

Apparent biogeographical trends in *Alexandrium* blooms for northern Europe: identifying links to climate change and effective adaptive actions

Kerstin Klemm^{a,b}, Allan Cembella^a, Dave Clarke^c, Caroline Cusack^c, Lars Arneborg^d, Bengt Karlsson^d, Ye Liu^d, Lars Naustvoll^e, Raffaele Siano^f, Sandra Gran-Stadniczenko^g, Uwe John^{a,b,*}

^a Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, Bremerhaven 27570, Germany

^b Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Ammerländer Heerstraße 231, Oldenburg 26129, Germany

^c Marine Institute, Rinville, Oranmore, Co. Galway, Ireland

^d Swedish Meteorological and Hydrological Institute, Research and development, oceanography, Sven Källfelts gata 15, Västra Frölunda, SE-426 71, Sweden

^e Institute of Marine Research, PO Box 1870 Nordnes, Bergen NO-5817, Norway

^f Ifremer, DYNCO, Plouzané 29280, France

^g Section for Aquatic Biology and Toxicology, Department of Biosciences, University of Oslo, P.O. Box 1066, Blindern, Oslo 0316, Norway

ARTICLE INFO

Editor by: Dr. C. Gobler

Keywords:

CoCliME

Climate change indicators

Climate services

Paralytic shellfish toxins

Regional climate model

Ecosystem function

ABSTRACT

The marine dinoflagellate *Alexandrium* Halim represents perhaps the most significant and intensively studied genus with respect to species diversity, life history strategies, toxigenicity, biogeographical distribution, and global magnitude and consequences harmful algal blooms (HABs). The socioeconomic impacts, environmental and human health risks, and mitigation strategies for toxigenic *Alexandrium* blooms have also been explored in recent years. Human adaptive actions based on future scenarios of bloom dynamics and shifts in biogeographical distribution under climate-change parameters remain under development and not yet implemented on a regional scale. In the CoCliME (Co-development of climate services for adaptation to changing marine ecosystems) project these issues were addressed with respect to past, current and anticipated future status of key HAB genera and expected benefits of enhanced monitoring. Data on the distribution and frequency of *Alexandrium* blooms related to paralytic shellfish toxin (PST) events from key CoCliME Case Study areas, comprising the North Sea and adjacent Kattegat-Skagerrak, Norwegian Sea, and Baltic Sea, and eastern North Atlantic marginal seas, were evaluated in a contemporary and historical context over the past several decades. The first evidence of possible biogeographical expansion of *Alexandrium* taxa into eastern Arctic gateways was provided from DNA barcoding signatures. Various key climate change indicators, such as salinity, temperature, and water-column stratification, relevant to *Alexandrium* bloom initiation and development were identified. The possible influence of changing variables on bloom dynamics, magnitude, frequency and spatial and temporal distribution were interpreted in the context of regional ocean climate models. These climate change impact indicators may play key roles in selecting for the occurrence and diversity of *Alexandrium* species within the broader microeukaryote communities. For example, shifts to higher temperature and lower salinity regimes predicted for the southern North Sea indicate the potential for increased *Alexandrium* blooms, currently absent from this area. Ecological and socioeconomic impacts of *Alexandrium* blooms and effects on fisheries and aquaculture resources and coastal ecosystem function are evaluated, and, where feasible, effective adaptation strategies are proposed herein as emerging climate services.

1. Introduction

Among the diverse microalgal genera causing harmful algal blooms (HABs), the dinoflagellate *Alexandrium* Halim is perhaps the best studied

with respect to species diversity, biogeographical distribution, bloom frequency and dynamics, toxin production and socioeconomic and ecological consequences of blooms. Mardones et al. (2020) reported that the highest number of harmful algal events reported in the Harmful

* Corresponding author at: Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, Bremerhaven 27570, Germany.
E-mail address: uwe.john@awi.de (U. John).

Algal Event Database (HAEDAT; <http://haedat.iode.org/>) from 1970 to 2020 were due to paralytic shellfish toxins (PSTs). Globally, the majority of these PST events (1974 separate events recorded) can be attributed to toxigenic *Alexandrium* species, although *Pyrodinium bahamense* Plate is a more frequent cause in Southeast Asia and the South Pacific. In many regions, particularly in the tropics and sub-tropics, PST events are not assignable to *Alexandrium* species, but this is not the case for northern Europe, where except for the eastern North Atlantic margin of Iberia, *Alexandrium* blooms are recognized as the exclusive cause of PST events.

Various comprehensive studies and global reviews of the genus *Alexandrium* (e.g., Anderson et al., 2012; Murray et al., 2021) have considered bloom dynamics of particular species and linked such toxigenic blooms as the cause of paralytic shellfish poisoning (PSP). Indeed, there are many excellent detailed studies on *Alexandrium* bloom dynamics (Anderson, 1997) and ecology and oceanography (Anderson et al., 2005), including life-history transitions (Richlen et al., 2016; Anderson et al., 2021). Such studies focussed on large-scale ecosystems in defined geographical regions, such as the Gulf of Maine and Bay of Fundy on the western Atlantic or the Chuckchi Sea off Alaska. In northern Europe, comparable studies on *Alexandrium* distribution, including benthic cysts linked to bloom frequency and magnitude, exist only for a few coastal embayments, e.g., for the Bay of Biscay off the coast of France (e.g., Klouch et al., 2016a; Lambert et al., 2018; Siano et al., 2021) and Cork Harbour in southern Ireland (Raine, 2014). This lack of comparable data has constrained the development of appropriate niche-models for broader scale applications in northern European seas in response to climate change.

Within the EU project CoCLiME (Co-development of Climate services for adaptation to changing Marine Ecosystems, <https://www.coclime.eu>), *Alexandrium* was identified as a key genus comprising several HAB species and a priority for developing knowledge on autecology and functional role in marine ecosystems in northern European and Arctic gateway waters. To date, there has been no published systematic analysis of *Alexandrium* bloom dynamics, species distribution or potential consequences in response to climate change at the regional scale for northern Europe. Recent publications (Bresnan et al., 2021; Karlson et al., 2021) have addressed the regional issues for northern Europe regarding paralytic shellfish toxin (PST) accumulation in shellfish, with toxins originating mostly from blooms of *Alexandrium* species, and associated human health and environmental consequences. These have been presented as HAB events over decadal time-scales, but with little consideration of the dynamics and biogeography of the causative species. Almost all northern European countries maintain a coastal monitoring program for both potentially toxigenic blooms of *Alexandrium* species and PST levels in shellfish. Such *Alexandrium* and PST data sets are designed to yield rapid response information for national regulatory compliance with EU norms on public health protections and sustainability of the aquaculture and shellfisheries sector. Unfortunately, the national data are not archived (and sometimes are not even accessible) for analysis and interpretation at the regional scale outside the regulatory framework. Within CoCLiME, monitoring and research data sets were integrated over decadal time-scales for defined case study regions. The objective was to identify trends in *Alexandrium* bloom magnitude and species distribution in the context of developing climate services, including i) future bloom projections from modeling scenarios related to climate change and environmental consequences; ii) identification of indicators for future risk assessments of shifts in bloom dynamics and biogeography; and iii) addressing the human dimension, effects on socioeconomic development and human and ecosystem health. The review herein provides a scientific analysis as a prerequisite for design and implementation of strategies for appropriate societal adaptive responses to potential enhanced risk of shellfish toxicity and degraded ecosystem function in regions subject to enhanced *Alexandrium* blooms in northern Europe and Arctic gateway waters.

