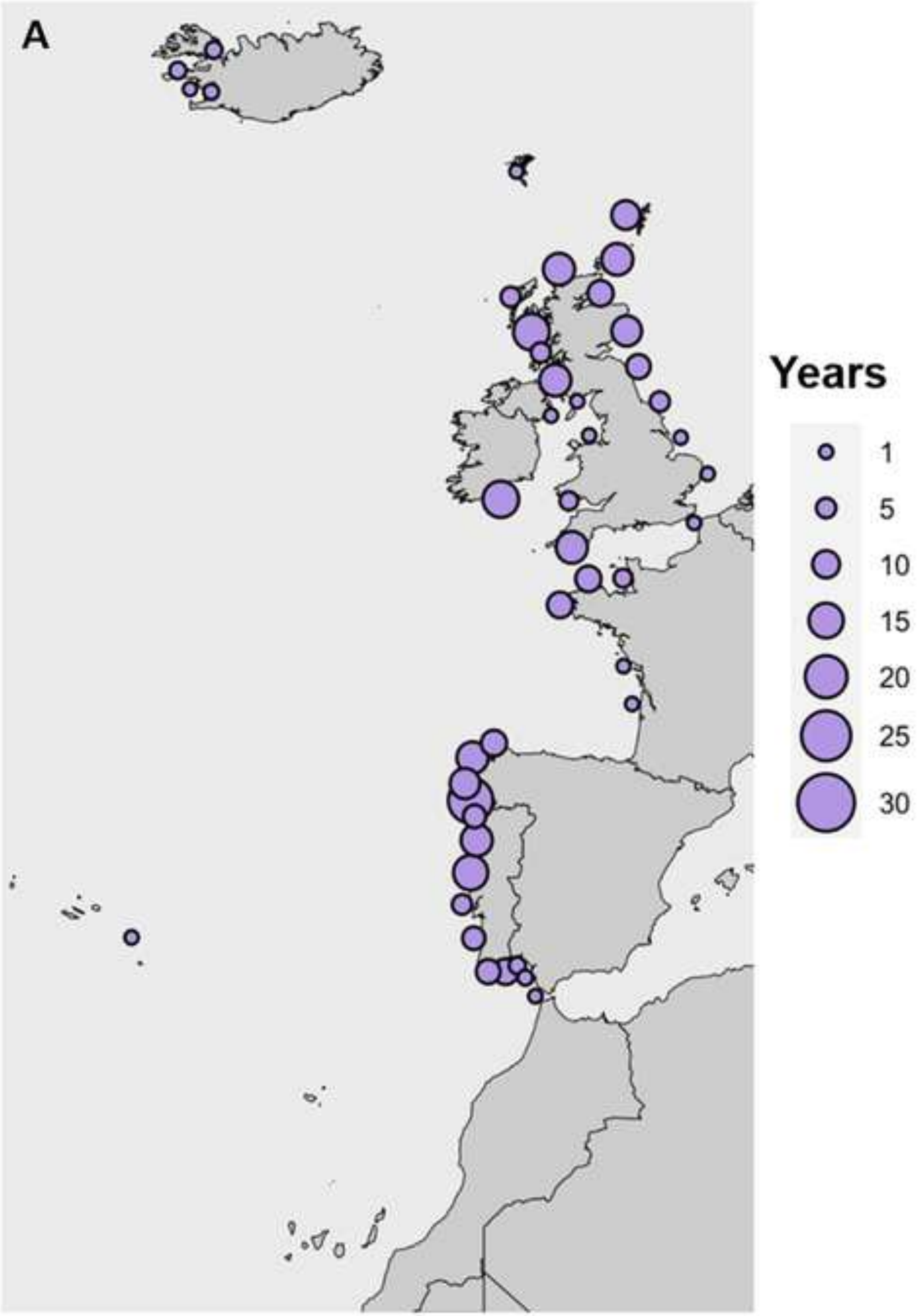

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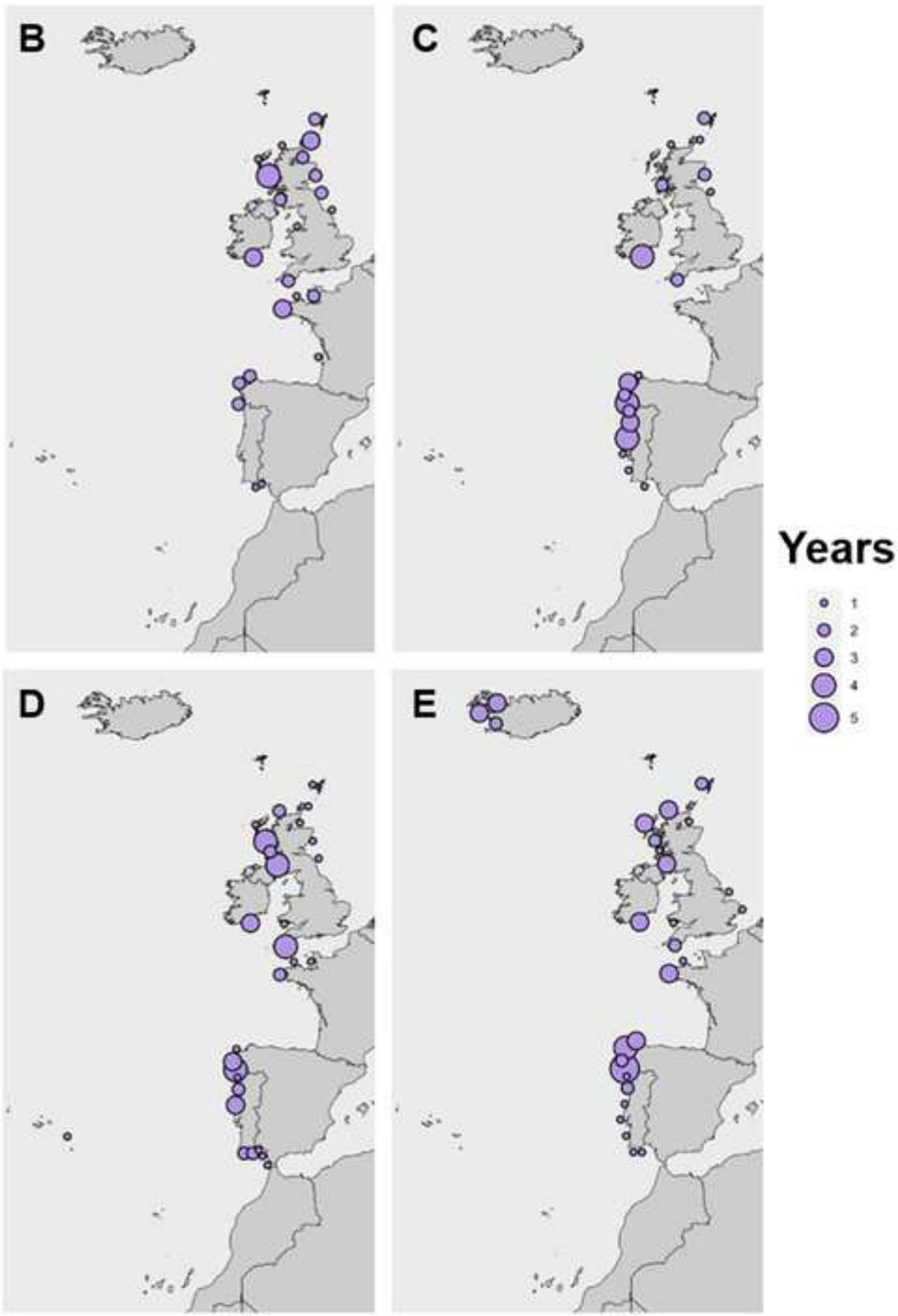

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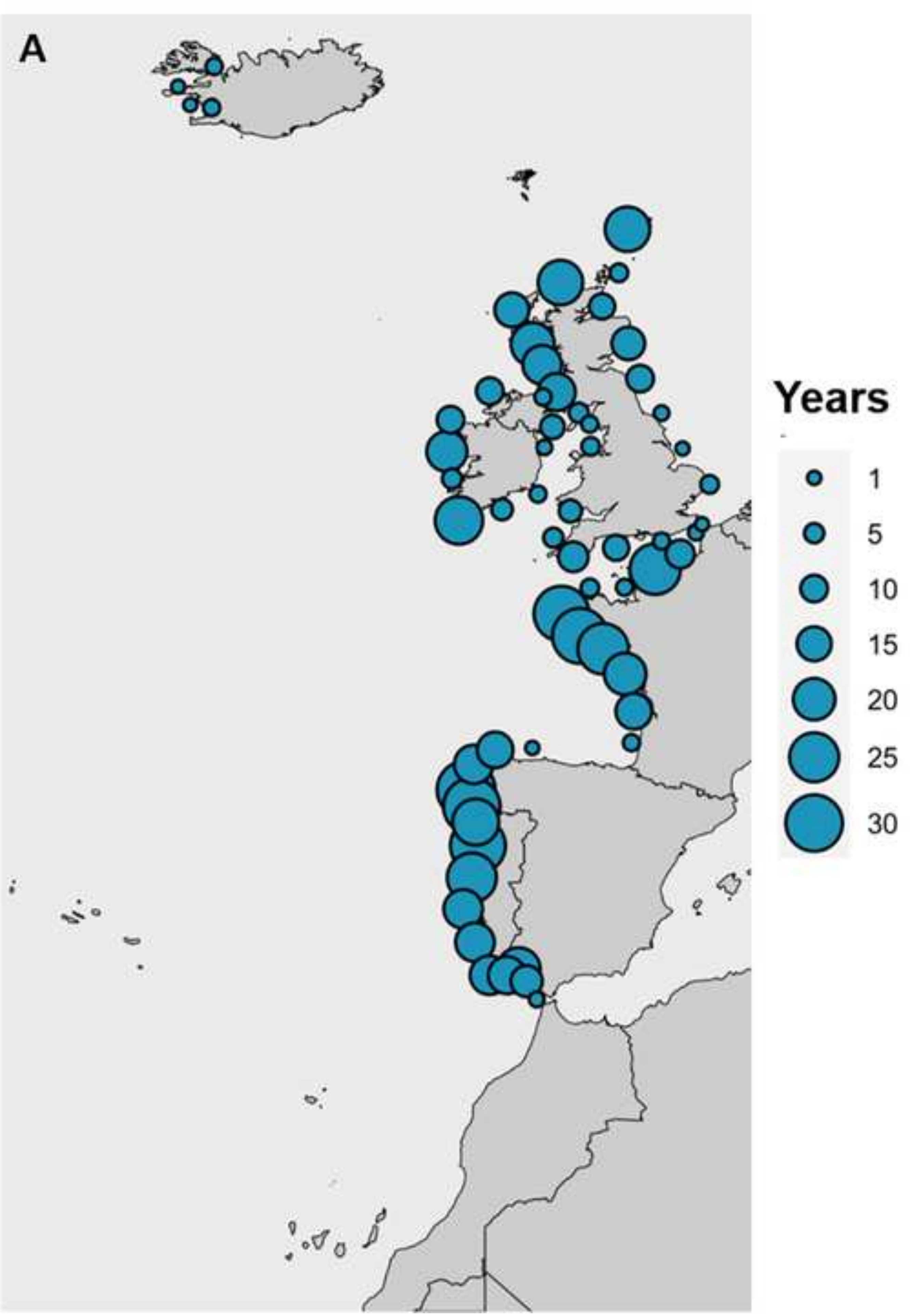

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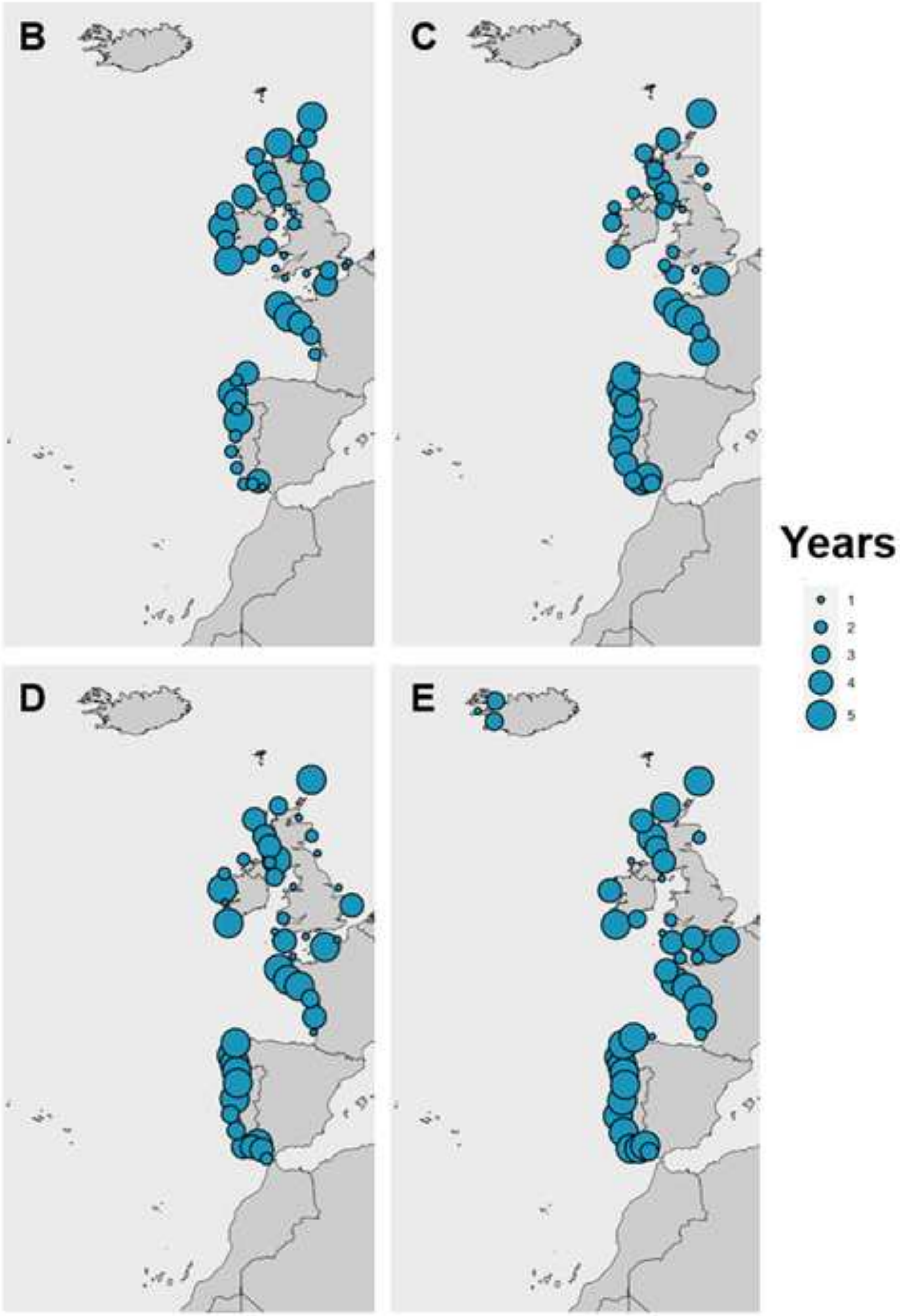