2. Overview of genus

2.1. Taxonomy and nomenclature

Species classified within the genus *Alexandrium* Halim may be found globally from polar seas to coastal and shelf seas at all latitudes, including from brackish water, and in tropical and subtropical lagoons (Tahvanainen et al., 2012; Van de Waal et al., 2015; Anderson et al., 2021; Liow et al., 2021). The type species *A. minutum* Halim was described from Alexandria harbor in Egypt (Halim, 1960), where it caused water discoloration ("red tide") but was not linked to harmful events at the time. Among the more than 30 recognized *Alexandrium* species registered in AlgaeBase (Guiry and Guiry, 2022) and the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Lundholm et al., 2009 onwards) approximately half are known to be toxigenic or have caused harmful events. These *Alexandrium* species are defined morphologically, but increasingly also on the basis of molecular signatures, toxigenicity, life-history (e.g., benthic cysts) and mating compatibility. The morphologically defined *A. tamarensis* species complex previously comprised *A. catenella* (Whedon & Kofoid) Balech, *A. fundyense* Balech, and *A. tamarensis* (Lebour) Balech, but without defined molecular profiles. After contentious taxonomic and nomenclatural revisions, consensus was finally achieved in assigning ribotype groups to named species (John et al., 2014; Litaker et al., 2018): *A. catenella* (Whedon & Kofoid) Balech (Group I), *A. mediterraneum* John (Group II), *A. tamarensis* (Lebour) Balech emend. John (Group III), *A. pacificum* Litaker (Group IV) and *A. australiense* Murray (Group V).

Phylogenetic analyses of *Alexandrium* inferred by maximum likelihood analysis of partial sequences of the LSU rRNA gene revealed three well-supported groups or species complexes: *Alexandrium ostenfeldii*, *A. minutum*, and *A. tamarensis* sub-groups (John et al., 2003, 2014; Lilly et al., 2007; Anderson et al., 2012). About a dozen species within the genus *Alexandrium* Halim have been found in northern European and eastern Arctic gateway waters (Table 1). Non-taxonomists are often challenged by the frequent and occasionally erroneous taxonomic and nomenclatural revisions of this genus (Cembella, 2018). This has also caused confusion in interpreting historical literature on biogeographical distribution and assignment of *Alexandrium* taxa to bloom and toxicity events for northern Europe. Even personnel trained for plankton identification have difficulties to correctly assign *Alexandrium* specimens to species (John et al., 2014; Litaker et al., 2018) in HAB monitoring programs. In the northern CoCLiME region, molecular taxonomic probes/assays are not routinely applied in any national monitoring program, but only for research, taxonomic validation, and for oceanographic field surveys. Hence frequent species misidentifications are almost certain (but unquantified) in national monitoring programs; most often taxonomic assignments from northern Europe are reliable only at the genus level or to the sub-group level (*Alexandrium ostenfeldii*, *A. minutum*, and *A. tamarensis* complexes).

These species reclassifications and nomenclatural revisions pose a challenge for correctly interpreting the history of PST events for northern Europe. According to nomenclatural precedence revision (Prud'homme van Reine, 2017) previous studies referring to *A. fundyense* Balech and current descriptions of this morphotype, genotype and toxin phenotype should now be assigned to *A. catenella* (Whedon & Kofoid) Balech. For northern Europe, this means that literature published between 1985 and 2017 on *A. fundyense* and on ribotype Group I, which is toxigenic and common in European waters, now refers to *A. catenella*. Finally, most PST events for the northern CoCLiME region have been attributed to *A. tamarensis* (Lebour) Balech, whereas the corresponding ribotype (Group III) is now regarded as *A. tamarensis* (Lebour) Balech emend. John and invariably non-toxigenic, at least with respect to PSTs (John et al., 2014).

Fortunately, almost without exception, the distribution of genus *Alexandrium* (Bresnan et al., 2021; Karlson et al., 2021) (Fig. 1a) can (with difficulty) be linked to historical PST events in northern Europe

Table 1

Alexandrium species and general biogeographical distribution in northern Europe and eastern Arctic gateway waters. Note that the toxigenic designation applies only to natural populations or cultured isolates of *Alexandrium* species found in the study area where at least one strain was confirmed to produce the particular toxin(s). This designation should not be inferred to conclude that the species is always (or even usually) or never toxigenic in the region. Toxigenicity refers only to defined groups of phycotoxins and does not include reference to unknown lysins, ichthyotoxins or other biologically active substances (allelochemicals) that may be produced by *Alexandrium* species. PSTs = paralytic shellfish toxins (or collectively “saxitoxins”); SPX = spirolides; GDA = goniiodomin A (GDA). NA = unknown, negative or unconfirmed toxigenicity in the region. STX = saxitoxin; GTX1,2,3,4 = gonyautoxins 1,2,3,4.

<i>Alexandrium</i> species	Taxonomic Synonyms	Known Toxigenicity	Biogeography	References
<i>Alexandrium affine</i> (Inoue & Fukuyo) Balech	<i>Protogonyaulax affinis</i> Inoue and Fukuyo 1985 <i>Episemicolon affine</i> (Inoue & Fukuyo) Gómez and Artigas 2019 <i>Alexandrium fukuyoi</i> Balech 1985	NA*	Bay of Biscay	Lassus et al., 2016
<i>Alexandrium andersonii</i> Balech		NA**	Bay of Biscay; South West Ireland	Lassus et al., 2016; Touzet et al., 2008
<i>Alexandrium catenella</i> (Whedon & Kofoid) Balech	<i>Gonyaulax catenella</i> Whedon and Kofoid 1936 <i>Protogonyaulax catenella</i> (Whedon & Kofoid) Taylor 1979 <i>Gessnerium catenellum</i> (Whedon & Kofoid) Loeblich III and Loeblich 1979 <i>Gonyaulax washingtonensis</i> Hsu 1967 <i>Alexandrium fundyense</i> Balech 1985 <i>Protogonyaulax fundyensis</i> (Balech) Gómez and Artigas 2019	GTX 2/3, GTX 1/4	Skagerrak*, North Sea*, Norwegian Sea*, Barents Sea*	[§] Norwegian Biodiversity information center
<i>Alexandrium concavum</i> (Gaarder) Balech	<i>Goniodoma concavum</i> Gaarder 1954 <i>Gonyaulax concava</i> (Gaarder) Balech 1967	NA	Bay of Biscay	Lassus et al., 2016
<i>Alexandrium gaarderae</i> Nguyen-Ngoc & Larsen		NA***	Bay of Biscay	Lassus et al., 2016
<i>Alexandrium kutnerae</i> (Balech) Balech	<i>Protogonyaulax kutnerae</i> (Balech) Sournia <i>Gonyaulax kutnerae</i> Balech	NA	Bay of Biscay	Lassus et al., 2016
<i>Alexandrium lei</i> Balech		NA****	Bay of Biscay	Lassus et al., 2016;
<i>Alexandrium margalefi</i> Balech		NA	Bay of Biscay	Tang et al., 2007 Lassus et al., 2016
<i>Alexandrium minutum</i> Halim	<i>Pyrodinium minutum</i> (Halim) Taylor 1976 <i>Alexandrium ibericum</i> Balech 1985 <i>Alexandrium lusitanicum</i> Balech 1985 <i>Alexandrium angustitabulatum</i> Taylor 1995	GTX 2/3, STX, GTX 1/4	English Channel, Bay of Biscay, Skagerrak, Kattegat, North Sea, Norwegian Sea, Barents Sea, Svalbard, South and West coasts Ireland	Cembella et al., 1987; Lassus et al., 2016; [§] Norwegian Biodiversity information center; Touzet et al., 2007; 2008
<i>Alexandrium ostenfeldii</i> (Paulsen) Balech & Tangen	<i>Goniodoma ostenfeldii</i> Paulsen 1904 <i>Gonyaulax ostenfeldii</i> (Paulsen) Paulsen 1949 <i>Heteraulacus ostenfeldii</i> (Paulsen) Loeblich III 1970 <i>Gessnerium ostenfeldii</i> (Paulsen) Loeblich and Loeblich III 1979 <i>Triadinium ostenfeldii</i> (Paulsen) Dodge 1981 <i>Protogonyaulax ostenfeldii</i> (Paulsen) Fraga and Sánchez 1985 <i>Gonyaulax phoneus</i> (Woloszyńska & Conrad) Loeblich & Loeblich <i>Glenodinium ostenfeldii</i> Paulsen 1903 <i>Pyrodinium phoneus</i> Woloszyńska and Conrad	STX, C1/C2, B1, GTX2/3; 13-desmethyl-SPX A and B and C; GDA	English Channel, Bay of Biscay, Skagerrak, Kattegat, Baltic Sea, North Sea, Wadden Sea, Norwegian Sea, Barents Sea, Svalbard, South and North coasts Ireland, Celtic Sea	Lassus et al., 2016; [§] Norwegian Biodiversity information center, [#] SMHI; Touzet et al., 2008; 2011

(continued on next page)

Table 1 (continued)

Alexandrium species	Taxonomic Synonyms	Known Toxicogenicity	Biogeography	References
	1939 <i>Goniaulax tamarensis</i> var. <i>globosa</i> Braarud 1945 <i>Gonyaulax dimorpha</i> Biecheler 1952 <i>Gonyaulax globosa</i> (Braarud) Balech 1971 <i>Gonyaulax trygve</i> Parke 1976 <i>Gonyaulax peruviana</i> Balech and de Mendiola 1977 <i>Protogonyaulax globosa</i> (Braarud) Taylor 1979 <i>Protogonyaulax phoneus</i> (Woloszyńska & Conrad) Taylor 1979 <i>Protogonyaulax peruviana</i> (Balech & Mendiola) Taylor 1979 <i>Protogonyaulax dimorpha</i> (Biecheler) Taylor 1979 <i>Alexandrium peruvianum</i> (Balech & Mendiola) Balech and Tangen 1985			
<i>Alexandrium pseudogonyaulax</i> (Biecheler) Horiguchi ex Yuki & Fukuyo	<i>Goniodoma pseudogonyaulax</i> Biecheler 1952 <i>Triadinium pseudogonyaulax</i> (Biecheler) Dodge 1981 <i>Gessnerium pseudogonyaulax</i> (Biecheler) Gómez and Artigas 2019	GDA	Bay of Biscay, Skagerrak, Kattegat, North Sea, Norwegian Sea, Barents Sea, Baltic Sea	Lassus et al., 2016; [§] Norwegian Biodiversity information center, [#] SMHI
<i>Alexandrium tamarense</i> (Lebour) Balech emend. U. John	<i>Gonyaulax tamarensis</i> Lebour 1925 <i>Gessnerium tamarensis</i> (Lebour) Loeblich III and Loeblich 1979 <i>Protogonyaulax tamarensis</i> (Lebour) Taylor 1979 <i>Gonyaulax tamarensis</i> var. <i>excavata</i> Braarud 1945 <i>Gonyaulax excavata</i> (Braarud) Balech 1971 <i>Protogonyaulax excavata</i> (Braarud) Taylor 1979 <i>Alexandrium excavatum</i> (Braarud) Balech and Tangen 1985 <i>Alexandrium tamarense</i> f. <i>excavatum</i> (Braarud) Konovalova 1993	NA	English Channel, Skagerrak, Kattegat, North Sea, South, West and Northeast coast Ireland, Norwegian Sea, Barents Sea, Svalbard	Lassus et al., 2016; [§] Norwegian Biodiversity information center, [#] SMHI; Touzet et al., 2008; Rathaille, Raine 2011
<i>Alexandrium taylori</i> Balech	<i>Gessnerium taylori</i> (Balech) Gómez and Artigas 2019	NA	Bay of Biscay	Lassus et al., 2016
<i>Alexandrium tamutum</i> Montresor, Beran & John		NA	Bay of Biscay, South & West coast Ireland	Lassus et al., 2016; Touzet et al., 2008

* a strain of *A. affine* from Vietnam was claimed to produce low levels of STX (Larsen, Nguyen Ngoc, 2004), but other studies from widespread global locations found that the species does not produce PSTs (Hallegraeff et al., 1991; Band-Schmidt et al., 2003; Stüken et al., 2011; Wang et al., 2006). PST toxicogenicity was not confirmed within the northern European study region.

** a strain of *Alexandrium andersoni* (CCMP2222) from the Gulf of Naples was reported to produce low levels of PSTs (Ciminiello et al., 2000; Frangopulos et al., 2004), but other studies based on CCMP2222 and other strains of *A. andersoni* have not detected these toxins (Sampedro et al., 2013; Stüken et al., 2011; Orr et al., 2011; Touzet et al., 2008). In any case, PST toxicogenicity was not confirmed within the northern European study region.

*** *Alexandrium gaarderae* reported as toxicogenic from Vietnam (Larsen, Nguyen-Ngoc, 2004), but not confirmed within northern European waters.

**** *Alexandrium leei* reported as potentially ichthyotoxic from Vietnam (Larsen, Nguyen-Ngoc (2004) and Singapore (Tang et al., 2007), but with no evidence of PST production in northern European populations.

[§] Norwegian Biodiversity information center (Norwegian BIC, <https://www.biodiversity.no/>). *Alexandrium tamarense*, *A. pseudogonyaulax*, *A. minutum* and *A. ostenfeldii* have been recorded along the whole Norwegian coast (Skagerrak, North Sea, Norwegian Sea, and Barents Sea) and (except *A. pseudogonyaulax*) also registered from Svalbard. The Norwegian monitoring program is based on light microscopy identification only; most likely, the identification of "*A. tamarense*" will also include *Alexandrium catenella*.

[#] Swedish national and regional monitoring programmes, data retrieved from the Swedish National Oceanographic Data center at the Swedish Meteorological and Hydrological Institute (SMHI), <https://sharkweb.smhi.se> and manually curated

The morphological characteristics were collated from original descriptions (e.g. Balech, 1971, 1985, 1995, as well as those in AlgaeBase (Guiry and Guiry 2022)).

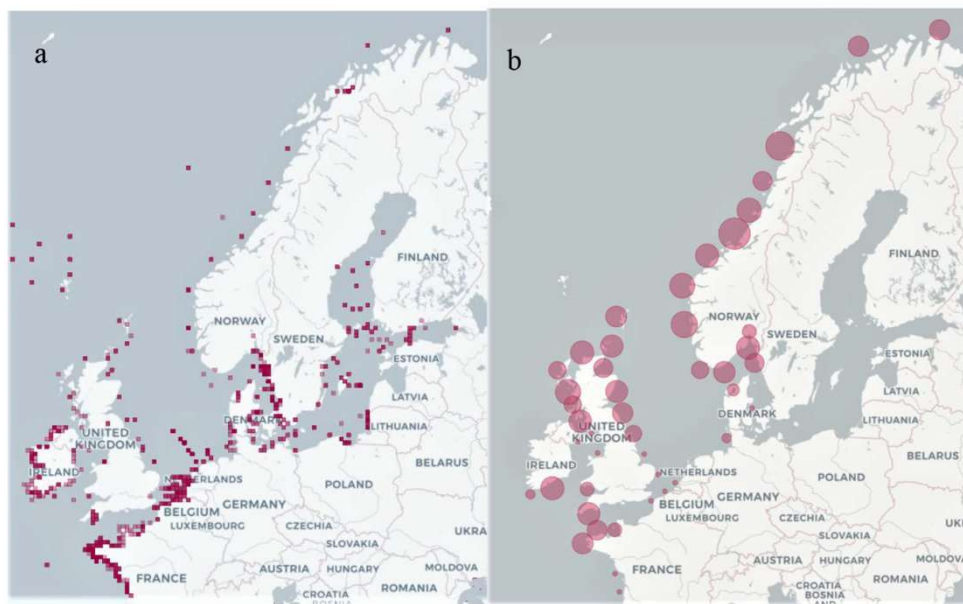


Fig. 1. Geographical distribution of a) records of *Alexandrium* species and b) PST events in northern Europe and the NE Atlantic margin region up to 2020 (maps derived from HAIS (<https://data.hais.ioc-unesco.org>) via HAEDAT/OBIS (<http://ipt.iobis.org/hab/resource?r=haedat>). Size of circles represents the number of events recorded for the region. An event is usually defined where PST toxin levels in seafood are above regulatory legislative levels or where precautionary closures of shellfish harvesting areas based on predefined thresholds of causative *Alexandrium* cells occur.