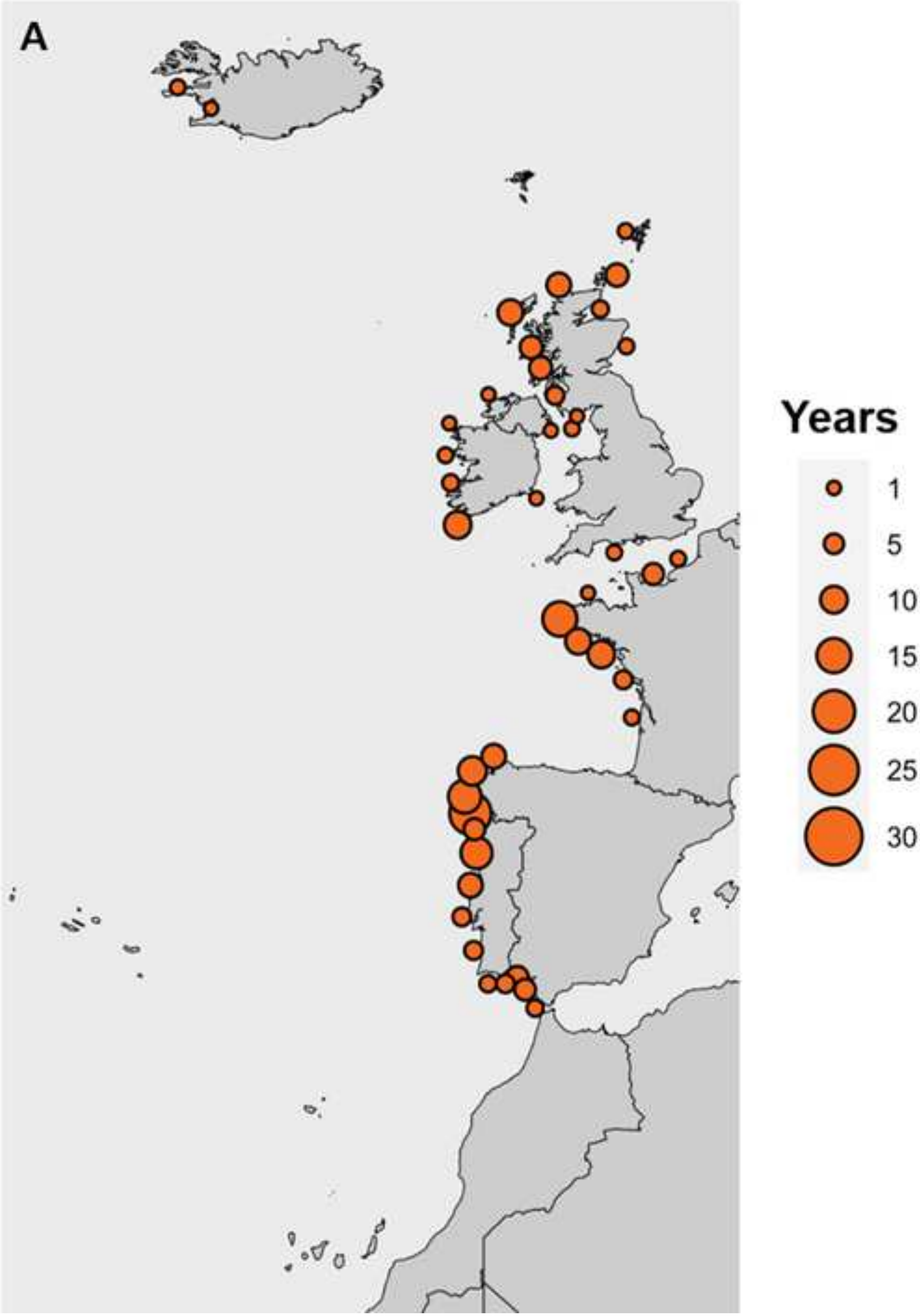
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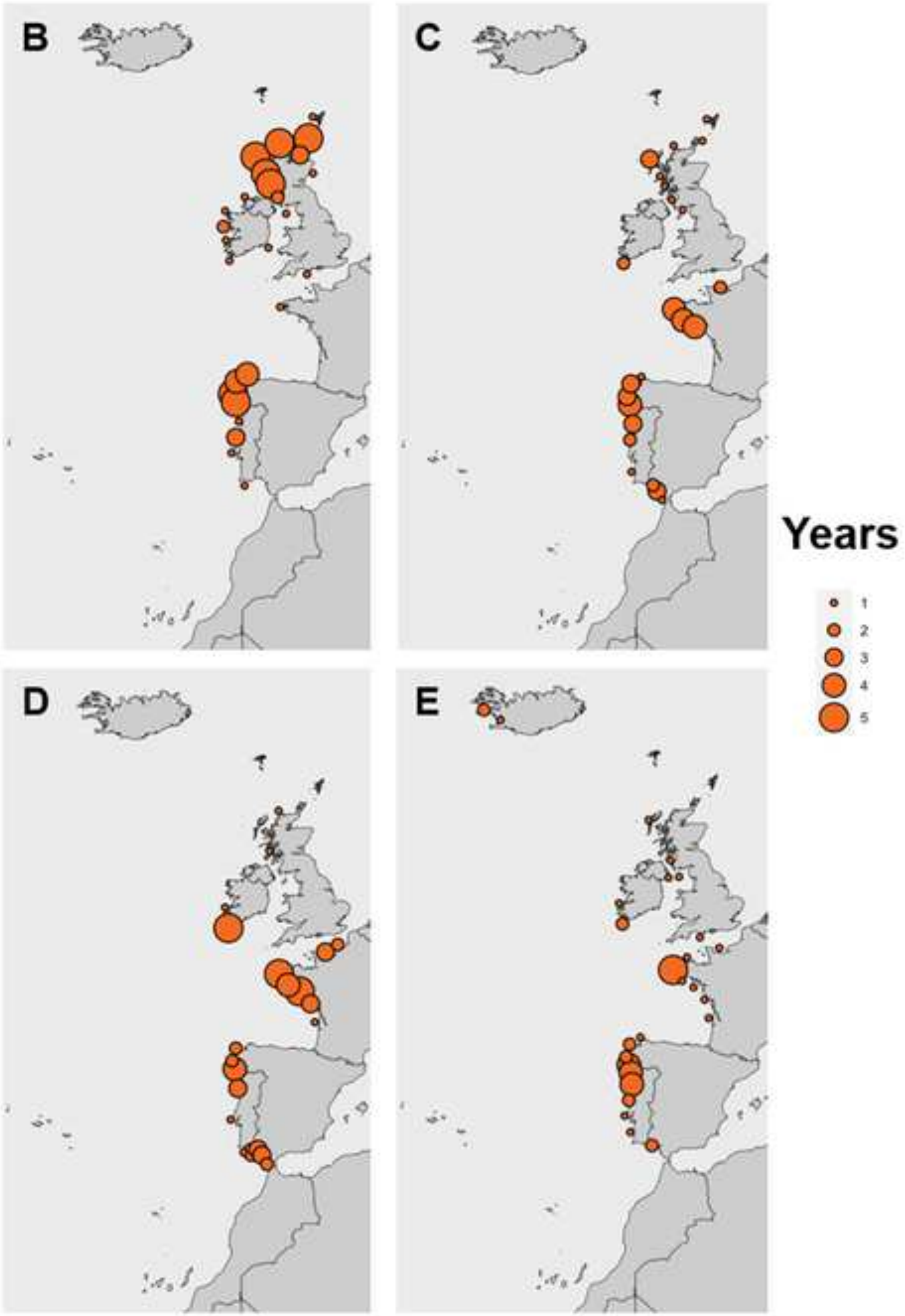

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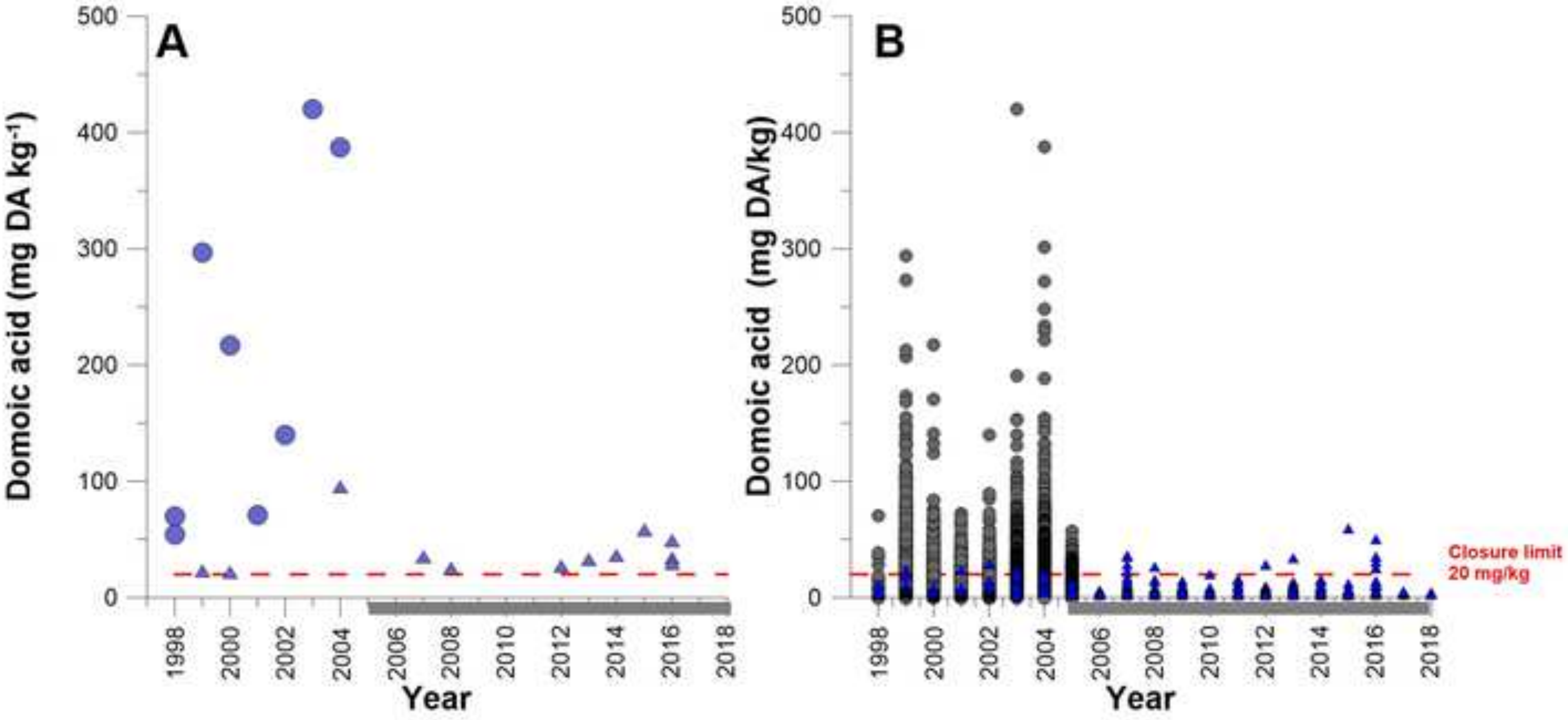