(Fig. 1b), i.e., north of Iberia. In fact, these PST events all fit within the known sub-groups of the *A. tamarense* (predominantly *A. catenella*), *A. minutum* and *A. ostenfeldii* species complexes. The *A. minutum* species complex (McCauley et al., 2009) is represented frequently along the eastern North Atlantic margin and in north European waters by members of the “global clade” as defined by the large subunit (LSU) rRNA sequences (Lilly et al., 2005). The related morphospecies *A. lusitanicum* Balech and *A. ibericum* Balech, first described from Iberia (Laguna Obidos, Portugal) (Balech, 1985), should be considered synonyms of *A. minutum* as recommended by Lilly et al. (2005).

Variation in sequences of different rRNA gene regions among global members of the *Alexandrium ostenfeldii* complex (including *A. peruvianum*) yielded division into six sub-groups, also reflecting differences in thecal plate morphology, toxin composition, ecophysiology and geographic distribution. Four of these groups (I, II, V and VI) are represented to a different extent among geographical regions and with highly variable cell abundances in European waters (Kremp et al., 2014; van de Waal et al., 2015).

Alexandrium pseudogonyaulax (Biecheler) Horiguchi ex Yuki and Fukuyo is an increasingly frequent member of the phytoplankton assemblage in northern Europe (Table 1) within the past decade. This species is found in many locations, but is particularly common and in high cell abundance in Danish fjords, along the Skagerrak-Kattegat (Krock et al., 2018) and in the southern Baltic proper (cited in Karlsson et al., 2021). There are no known unresolved taxonomic issues and its distinctive morphology allows for reliable separation by light microscopy of *A. pseudogonyaulax* from other *Alexandrium* taxa in routine monitoring programs in northern Europe.

2.2. Toxicogenicity, human health and environmental consequences

The biogeography and bloom dynamics of *Alexandrium* species have been a major concern for a century in northern Europe. Many PSP events were attributed to *A. tamarense*, described from the Tamar River in southern England as *Gonyaulax tamarensis* Lebour (1925). Subsequent molecular and toxicological studies clarified that *A. tamarense* (Group III) is nontoxic and that the species of the *A. tamarense* species complex responsible for PSP production in North Europe is what is now called *A. catenella* (Group I).

Globally, about half of the described *Alexandrium* species are known to produce PSTs, at least in certain strains and populations (Anderson

et al., 2012; Cembella and Durán-Riveroll, 2021; Murray et al., 2021). For example, both toxic and nontoxic strains of *A. minutum* have been isolated from Irish coastal waters (Touzet et al., 2007). Toxicogenicity of *Alexandrium* species can vary widely among and within species, even among geographical populations (Anderson et al., 2012). Biosynthetic capacity for PSTs is widely shared among almost all of the frequently occurring *Alexandrium* species forming blooms in northern European waters (Table 1), with non-toxicogenic *A. tamarense* John et al., 2014) and *A. tamutum* (Montresor et al., 2004) as notable exceptions. Biosynthesis of PSTs in *Alexandrium* is long recognized as regulated by both intrinsic and environmental factors (e.g., Cembella, 1998). The genetic basis for PST production in *Alexandrium* is now reasonably well understood. Variation in toxin composition among strains can be accounted for by the presence/absence and regulation of key biosynthetic genes (Murray et al., 2021).

Paralytic shellfish toxins (PSTs) comprise a suite of >50 naturally occurring guanidinium neurotoxins, the most potent being carbamoyl derivatives, such as saxitoxin (STX) and neosaxitoxin (neoSTX) (reviewed in Cembella and Durán-Riveroll, 2021). These PSTs (or “saxitoxins”) block conductance through sodium ion channels of nerve cells causing neuromuscular paralysis in vertebrates (Kao, 1993; Van Dolah, 2000; Durán-Riveroll et al., 2018) and in severe cases can cause death.

Bioaccumulation of PSTs can occur in a wide diversity of marine organisms, directly by ingesting toxicogenic dinoflagellates such as *Alexandrium* or via food web transfer, including mollusks, crustaceans, starfish, octopus, fish, turtles, marine mammals and sea birds (Cembella and Durán-Riveroll, 2021). Toxicogenic *Alexandrium* blooms can also have severe impacts on marine ecosystems (Anderson, 1997; Llewellyn et al., 2006), causing localized disruption of ecosystem functioning and mortality of marine fauna via food web transfer.

Some *Alexandrium* species also produce other bioactive compounds such as the cyclic imine spirolides (SPX) and gymnodimines (GYM), or goniodomin (GD) (Long et al., 2021; Murray et al., 2021). These bioactive metabolites can be “toxic” in some circumstances, but are considered as emerging toxins for further toxicological study. As such, these emerging toxins are not currently subjected to regulation in seafood within the European Union. Certain *Alexandrium* strains also produce extracellular secondary metabolites that are membrane-destructive (“lysins”) or other allelochemicals against a wide range of microplanktonic species (Tillmann and John, 2002). These lytic substances

can negatively affect or suppress growth of surrounding microbial communities (Weissbach et al., 2010), phytoplankton (Fistarol et al., 2004) and co-occurring heterotrophic protists (John et al., 2015), but defined chemical structures remain unknown. To date no harmful or toxic events have been associated specifically with these bioactive compounds in natural blooms of *Alexandrium* in northern Europe.

2.3. Molecular tools for *Alexandrium* species identification

Species of the genus *Alexandrium* are morphologically quite similar, but can usually be discriminated based on fine details of thecal plate tabulation and structure, cell size, cyst type and shape, and the formation (or not) of chains of cells. Many of these minute thecal characters are very difficult to resolve by light microscopy (LM) of whole cells even equipped with phase-contrast or contrast interference (Nomarski) optics. Fluorescence microscopy coupled with thecal staining (e.g., with the optical brightener Andersen et al., 2010), and/or iodine staining and dissection of thecal plates, is sometimes necessary. Scanning electron microscopy (SEM) can also be applied to distinguish species based on their thecal morphology. Critical morphotaxonomic observations of individual whole cells by LM and SEM is impractical for routine species identification, especially for surveillance programs when demand for results is time-sensitive.

The application of species discriminant molecular detection methods for *Alexandrium* has been well established over the last two decades (e.g., John et al., 2003; 2005; Murray et al., 2011; Toebe et al., 2013; Ruvindy et al., 2018; Hatfield et al., 2019; Geffroy et al., 2021). *Alexandrium* species have been defined and discriminated recently (e.g., Murray et al., 2014; Lim et al., 2015; Branco et al., 2020), by combining morphological analysis with DNA sequencing of selected target gene regions. Sequence variation in regions of the large subunit (LSU) rRNA, internal transcribed spacer (ITS/5.8S) and/or the small subunit (SSU) rRNA (John et al., 2014; Murray et al., 2021) has revealed cryptic undescribed diversity within this genus.