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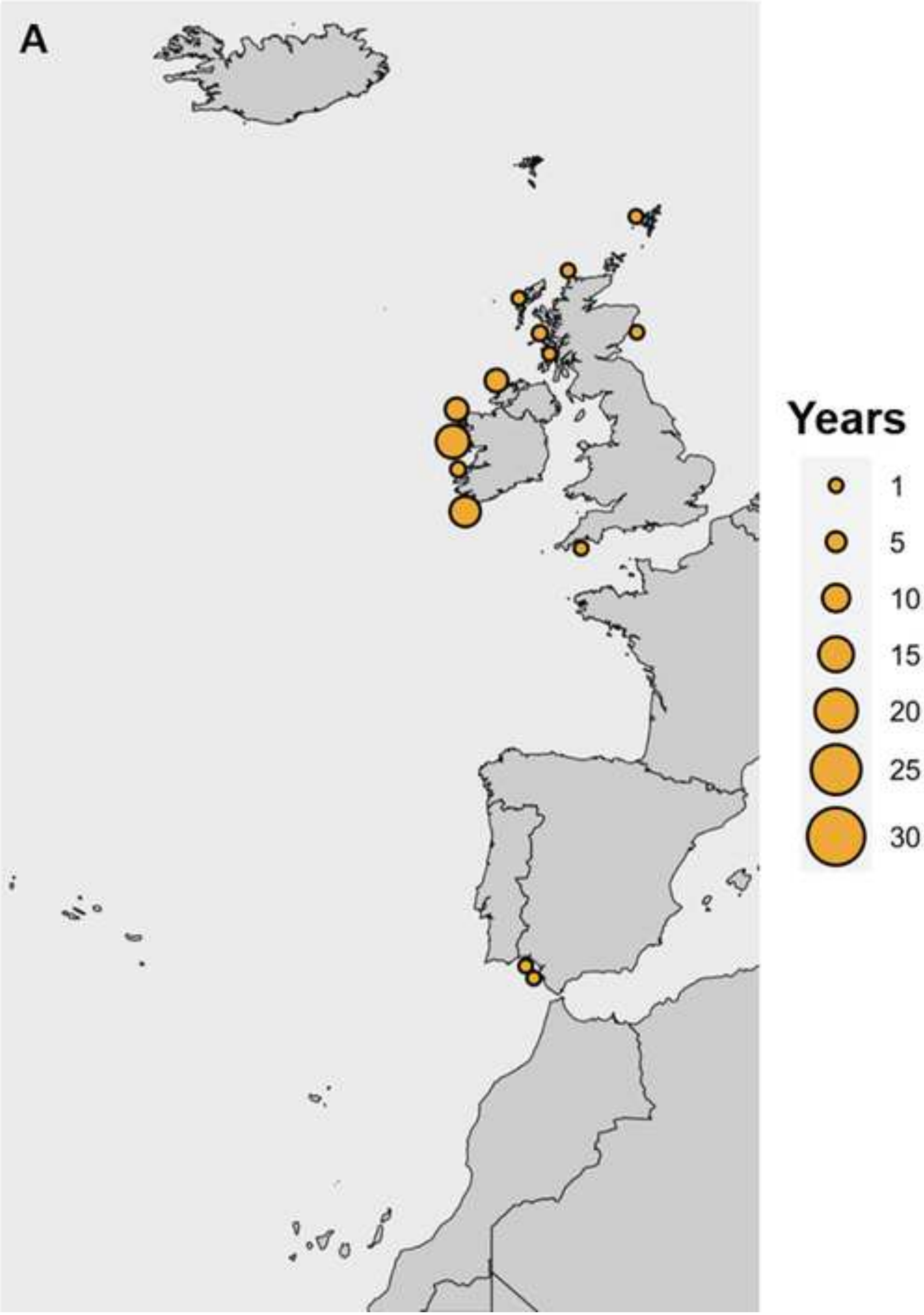

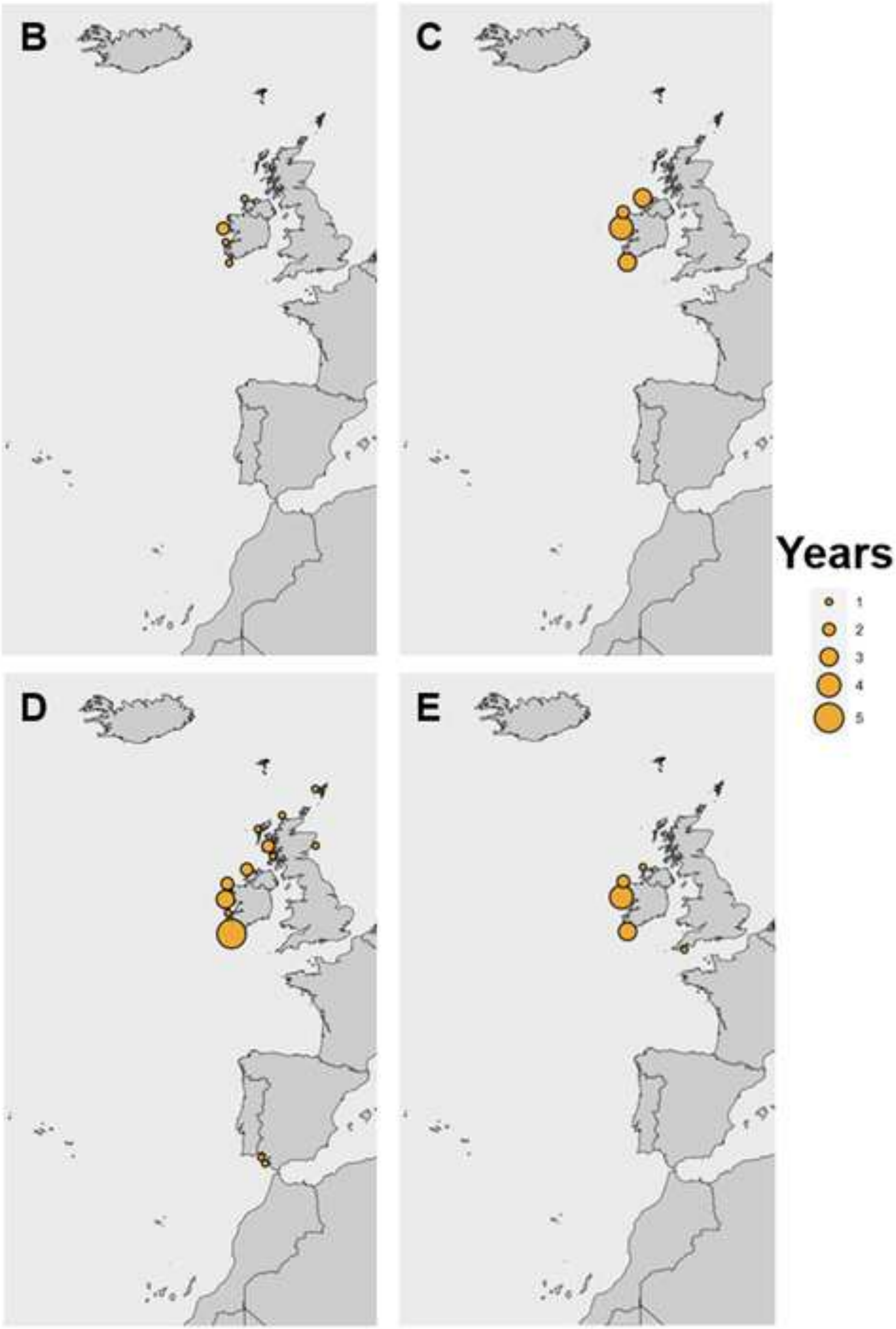
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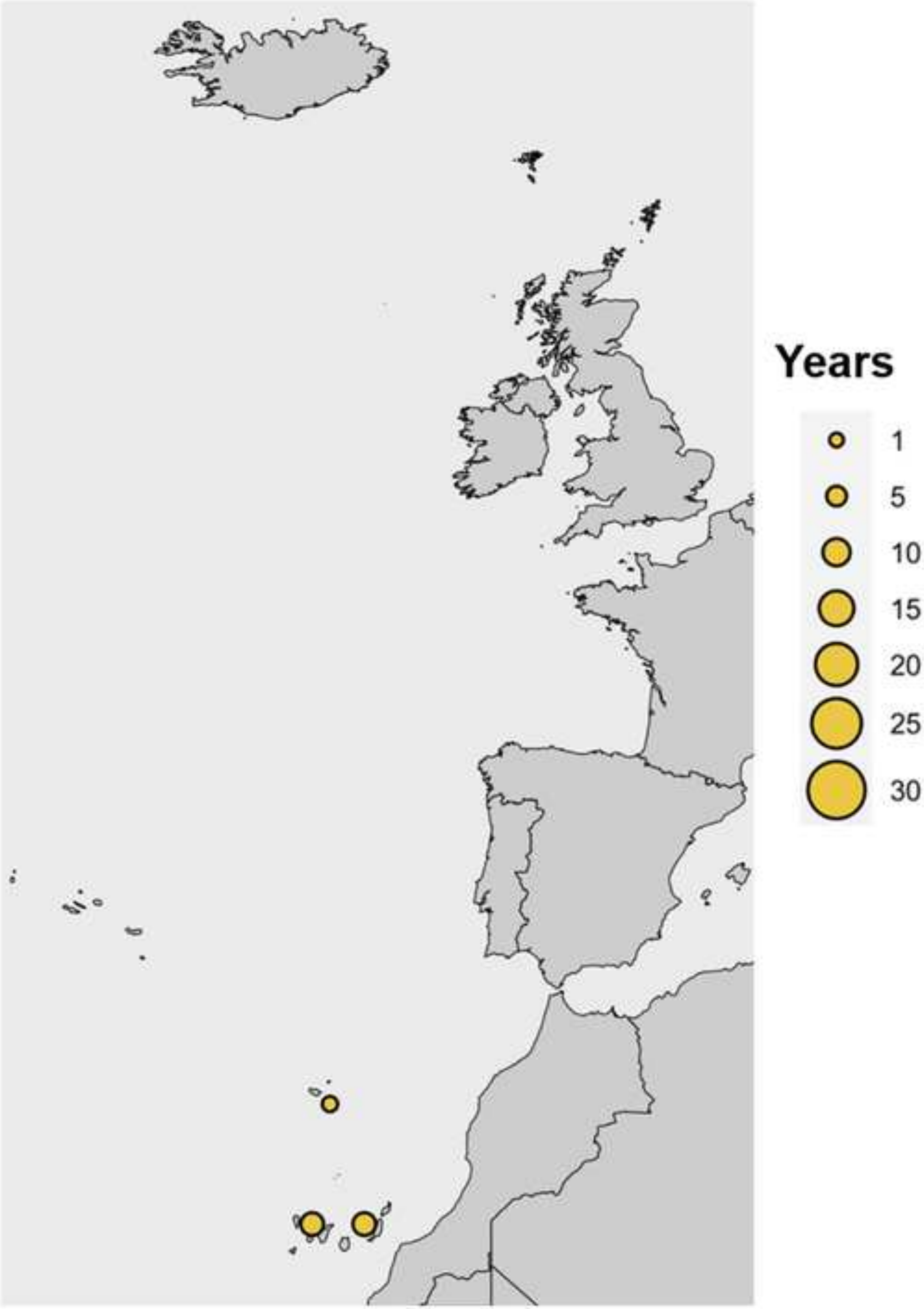

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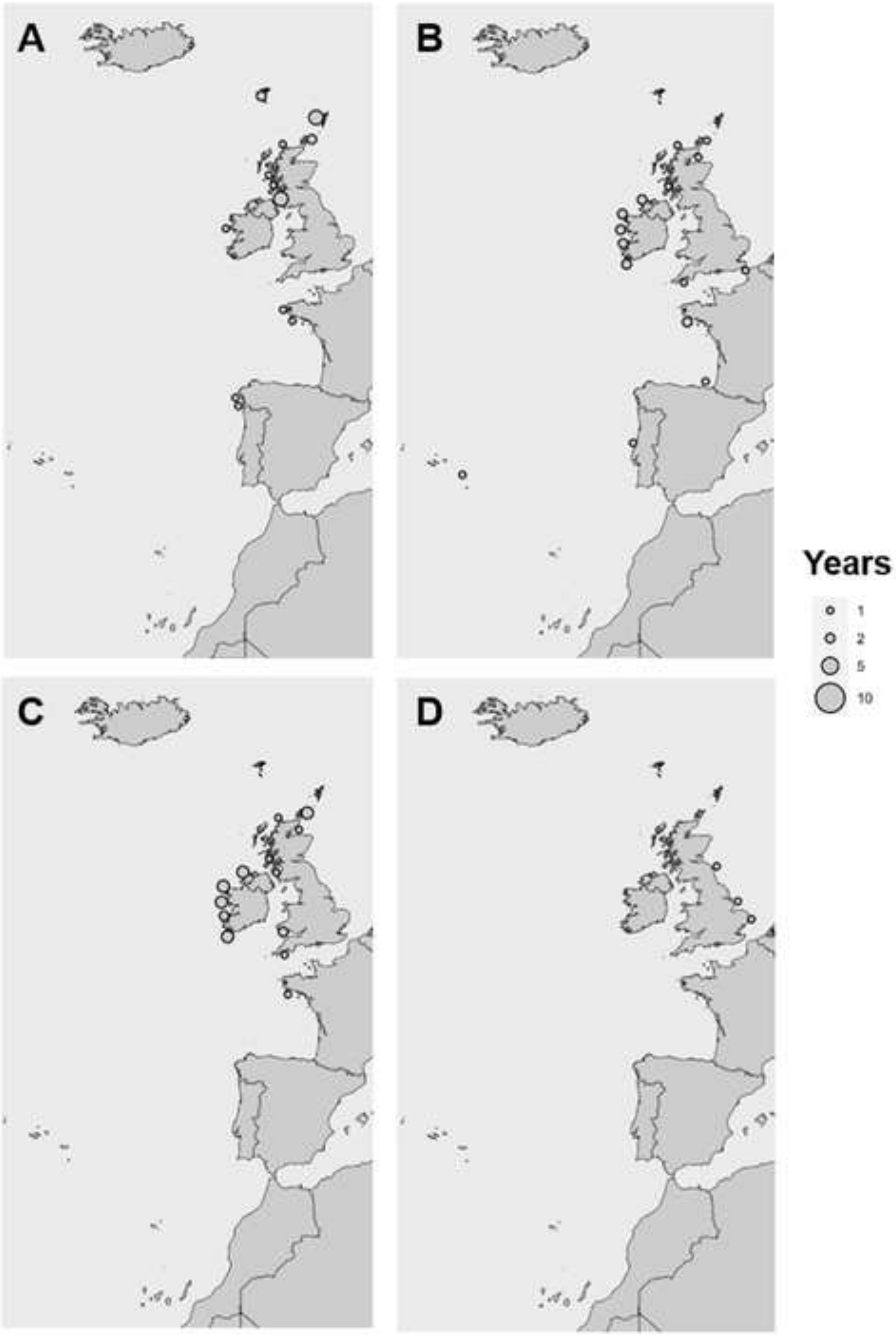

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Table 1A. Shellfish toxin monitoring metadata for countries involved in this study

Country	**Method Details**	**Shellfish Marine Biotoxin Group**					
		Amnesic Shellfish Toxins	**Azaspiracid Shellfish Toxins**	**Diarrhetic Shellfish Toxins**	**Paralytic Shellfish Toxins**	**Yessotoxins**	**Ciguatoxins**
France	Method/Ref	HPLC Quilliam et al., 1995	LC-MS/MS EURLMB Reference	Mouse Bioassay Yasumoto (1978, 1984)	Mouse Bioassay AOAC 959.08	LC-MS/MS EURLMB Reference	Not Analysed
	Result Unit	mg DA kg^{-1}	µg AZA1 eq. kg^{-1}	Positive/Negative	µg STXdi-HCl eq. kg^{-1}	mg YTX eq. kg^{-1}	
	Dates	1999 - current	2009 - current	1987-2009	1988-current	2009 - current	
	Method/Ref			LC-MS/MS EURLMB Reference	HPLC EN 14526		
	Result Unit			µg OA eq. kg^{-1}	µg STXdi-HCl eq. kg^{-1}		
	Dates			2010 - current	During 2021		
Ireland	Method/Ref	HPLC Quilliam et al., 1995	LC-MS/MS Quilliam et al., 2001 modified by Hess et al., 2003	Rat Bioassay Kat et al., (1983)	Mouse Bioassay AOAC 959.08	LC-MS/MS EURLMB Reference	Not Analysed
	Dates	1999 - current	2001-2010	1990-1998	1999-2011	2012 - current	
	Result Unit	µg DA g^{-1}, mg DA kg^{-1}	µg AZA1 eq. g^{-1}	Negative (-) / Toxicity (±, +, ++, +++)	µg STXdi-HCl eq. 100g^{-1} µg STXdi-HCl eq. kg^{-1}	mg YTX eq. kg^{-1}	
	Method/Ref		LC-MS/MS EURLMB Reference	Mouse Bioassay Yasumoto (1978, 1984)	HPLC/UPLC-FLD AOAC 2005.06		
	Dates		2009 - current	1999-2011	2011-current		
	Result Unit		µg AZA1 eq. g^{-1}	Positive/Negative	µg STXdi-HCl eq. kg^{-1}		
	Method/Ref			LC-MS/MS Quilliam et al., 2001 modified by Hess et al., 2003			
	Dates			2001-2010			
	Result Unit			µg OA eq. g^{-1}			
	Method/Ref			LC-MS/MS EURLMB Reference			