Basic semi-quantitative information on the presence of *Alexandrium* genotypes in natural plankton assemblages may be readily obtained by DNA barcoding of selected regions of the SSU- (18S) and LSU (28S) rRNA gene. Operationally, this approach has frequently been combined with application of species-specific qPCR probes and amplicon sequencing designed for regional populations. Now that the biosynthetic genes for STX and other PSTs have been discovered in *Alexandrium*, targeted probes for the STX-biosynthetic genes have identified these elements in natural population and cultured isolates of *Alexandrium* species (Murray et al., 2015; Murray et al., 2019). Given the frequent occurrence of non-toxicogenic *Alexandrium* species and strains in global populations, detection of toxin-specific genes provides a valuable insight into toxin risk associated with particular blooms. These molecular approaches are typically applied in research mode to resolve taxonomic, phylogenetic and toxicogenic issues or for species confirmation. As yet, they are not deployed for broad-scale routine monitoring of *Alexandrium* genotypes in plankton for northern Europe.

2.4. Species biogeography and distribution of toxic events among case study areas

Alexandrium species are highly adaptive to colonize and proliferate in diverse coastal habitats and shelf regions around the world (Murray et al., 2021). This pattern of high infraspecific diversity within *Alexandrium* (e.g., Tillmann et al., 2009; Kremp et al., 2014) is also noteworthy for populations from northern Europe and eastern Arctic gateway waters. The frequency and biogeographical occurrence of *Alexandrium* blooms are influenced by biological and environmental factors, which interplay to varying degrees under different environmental regimes. For example, *A. ostenfeldii* is commonly found in vastly different environments from inland freshwater lagoons in the Netherlands (Van de Waal et al., 2015) and brackish waters of the northern Baltic Sea (Kremp et al.,

2009; Tahvainen et al., 2012) to a highly dynamic open coastal fjord system along the Norwegian coast (Aasen et al., 2005).

Toxicogenic *Alexandrium* blooms have been observed in marine waters across all CoCliME Case Study areas, including the North Sea, Norwegian Sea/Barents Sea and NE Atlantic Ocean, but high cell density blooms (e.g., $>10^5$ cells L⁻¹) are rarely noted. Biogeographical studies on the distributional range of *Alexandrium* are based primarily on cell morphological analysis, but often include DNA sequence confirmation and less frequently benthic cyst identification and quantitation, and in some cases even PST composition of natural populations. Such biogeographical information is available from the NE Atlantic margin, northern Europe including the Baltic Sea, sub-Arctic islands and archipelagos to polar latitudes, including Iceland and Greenland (Richlen et al., 2016) and more recently Svalbard.

The history of documented PST events in northern Europe begins in 1901 (Karlsen et al., 2021), but anecdotal reports of human illness in Scandinavia with symptoms of PSP after shellfish consumption precede this record by centuries. Presumably, all of these PST events were caused by *Alexandrium* blooms, although the causal link to particular species was only established several decades ago. In some cases, *Alexandrium* blooms have been linked to specific harmful (PST) events within the CoCliME region of northern Europe (reviewed in Bresnan et al., 2021; Karlsen et al., 2021). To date, high magnitude *Alexandrium* blooms are unknown in the Arctic and sub-arctic.

The most common *Alexandrium* species reported in monitoring and field research programs in northern Europe and Arctic gateway waters are: *A. catenella* (Whedon & Kofoid) Balech and *A. minutum* Halim, but *A. ostenfeldii* (Paulsen) Balech & Tangen, *A. pseudogonyaulax* (Biecheler) Horiguchi ex Yuki & Fukuyo, *A. tamarense* (Lebour) Balech emend John and *A. tamutum* Montresor, Beran & John also frequently occur (Table 1). In northern Europe, *A. catenella* was often previously recorded as *A. tamarense* or *A. fundyense*, and originally as *Gonyaulax tamarensis* Lebour or later as *G. excavata* (Braarud) Balech. Confusingly, *A. ostenfeldii* from Scandinavia was considered conspecific with *G. tamarensis* but as a larger rounder morphotype (*G. tamarensis* var. *globosa* Braarud).

High cell density toxicogenic blooms of *A. ostenfeldii* are thus far only known from northern parts of the Baltic Sea, primarily the Åland Archipelago (Kremp et al., 2009; Hakanen et al., 2012), one inner coastal area in the Netherlands (van de Waal et al., 2015), a single fjord on the Norwegian west coast (Norwegian Biodiversity information center) and in the Limfjord (Denmark) (Kremp et al., 2019). In Ireland, low cell densities of SPX-producing *A. ostenfeldii* have been found in Cork Harbour in the South (Touzet et al., 2011), and in Bantry Bay and Lough Swilly along the SW and North coasts, respectively (Touzet et al., 2008).

Recent findings document that *A. pseudogonyaulax* has become a prominent member of the post-spring bloom phytoplankton community over the past decade in the study area, including the Skagerrak-Kattegat and southern Baltic Sea, whereas *A. ostenfeldii* may be declining in relative abundance (Karlsen et al., 2021). Analyses of long-term decadal monitoring data from the Limfjord, Denmark confirmed a recent shift to *A. pseudogonyaulax* dominance. In fact, *A. pseudogonyaulax* has also become more common in the inner part of the Skagerrak coast of Norway for more than a decade. The species occurred only during summer (July to August) in Norwegian waters, at first only sporadically (years 2001 to 2011), but since 2016 blooms of this species have become an annual event (Naustvoll, unpubl.; Karlsen et al., 2021).

There are few published studies on the distribution of fossil and recent *Alexandrium* cyst deposits in sediments from northern Europe, as evidence of regime shifts or climate change effects on biogeography. Palaeoecological studies in the Bay of Brest (Brittany, France) based on metabarcoding analyses of sedimentary ancient DNA showed a recent shift in dinoflagellate genus abundance from *Gonyaulax* to *Alexandrium*. After the Second World War, in particular, this genus shift towards *Alexandrium*, up to and including the 1980s, may be related to intensification of agriculture that suffered in the coastal area during the war

period (Siano et al., 2021).

The PST events in the northern CoCliME region have been referenced for several decades in the Harmful Algal Event Database (HAEDAT; <http://haedat.iode.org/>), and can be mapped according to location and frequency (Fig. 1b). In the NE Atlantic, the major producers of PSTs are *A. catenella*, *A. minutum*, and to a lesser regional extent *A. ostenfeldii*. In most cases where a link to species-specific dinoflagellate blooms can be established, such PST events are associated with toxigenic populations of *A. catenella* or *A. minutum*. *Alexandrium catenella* is responsible for the majority of toxic bloom events in the North Sea and adjacent waters such as the Norwegian Sea. This *Alexandrium* species blooms regularly in Norwegian waters, causing PST-contaminated shellfish recurring annually, especially along the mid- and west-coast of Norway. In northern Norway *Alexandrium* blooms and PST accumulation above regulatory levels in blue mussels (*Mytilus edulis*) have also occurred more or less annually for the last 20 years, with a typical peak from late June to late August. Along the Skagerrak coast of Sweden, PSTs derived from *Alexandrium* blooms (when confirmed usually of *A. catenella*) have contaminated blue mussels, mainly from April to May, leading to mussel harvesting closures in 2014, 2015 and 2017 (Persson et al., 2020).

In contrast to Scandinavian waters, toxigenic *Alexandrium* blooms in NE Atlantic regions, e.g., occurring from north of the Bay of Biscay to Brittany in France, and the south coast of Ireland, are usually dominated by *A. minutum*. These bloom events have led to sporadic closures of shellfish harvesting due to PST levels exceeding the commission regulation (EC) regulatory limit over the past several decades, but tend not to be persistent (HAEDAT; Bresnan et al., 2021). Regular blooms of *A. minutum* are observed in different estuarine ecosystems of Brittany, but only occasionally lead to shellfish harvest closures (Chapelle et al., 2015; Guallar et al., 2021). In the Bay of Brest, *A. minutum* has become progressively more invasive during the late 20th Century, especially after the 1980s (Klouch et al., 2016a; Lambert et al., 2018). Now the species is present throughout the whole bay (Klouch et al., 2016b).