	Dates			2009 - current			
	Result Unit			µg OA eq. g^{-1}			
Portugal	Method/Ref	HPLC Quilliam et al., 1995	LC-MS/MS EURLMB Reference	Mouse Bioassay Yasumoto (1978, 1984)	Mouse Bioassay AOAC 959.08	LC-MS/MS EURLMB Reference	Not Analysed
	Dates	1997 - current	2017-current	1987-2003	1986-2003	2017-current	
	Result Unit	mg DA kg^{-1}	µg AZA1 eq kg^{-1}	Positive/Negative	µg STXdi-HCl equivalents kg^{-1}	mg YTX eq. kg^{-1}	
	Method/Ref			LC-MS/MS Vale & Sampayo (2003)	HPLC Lawrence et al. 1995 modified by Vale & Sampayo 2001		
	Dates			2003-2013	2003-2008		
	Result Unit			µg OA eq. kg^{-1}	µg STXdi-HCl equivalents kg^{-1}		
	Method/Ref			LC-MS/MS EURLMB Reference	HPLC AOAC 2005.06		
	Dates			2013-current	2008-current		
	Result Unit			µg OA eq. kg^{-1}	µg STXdi-HCl equivalents kg^{-1}		
United Kingdom	Method/Ref	HPLC Quilliam et al., 1995	LC-MS/MS EURLMB Reference	Mouse Bioassay Yasumoto (1978, 1995)	Mouse Bioassay AOAC (959.08)	LC-MS/MS EURLMB Reference	Not Analysed
	Dates	1998 - current	2011-current	1992-2011	1990-2008	2011-current	
	Result Unit	µg DA g^{-1}, mg DA kg^{-1}	µg AZA1 eq. kg^{-1}	Positive/Negative	µg STX eq. 100g^{-1}	mg YTX eq. kg^{-1}	
	Method/Ref			LC-MS/MS EURLMB Reference	HPLC AOAC 2005.06 modified by Turner et al., 2009		
	Dates			2011-current	2008-current		
	Result Unit			µg OA eq. kg^{-1}	µg STXdi-HCl eq. kg^{-1}		

Iceland	Method/Ref	HPLC Quilliam et al., 1995	LC-MS/MS Quilliam et al., 2001 modified by Hess et al., 2003	LC-MS/MS Quilliam et al., 2001 modified by Hess et al., 2003	Mouse Bioassay AOAC 959.08	LC-MS/MS EURLMB Reference	Not Analysed
	Dates	2004 - current	2005-2010	2005-2010	2009-2011	2012 - current	
	Result Unit	mg DA kg^{-1}	µg AZA1 eq. g^{-1}	µg OA eq. g^{-1}	µg STXdi-HCl eq. 100g^{-1} µg STXdi-HCl eq. kg^{-1}	mg YTX eq. kg^{-1}	
	Method/Ref		LC-MS/MS EURLMB Reference	LC-MS/MS EURLMB Reference	HPLC AOAC 2005.06		
	Dates		2009 - current	2009 - current	2011-current		
	Result Unit		µg AZA1 eq. g^{-1}	µg OA eq. g^{-1}	µg STXdi-HCl eq. kg^{-1}		
Spain	Method/Ref	HPLC AOAC 991.26	LC-MS/MS EURLMB Reference	Mouse Bioassay Yasumoto (1978, 1984)	Mouse Bioassay AOAC 959.08	LC-MS/MS EURLMB Reference	Neuroblastoma (neuro-2a) cell-based assay (CBA) Caillaud et al., 2012
	Dates	1994-current	2009-current	1980-2013	1977-2020	2009-current	2011
	Result Unit	µg DA g^{-1}, mg DA kg^{-1}	µg AZA1 eq. kg^{-1}	Positive/Negative	µg STXdi-HCl eq. 100g^{-1} µg STXdi-HCl eq. kg^{-1}	mg YTX eq. kg^{-1}	Positive/Negative
	Method/Ref			LC-MS/MS EURLMB Reference	HPLC / UPLC-FLD AOAC 2005.06		
	Dates			µg OA eq. kg^{-1}	2020-current		
	Result Unit			2009-current	µg STXdi-HCl eq. kg^{-1}		

DA – Domoic Acid, OA - Okadaic Acid, STX – Saxitoxin, YTX – Yessotoxin, AZA - Azaspiracid

Note: The Mouse Bioassay (Yasumoto 1978, 1984) was used in many countries as the reference method for the detection of DSTs up until end 2014 when legislation 15/2011 (amending 2074/2005) came into effect in 2011. This enabled the use of LC-MS/MS methods (EURLMB Reference method) as the official reference method of analysis. The mouse bioassay methodology (depending on method type, observation time, symptomology of mortality and time of death) was also observed to co-extract other toxins in addition to DSTs, including AZAs, YTXs and Cyclic Imines including Spirolides (SPX).

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Table 1B. Regulatory shellfish toxin limits

Toxin group	**Abbreviation**	**Maximum permissible limit**
Paralytic Shellfish Toxins	PSTs	800 micrograms of Saxitoxin di-HCl equivalent per kilogram (µg STXdi-HCl eq. kg^{-1})
Diarrhetic Shellfish Toxins (Okadaic Acid, Dinophysistoxins and Pectenotoxins[1] together)	DSTs	160 micrograms of Okadaic Acid equivalents per kilogram (µg OA eq. kg^{-1})
Amnesic Shellfish Toxins	ASTs	20 milligrams of Domoic Acid per kilogram (mg DA kg^{-1})
Azaspiracid Toxins	AZTs	160 micrograms of Azaspiracid equivalents per kilogram (µg AZA1 eq. kg^{-1})
Yessotoxins	YTXs	3.75 milligrams of Yessotoxin equivalent per kilogram (mg YTX eq. kg^{-1})

[1] –Pectenotoxins is due to be deregulated and removed from legislation

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Table 2: Incidents of human illness with paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP) Azaspiracid poisoning (AZP) and ciguatera poisoning (CP).

Country	syndrome	illness/deaths	year	reference
Spain				
	PSP	63/0	1976	DXSP, 1996
	PSP	17/0	1986	DXSP, 1996
	PSP	27/0	1993-1994	DXSP, 1996
	DSP	5,000/0	1981	DXSP, 1996
	DSP	1/0	1982-1994	DXSP, 1996
	DSP	61/0	1995	DXSP, 1996
	DSP	1 outbreak/0	1999	DXSP, 1999
	DSP	2 outbreaks/0	2000-2006	DXSP, 2005; Granado et al., 2007
	DSP	4 outbreaks/0	2012	DXSP, 2012a,b
	DSP	6 families/0	2015	regional press
	DSP	1 family/0	2016	DXSP, 2016a
	ASP	2/0	2014	DXSP, 2014; Pazos et al., 2016
	CP	5/0	2004	Pérez-Arellano et al., 2005
	CP	115/0	2008-2018	SVEICC, 2020
Portugal				
	PSP	100/6	1946	Correia 1946
	PSP	21/1	1955	Pinto and Silva 1956
	PSP	9/0	1994	Carvalho et al., 1998
	PSP		2007	Rodrigues et al., 2008
	PSP	4/0	2012	Santos et al., 2014
	PSP	2/0	2018	de Carvalho et al., 2019
	DSP	18/0	1998	Vale 2020
	DSP	13/0	2002	Vale 2020

	DSP	30/0	2012	Vale 2020
	CP	7/0	2008	Gouveia et al., 2009
	CP	12/0	2012	Costa 2017
	CP	7/0	2015	Cebadera-Miranda et al., 2018
France				
	DSP	3450/0	1983	Lassus et al, 2016
	DSP	2070/0	1984	Lassus et al, 2016
	DSP	10/0	2009	Santé Publique France
	DSP	12/0	2012	Santé Publique France
	DSP	12/0	2013	Santé Publique France
	DSP	4/0	2014	Santé Publique France
	DSP	5/0	2015	Santé Publique France
	DSP	71/0	2016	Santé Publique France
	DSP	18/0	2017	Santé Publique France
	DSP	95/0	2018	Santé Publique France
UK	PSP	30/2	1827	Ayres 1975
	PSP	‘several’/1	1858	Ayres 1975
	PSP	3/1	1888	Ayres 1975
	PSP	2/1	1904	Ayres 1975
	PSP	19/1	1909	Ayres 1975
	PSP	3/0	1958	Ayres 1975
	PSP	78/0	1968	Ayres 1975
	DSP	49/0	1997	Hinder et al., 2011
	DSP	159/0	2006	McDougall and Midgley 2011
	DSP	70/0	2013	Whyte et al., 2013
	DSP	6/0	2019	Young et al., 2019
Ireland				
	PSP	4/3	1872	Ayres 1975

	PSP	7/5	1890	Ayres 1975
	AZP	8/0	1995	McMahon & Silke 1996
	AZP	20-24/0	1997	Twiner et al. 2008
	AZP	10/0	1998	Twiner et al. 2008
	AZP	20-30/0	1998	Twiner et al. 2008
	AZP	12-16/0	2000	Twiner et al. 2008
	AZP	2/0	2008	Klontz et al. 2009
	AZP	219/-	2008	EFSA 2008
	AZP	15/0	2013	RASFF 2013a
	AZP	112/-	2013	RASFF 2013b
	DSP	132/0	2012	RASFF 2012
	DSP	3/0	2014	RASFF 2014
	DSP	31/0	2016	RASFF 2016
Faroe Islands				
	PSP	4/0	1984	Mortensen 1985

Table

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Table 3. Maximum toxin levels measured in different shellfish species from the study area and date of occurrence

Shellfish	ASTs mg kg^{-1}		AZTs µg kg^{-1}		DSTs µg kg^{-1}		PST µg kg^{-1}		YTX mg kg^{-1}	
Ireland										
Cerastoderma edule	9	29/05/2014	240	05/10/2015	790	02/09/2014	<LOD	-	<LOD	-
Crassostrea gigas	152	26/04/2005	340	26/11/2012	270	27/08/2012	323	01/07/2015	<LOD	-
Mytilus edulis	444.9	25/04/2005	8970	01/10/2005	6820	23/09/2014	1174	26/06/2017	<LOQ	-
Ostrea edulis	7	26/08/2003	200	22/08/2012	70	05/12/2005	<LOQ	-	<LOD	-
Pecten maximus (R)	1379	09/02/2004	320	30/09/2015	4620	20/10/2014	62	09/07/2017	<LOQ	-
Ruditapes philippinarum	34	15/05/2012	250	11/07/2012	60	10/12/2002	<LOD	-	<LOD	-
Spisula solida	54	09/05/2017	340	11/07/2012	650	06/08/2012	<LOD	-	<LOD	-
France										
Cerastoderma edule	-	-	-	-	11755	09/04/2012	3300	30/06/1998	-	-
Crassostrea gigas	110	01/04/2014	45	30/05/2011	1041	12/12/2012	7360	13/08/2001	-	-
Mytilus edulis	221	22/04/2014	53	12/06/2017	24862	22/04/2012	11664	16/04/2012	1.72	11/07/2018
Pecten maximus (TF)	861	21/04/2014	16	13/03/2017	2305	03/09/2014	543	25/07/2012	-	-
Pecten maximus (DG)	2269	24/09/2007								
Ruditapes decussatus	-	-	-	-	842	25/06/2013	5740	12/11/2001	-	-
Ruditapes philippinarum	123	19/04/2010	-	-	2278	22/04/2012	-	-	-	-
UK										
Aequipecten opercularis	7	15/06/2016	<LOQ	-	517	15/06/2016	<LOQ		1.9	15/06/2016
Cerastoderma edule	33	12/08/2013	157	06/01/2014	154	20/06/2016	1678	12/06/2012	<LOQ	-
Crassostrea gigas	8	01/05/2012	237	23/05/2013	682	03/09/2013	4392	28/04/2015	<LOQ	-
Ensis spp.	34	14/06/2016	26	07/08/2012	231	05/06/2012	33060	26/08/1998	<LOQ	-
Mytilus spp.	58	06/07/2015	626	11/10/2011	6950	17/07/2013	43050	27/07/1995	3.0	11/06/2012
Ostrea edulis	7	11/06/2001	<LOQ	-	61	27/07/2016	<LOQ	-	<LOQ	-
Pecten maximus (TF)	1294	03/10/2004	<LOQ	-	264	18/07/2012	2321	19/08/2015	-	-

Pecten maximus (G)	420	30/11/2003	<LOQ	-	<LOQ	-	1222	23/06/2014	-	-
Spisula solidissima	11	06/05/2014	209	14/08/2012	534	05/08/2014	2152	25/06/2013	<LOQ	-
Spain										
Acanthocardia tuberculata	24	29/04/2014	<LOQ	-	< LOQ	-	22150	17/01/2000	< LOQ	-
Aequipecten opercularis	63.	14/04/2015	< LOQ	-	3180	21/05/2014	9050	12/11/2014	< LOQ	-
Callista chione	166	05/02/2001	< LOQ	-	<LOQ	-	9500	26/01/2000	< LOQ	-
Cerastoderma edule	195	11/04/2000	90	03/08/2009	5448	07/07/2015	18500	10/08/2015	0.094	06/07/2016
Chamelea gallina	103	23/01/2001	575	20/07/2009	471	25/03/2019	15710	24/01/2000	< LOQ	-
Crassostrea gigas	107	27/05/2010	< LOQ	-	118	08/07/2020	9810	16/08/2012	< LOQ	-
Donax trunculus	75	13/04/2009	284	20/07/2009	2278	13/08/2018	17300	19/01/2000	< LOQ	-
Ensis ensis	150	10/04/2015	< LOQ	-	855	29/04/2016	4690	20/12/2005	< LOQ	-
Ensis siliqua	95	18/03/2004	< LOQ	-	1907	06/07/2015	6100	23/08/2000	< LOQ	-
Haliotis tuberculata	< LOQ	-	< LOQ	-	< LOQ	-	7300	02/01/2006	< LOQ	-
Mimachlamys varia	2	28/09/2020	< LOQ	-	333	19/09/2016	>15000	30/10/2012	< LOQ	-
Mytilus galloprovincialis	248	27/04/2005	< LOQ	-	12744	04/09/2014	40800	12/12/2005	1.434	18/08/2014
Ostrea edulis	17	19/04/2000	< LOQ	-	166	05/04/2016	1280	22/12/2005	< LOQ	-
Pecten maximus	980	13/02/2001	< LOQ	-	1271	10/07/2015	14460	22/08/2000	< LOQ	-
Polititapes rhomboides	228	08/04/2015	< LOQ	-	900	13/08/2014	16000	19/12/2005	< LOQ	-
Ruditapes decussatus	73	27/05/2010	< LOQ	-	425	10/10/2013	1890	03/06/2004	< LOQ	-
Ruditapes philippinarum	188	03/10/2008	< LOQ	-	994	10/10/2013	13800	15/12/2005	< LOQ	-
Scrobicularia plana	13	20/03/2017	< LOQ	-	< LOQ	-	400	30/10/2014, 24/11/2015	< LOQ	-
Venerupis corrugata	316	21/09/2004	< LOQ	-	1091	23/06/2015	10950	07/12/2005	< LOQ	-
Venus verrucosa	74	06/05/2003	< LOQ	-	< LOQ	-	3320	13/12/2011	< LOQ	-
Portugal										
Cerastoderma edule	82	Jun/Jul 2004	27	22/08/2016	888	Aug/Sep 2004	3174	09/01/2017	0.1	Aug/2020
Crassostrea gigas	56	25/3/2019	< LOQ	-	510	2/9/2019	4892	10/01/2017	0.1	02/05/2016
Donax spp.	-	-	27	29/08/2016	1990	Aug/Sep 2004	9800	29/09/1995	< LOQ	-

Mytilus edulis	102	07/04/2015	43	24/08/2016	1945	07/06/2016	59990	19/10/1995	0.9	06/07/2020
Ruditapus decussatus	72.	Jun/Jul 2004	< LOQ	-	667	22/10/2019	33600	26/10/1995	<LOQ	-
Solen sp.	37	Jun/Jul 2004	< LOQ	-	673	20/05/2019	2300	17/10/1995	<LOQ	-
Spisula solida	86	8/4/2015-	< LOQ	-	858	Aug/Sep 2004	2749	25/09/2018	0.2	08/06/2016