Similarly, in the coastal waters of Ireland, *A. minutum* is also the main cause of toxic blooms linked to PST events, and responsible for occasional shellfish farm closures. Prior to 2019, and since PST monitoring commenced in the 1990s, PST closures on an annual basis were confined to one location - Cork Harbour on the South coast. Historically, this shellfish production area was the only site to experience near annual closures for mussel (*Mytilus edulis*) harvest due to PSTs above regulatory levels, typically for one to two weeks at the end of June (Bresnan et al., 2021). Today, PST events are known to occur in Cork Harbour and Castlemaine Harbour (SW coast), both are semi-enclosed embayments, suggesting that habitat type is important in bloom retention and dynamics. Extensive studies into *Alexandrium* blooms in Cork Harbor have revealed that both tidal influences and the stability of the water column may be predictable indicators for the timing of these blooms (Raine, 2014).

2.5. Ecology

Alexandrium species have a complicated life history, which includes alternation of haploid and diploid phases after sexual recombination, and may include a benthic resting stage (Anderson and Wall, 1978; Brosnahan et al., 2010; Lau et al., 2017). The benthic phase cells, known as sexual cysts (or hypnozygotes) can form a vast repository “seed bed” as inoculum for initiation of subsequent blooms (Brosnahan et al., 2020), usually after an obligate dormancy period. Approximately half of all described *Alexandrium* species have a known benthic resting stage (Bravo et al., 2006), including most (>70%) of PST-producing species. In fact, perhaps all *Alexandrium* taxa are capable of producing sexual cysts but the confirmatory life cycle studies have not been completed for many species.

The rate and quantity of formation, sedimentation and hatching of *Alexandrium* cysts are key life stage processes for determining the population dynamics (Anderson et al., 2005; Alpermann et al., 2009; Kremp

et al., 2009; A. 2019). Cyst germination has been identified as a major trigger of bloom initiation for *Alexandrium* (Anderson et al., 2012), as opposed to initiation from residual populations of vegetative cells overwintering in the water column. In marine environments, sexual cyst formation serves as a crucial component for survival and an important life-cycle strategy, especially since vegetative growth is restricted seasonally to a short time each year (Ellegaard and Ribeiro, 2018). These dormant resting cysts may stay viable for more the 150 years (Delebecq et al., 2020) but often germinate only under favorable environmental conditions, e.g., when water temperature (surface as well as bottom), light and nutrient regimes are suitable (Brosnahan et al., 2020).

Alexandrium cyst beds have been extensively mapped along the western Atlantic margin, including estuaries and embayments of the Gulf of St. Lawrence, Bay of Fundy and the Gulf of Maine (e.g., Castell-Perez et al., 1998; Matrai et al., 2005; Stock et al., 2005; Martin et al., 2014; Anderson et al., 2014; Bucci et al., 2020), and recently for the Alaskan coast of the Chuckchi Sea (Anderson et al., 2021). In northern Europe, the biogeographical extent of *Alexandrium* cyst beds has been determined only locally and sporadically for a few coastal zones. Dense *Alexandrium* cyst beds have been associated with areas where regular blooms occur, such as in Bay of Brest (Brittany, France) (Klouch et al., 2016b), Baltic Sea (Kremp et al., 2019) and Kattegat (Persson et al., 2000). Such studies highlight the importance of the benthic-pelagic coupling in the phenology of *Alexandrium* species (Lewis et al., 2018), and indicate that the fundamental processes are likely similar along the western and eastern North Atlantic coasts. Unfortunately, detailed biogeographical maps of recent *Alexandrium* cyst distributions are lacking, even for areas of northern Europe where *Alexandrium* blooms and associated PST events are common, particularly for the coastal zones of Norway, Ireland and the U.K.

2.6. Nutritional strategies

Alexandrium blooms in coastal zones might experience strong shifts in macronutrient availability and supply ratios, but detailed monitoring and process descriptions are rare (for example see Whitehead and Crossman, 2012). Yet there is no convincing evidence that *Alexandrium* has special adaptations for macronutrient acquisition or sequestration, or responses to anthropogenic eutrophication or altered supply ratios (Davidson et al., 2014), that would distinguish this genus from other large-celled primarily photosynthetic HAB dinoflagellates. In fact, at the generic level *Alexandrium* is not an apparently formidable nutrient competitor based on classic nutrient dynamics. Nevertheless, nutritional strategies among and within *Alexandrium* species are diverse, and include the ability to utilize a wide range of dissolved inorganic and organic substrates, and in some cases even particulate nutrients. Among the more than 30 described *Alexandrium* species, only six are known mixotrophs (Anderson et al., 2012), but it is likely that many other species (perhaps all) are cryptic mixotrophs (reviewed in Tillmann and Hansen, 2009; Flynn et al., 2019; Murray et al., 2021).

Little is known about species- or strain-specific adaptation to nutrient acquisition that is relevant to natural *Alexandrium* blooms in northern Europe. In an exceptional study, single-cell analyses performed by microfluidics on *A. minutum* strains revived from sediments of different ages advance the hypothesis that the species may have adapted to the contemporary apparent growth-limiting phosphorus concentrations in the Bay of Brest (France) (Girault et al., 2021). This has been attributed to the capacity of the species to rely upon internal stocks of phosphorus.

At high cell densities *Alexandrium* blooms may become growth-limited by nutrients, such as nitrogen (N) and/or phosphorus (P) (Elser et al., 2007; Labry et al., 2008; Girault et al., 2021). Under certain circumstances, over seasonal cycles these nutrients can be resupplied by the input of nutrient-rich waters from riverine discharge (Anderson et al., 2002). Alternative resupply may arise from mixing of deep waters induced by storms, upwelling, frontal systems and/or longshore

transport (Franks and Anderson, 1992; Purz et al., 2021). In any case, macronutrient dynamics cannot fully account for species dominant or virtually monospecific *Alexandrium* blooms that occasionally form in coastal embayments and estuarine waters, including in northern Europe (Hattenrath-Lehmann et al., 2015; Wells et al., 2015).

3. Potential effects of climate change on *Alexandrium* blooms

3.1. Recent changes in distribution magnitude and frequency of blooms

In recent years, the reported global distributional range of *Alexandrium* has expanded towards the poles (Anderson et al., 2021 and references therein). However, this apparent geographical expansion and the putative relationship to global ocean warming is not well documented for *Alexandrium* species for warm waters and temperate areas, nor particularly for Arctic seas (Anderson et al., 2021).

In any case, in northern Europe *Alexandrium* species are known from an ever-expanding distributional range in NE Atlantic marginal seas and throughout northern Europe and linked gateway seas to the eastern Arctic. Members of this genus are commonly found throughout the region albeit most frequently at arbitrarily defined “sub-bloom” ($<10^4$ cells L^{-1}) cell densities. In fact, absence of high magnitude *Alexandrium* populations is rather characteristic in northern European waters. Along the Wadden Sea coast of Germany and in the southern German Bight where significant cell densities ($>10^2$ cells L^{-1}) of *Alexandrium* are rarely recorded, strong deep mixing, the presence of an extensive sandy bottom and large exposed tidal flats during low-tide may preclude establishment of benthic cyst beds for recruitment.

In northern Europe, new reports of toxigenic *Alexandrium* blooms from areas with no history of PST indicate the likelihood that such blooms may be spreading to new habitats. If this apparent spreading could be related to changes in ocean temperature, wind systems and nutrient availability, with historical support from sediment cores of these areas, this would help to interpret expansion processes of extant *Alexandrium* populations over time (Klouch et al., 2016a; Lambert et al., 2018; Shaw et al., 2019; Siano et al., 2021). Nevertheless, as elsewhere, it is not possible to rule out the fact that such “new findings” arise from improved technology and enhanced surveillance programs for *Alexandrium* blooms. In most global regions, decadal time-series data on *Alexandrium* blooms and cyst distributions are inadequate to resolve the issue. This is particularly true for northern Europe even for most locations with a decadal history of *Alexandrium* blooms and/or PST events.