< LOD: < Limit of Detection

<LOQ: < Limit of Quantification

TF: Total Flesh

DG: Digestive gland

G: Gonad

R: Remainder tissue

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Table 4: Harmful microalgal species from the IOC taxonomic list and species associated with fish and benthic mortality events from the study area

Species	**ES**	**PT**	**FR**	**IE**	**GB**	**FO**	**IS**
PST events							
Alexandrium andersonii Balech, 1990	●			●			
Alexandrium catenella (Whedon & Kofoid) Balech, 1985					◆	◆	◆
Alexandrium minutum Halim 1960	◆	◆	◆	◆	◆		
Alexandrium ostenfeldii (Paulsen) Balech and Tangen 1955	●		●	●	●	●	
Centrodinium punctatum (Cleve) F.J.R.Taylor, 1976	●		●				
Gymnodinium catenatum H.W. Graham 1943	◆	◆					
DST events							
Dinophysis acuminata Claparède & Lachmann, 1859	◆	◆	◆	◆	◆	◆	◆
Dinophysis acuta Ehrenberg, 1859	◆	◆	◆	◆	◆	◆	◆
Dinophysis caudata Saville-Kent 1881	◆	●	●	●	●[1]	●	
Dinophysis fortii Pavillard 1924	●	●	●	●	●[1]		
Dinophysis infundibulum J.Schiller 1928	●				●[1]		
Dinophysis norvegica Claparède & Lachmann, 1859	●[2]		●	●	●	●	●
Dinophysis ovum (F.Schütt) T.H.Abé	◆	◆		●	●		
Dinophysis sacculus F.Stein, 1883	●	●	●				
Dinophysis tripos Gourret, 1883	●	●	●	●	●[3]		
Phalacroma rotundatum (Claparéde & Lachmann) Kofoid & J.R.Michener, 1911	●	●	●	●	●		

Species	**ES**	**PT**	**FR**	**IE**	**GB**	**FO**	**IS**
Prorocentrum hoffmannianum M.A.Faust, 1990	●						
Prorocentrum lima (Ehrenberg) F.Stein, 1878	●	●	●	●	●		
Prorcentrum rhaythmum A.R.Loeblich III, Sherley & Schmidt, 1979		●					
AZT events							
Amphidoma languida Tillmann, Salas & Elbrachter, 2012	◆			●	●		
Azadinium dexteroporum I.Percopo & A.Zingone, 2013							
Azadinium poporum Tillmann & Elbrächter, 2011	●		●	●	●		
Azadinium spinosum Elbrächter & Tillmann, 2009	●			◆	●		
YTX							
Gonyaulax spinifera (Claparède & Lachmann) Diesing, 1866		●	●	●	●		
Lingulodinium polyedra (F.Stein) J.D.Dodge, 1989		●	●	●	●		
Protoceratium reticulatum (Claparède & Lachmann) Bütschli 1885	●	●	●	●	●		
Pinnatoxin							
Vulcanodinium rugosum Nézan & Chomérat, 2011	●		●				
Other							
Prorocentrum cordatum (Ostenfeld) J.D.Dodge, 1975					●		

Species	ES	PT	FR	IE	GB	FO	IS
AST events							
Halamphora coffeaeformis (C.Agardh) Levkov, 2009	●				●		
Pseudo-nitzschia abrensis Pérez-Aicua & Orive, 2013	●						
Pseudo-nitzschia australis Frenguelli, 1939	◆	◆	◆	◆	◆		
Pseudo-nitzschia brasiliana N.Lundholm, G.R.Hasle & G.A.Fryxell, 2002	●						
Pseudo-nitzschia caciantha Lundholm, Moestrup & Hasle, 2003					●		
Pseudo-nitzschia calliantha Lundholm, Moestrup & Hasle, 2003	●	●			●		
Pseudo-nitzschia cuspidata (Hasle) Hasle, 1993		●			●		
Pseudo-nitzschia delicatissima (Cleve) Heiden, 1928	●	●	●	●	●		
Pseudo-nitzschia fraudulenta (Cleve) Hasle, 1993	●	●	●	●	●		
Pseudo-nitzschia hasleana Lundholm, 2012	●						
Pseudo-nitzschia multiseries (Hasle) Hasle, 1995	●	●	●	●	◆		
Pseudo-nitzschia multistriata (Takano) Takano, 1995	●	●	●				
Pseudo-nitzschia plurisecta Orive & Pérez-Aicua, 2013	●		◆				
Pseudo-nitzschia pseudodelicatissima (Hasle) Hasle, 1993	●	●	◆[4]	●	●		◆[4]
Pseudo-nitzschia pungens (Grunow ex Cleve) G.R.Hasle, 1993	●	●	◆	●	●		
Pseudo-nitzschia seriata (Cleve) H.Peragallo, 1899				●	◆		◆
Pseudo-nitzschia subpacifica (Hasle) Hasle, 1993	●	●			●		

Species	ES	PT	FR	IE	GB	FO	IS
CP							
Gambierdiscus australes Chinian & M.A.Faust, 1999	◆						
Gambierdiscus belizeanus M.A.Faust, 1995	●						
Gambierdiscus caribaeus Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester, 2009	●						
Gambierdiscus carolinianus Litaker, Vandersea, M.A.Faust, Kibler, W.C.Holland & P.A.Tester, 2009	●						
Gambierdiscus excentricus S.Fraga, 2011	◆	◆					
Gambierdiscus silvae S.Fraga & F.Rodríguez, 2014	●						
Palytoxins							
Ostreopsis heptagona D.R.Norris, J.W.Bomber & Balech, 1985		●					
Ostreopsis cf. ovata Fukuyo, 1981		●	●				
Ostreopsis cf. siamensis Johs.Schmidt, 1901	●	●	●				
Ichthyotoxins and other fish killing/benthic mortality mechanisms							
Karenia brevis (C.C.Davis) Gert Hansen & Moestrup, 2000					●[1]		
Karenia concordia F.H.Chang & K.G.Ryan, 2004		●					
Karenia mikimotoi (Miyake & Kominami ex Oda) Gert Hansen & Moestrup, 2000	●	●	◆	◆	◆		
Karenia papilionacea A.J.Haywood & K.A.Steidinger, 2004	●	●	●				
Karlodinium armiger Bergholtz, Daugbjerg & Moestrup, 2006	●						

Karlodinium gentienii E.Nézan, N.Chomérat & R.Siano, 2014			●				

Species	**ES**	**PT**	**FR**	**IE**	**GB**	**FO**	**IS**
Karlodinium veneficum (D.Ballantine) J.Larsen, 2000	●				●[5]		
Margalefidinium polykrikoides (Margalef) F.Gómez, Richlen & D.M.Anderson, 2017	●						
Polykrikos hartmannii W.M.Zimmermann, 1930	●	●					
Takayama cladochroma (J.Larsen) M.F.Salas, Bolch & Hallegraeff, 2003		●					
Chattonella antiqua (Y.Hada) C.Ono, 1980	●						
Chattonella marina (Subrahmanyan) Hara & Chihara, 1982			●				
Pseudochattonella farcimen (Eikrem, Edvardsen & J.Throndsen) Eichrem 2009	●						
Vicicitus globosus (Y.Hara & Chihara) F.H.Chang 2012	●						
Fibrocapsa japonica S.Toriumi & H.Takano, 1973	●	●	●	●	●		
Heterosigma akashiwo (Y.Hada) Y.Hada ex Y.Hara & M.Chihara, 1987	●	●	◆	◆	◆		◆
“Flagellate X”				◆	◆		
Prymnesium calathiferum Chang & Ryan, 1985	●						
Prymnesium parvum N. Carter, 1937	●						
Prymnesium polylepis (Manton & Parke) Edvardsen, Eikrem & Probert, 2011					●		

Species	ES	PT	FR	IE	GB	FO	IS
Other species/genera associated with mortality events							
Chaetoceros calcitrans (Paulsen) Takano, 1968		●					
Octactis speculum (Ehrenberg) F.H.Chang, J.M.Grieve & J.E.Sutherland 2017	●						
Mesodinium rubrum (Lohmann, 1908)		●					
Lepidodinium chlorophorum (M.Elbrächter & E.Schnepf) Gert Hansen, Botes & Salas, 2007			●				
Phaeocystis sp. Lagerheim, 1893					●		

◆ Dominant species associated with events

●[1] Recorded in Parke and Dixon 1976

●[2] Single record

●[3] Infrequently observed

●[4] Identified before Lundholm et al., 2003

●[5] Isolated by Parke and Dixon 1950