Recent results from CoCLiME oceanographic cruises around Svalbard show the presence of *Alexandrium* species in the eastern Arctic (Fig. 2). The large geographical distance to the north coast of Norway makes frequent recruitment via advection of established *Alexandrium* populations rather unlikely. In this case, the presence of *Alexandrium* in the Svalbard archipelago cannot yet be confirmed as a recent introduction or remnant of relict endemic populations.

In few areas of the western Atlantic, e.g., the Bay of Fundy in Atlantic Canada and the Gulf of Maine (Anderson et al., 2014) such detailed information is available and has been effectively modelled for bloom dynamics. Similar efforts from the Bay of Brest and the surrounding area (Klouch et al., 2016a; Lambert et al., 2018; Siano et al., 2021) point the way to future pilot modeling studies for northern Europe based on time-series data.

3.2. Future projections of *Alexandrium* blooms

Hitherto, population dynamic models were scarce and ecological niche models remain completely unavailable for *Alexandrium* blooms within the northern CoCLiME Case Study area, comprising the greater North Sea, Norwegian Sea and NE Atlantic marginal seas. Several identified environmental factors defined as climate change indicators (e.g., stratification, sea surface temperature (SST) and salinity) are known drivers of *Alexandrium* bloom initiation, development and expansion,

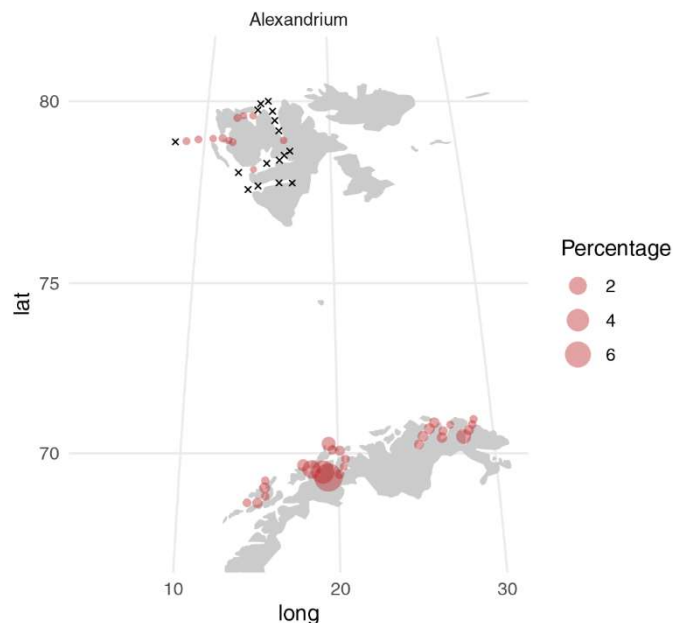


Fig. 2. Relative abundance (% total ASV reads) of *Alexandrium* molecular signatures in the vicinity of Svalbard archipelago (sampled in 2017) and Norway (sampled in 2019) based on rRNA gene sequencing. The number of *Alexandrium* signatures (“sequence reads”) was normalized against the total number for dinoflagellates and presented as a percentage of all dinoflagellate taxa detected. Red dots indicate relative abundance at stations sampled and the black “x” symbol denotes stations where *Alexandrium* was not detected.

and thus provide a good foundation for modeling future bloom scenarios. As these environmental factors can have major influences on the distribution, frequency and magnitude of *Alexandrium* blooms, two future Representative Concentration Pathway (RCP 4.5 and 8.5) scenarios were investigated with regional ocean climate model projections for the Baltic Sea and North Sea linked with the coupled atmosphere-ocean model RCA4-NEMO (Gröger et al., 2019). A Representative Concentration Pathway (RCP) is a greenhouse gas concentration (not emissions) trajectory adopted by the Intergovernmental Panel on Climate Change (IPCC); four alternative pathways were used for climate modeling and research outlined in the Fifth Assessment Report (AR5) (IPCC, 2014). The pathways describe different climate futures, all of which are considered possible depending on the volume of greenhouse gases (GHG) emitted in the years to come. The RCPs – originally RCP2.6, RCP4.5, RCP6, and RCP8.5 – are labelled after a possible range of radiative forcing values in the year 2100 (2.6, 4.5, 6, and 8.5 W/m^2 , respectively). This modeling approach was adopted herein to present the ensemble means of these RCP4.5 and RCP 8.5 projections for the southern North Sea and adjacent Kattegat-Skagerrak (Fig. 3a and b).

There is no clear apparent trend in *Alexandrium* bloom frequency or magnitude on a latitudinal gradient for the Norwegian coast (Fig. 4) and the Baltic Sea or Kattegat/Skagerrak region over the past two decades (Fig. 5). Higher *Alexandrium* bloom magnitude at latitude 55° and 57°N in the Baltic in 2005 are not repeated in subsequent years and do not match the apparently stable pattern for the Kattegat/Skagerrak. The consistent magnitude throughout the time-series for the Kattegat/Skagerrak does reflect generally higher cell density blooms for latitude $>55^{\circ}N$. For the adjacent Norwegian coast, annual variation in bloom magnitude since 2000 also appears stochastic, except for the occasional tendency for *Alexandrium* “hot spots” between 62 and 63°N. But this observation is derived from monitoring of a single station and cannot be interpreted further.

Future estimated rising sea surface temperatures will tend to favor growth of *Alexandrium*, including in areas where blooms are unrecorded historically. If these regime shifts favor cyst deposition and maintenance

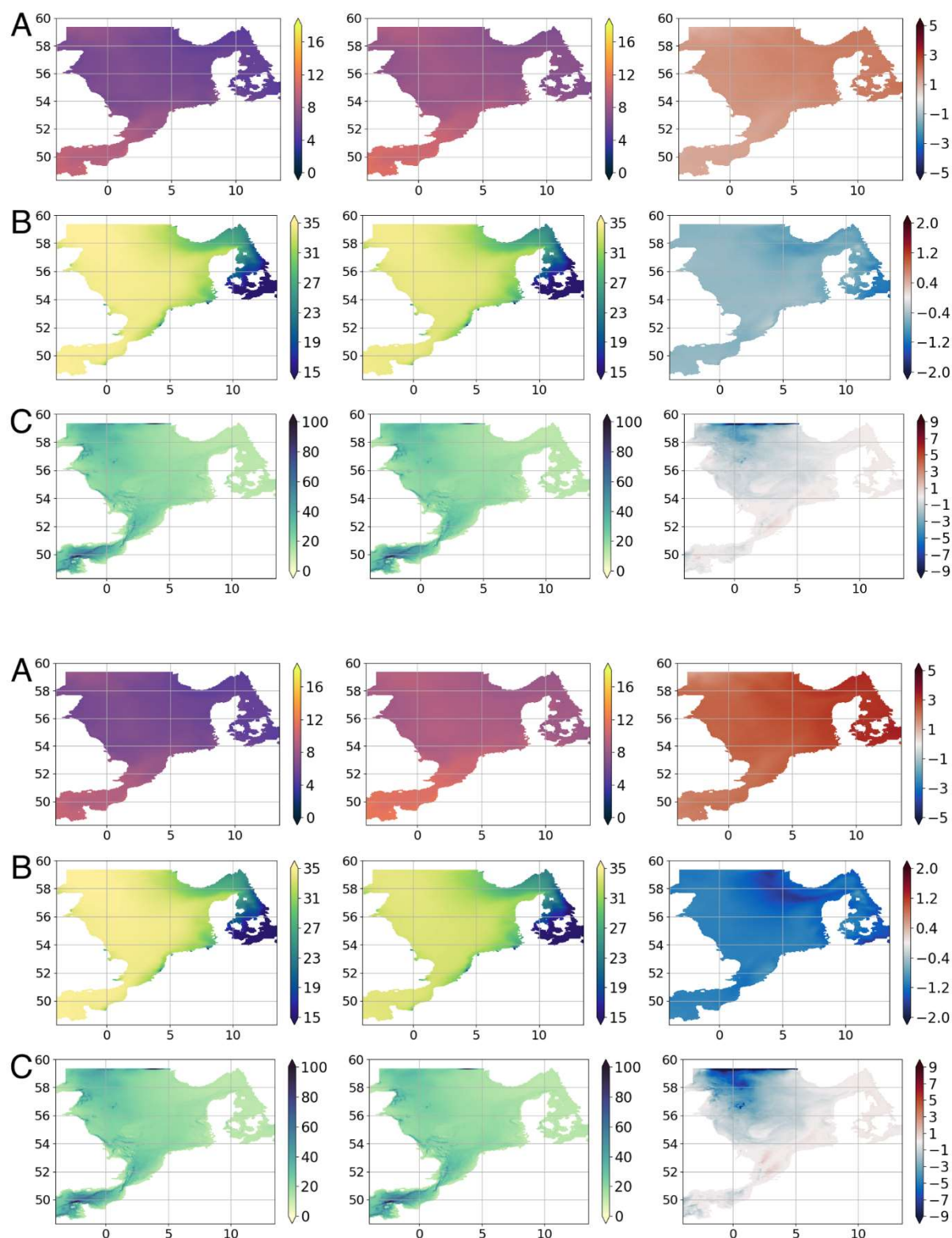


Fig. 3. Simulations from SMHI regional climate model RCA4-NEMO representing ensemble means of five regionalized global climate model projections for climate forcing functions defined as RCP4.5 (Fig. 3a) or RCP8.5 (Fig. 3b). A: spring surface temperature (°C); B: spring surface salinity (salt content) (g kg⁻¹); C: spring mixed layer depth (MLD) (m). Panels left: conditions during period 1970–1999; middle: predicted conditions during period 2070–2099; and right: difference between periods.

of seed beds in these expansion areas and future conditions continue to facilitate the vegetative growth of *Alexandrium* populations, enhanced vigilance and surveillance initiatives will become more critical in the southern North Sea. The ocean climate model shows that seawater

salinity will tend to decrease in the region. The projected freshening of the North Sea is consistent with earlier findings (e.g., Schrum et al., 2016). The anticipated freshening will result from a generally intensified water cycle in the atmosphere leading to more precipitation at mid- to

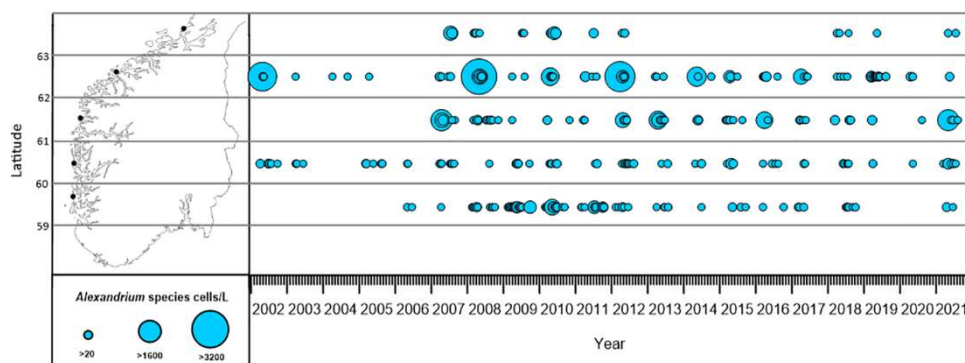


Fig. 4. Temporal and spatial distribution of maximum *Alexandrium* cell abundances displayed on a latitudinal gradient (per °N). Bubble plots represent the highest maximum cell densities (>20 cells L^{-1}) calculated from selected sites along the Norwegian west monitored in the Norwegian National Monitoring Program from 2001 to 2021.

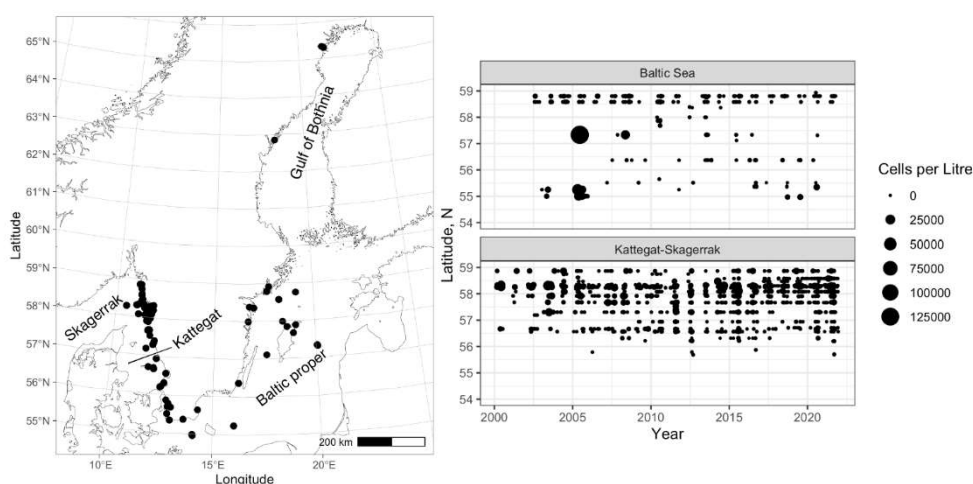


Fig. 5. Temporal and spatial distribution of maximum *Alexandrium* cell abundances displayed on a latitudinal gradient (per °N) for the Kattegat/Skagerrak and Baltic Sea proper and Gulf of Bothnia. Bubble plots represent the highest maximum cell densities (first interval $>0 - 25,000$ cells L^{-1}) calculated from selected sites monitored in the Swedish National Monitoring Program from 2000 to 2021.

high latitudes (see Gröger et al., 2019 for detailed discussion). This sharpened salinity gradient and reduced salinity in the coastal zones should benefit *Alexandrium* species that can grow over a wide range of salinities (Grzebyk et al., 2003; Kremp et al., 2009), perhaps particularly in the Wadden Sea, Skagerrak/Kattegat and in the Baltic Sea. In any case, it is premature to link warming, freshening, and increased stratification predicted from the RCP modeling (Fig. 3a and b) directly with decadal patterns of *Alexandrium* bloom magnitude (Figs. 4 and 5) in this region.

Further important environmental drivers that may influence the growth of *Alexandrium* include changed mixed layer depths (Fig. 3a and b). Deepened mixed layer depth in spring in the southern and eastern parts of the North Sea and shallowing spring mixed layers in the northern and central North Sea will cause a progressive shift in nutrient availability and growth conditions in these areas. In central areas with shallowing mixed layers, inorganic nutrients in the surface layer will be depleted at a faster rate, which favors growth of *Alexandrium* over potential diatom competitors. Admittedly, this effect is not restricted to *Alexandrium* among the dinoflagellates, particularly those capable of diurnal vertical migration and mixotrophy. Further aspects are related to changes in total organic carbon (TOC) transport to coastal areas. Increased organic loading could be beneficial for mixotrophic species, including many *Alexandrium* taxa, in competition for N and P, when depleted in the surface layer.

Gradient boosting models (GBM) have been developed for *Alexandrium* species based on cell presence/absence combined with

temperature, salinity and a water column stratification index (Yamanaka et al., this special issue). The regional GBM modeling approach herein is based on weekly data of *Alexandrium* cell counts from the Irish national monitoring program (see Yamanaka et al. this special issue, for details on model construction and design). This HAB climate service prototype was developed with physical environmental variables from a numerical ocean model (Nagy et al., 2021; this special issue) and applied to provide future probability estimates of *Alexandrium* cell presence. The CoCliME climate service PPM (presence/absence probability model) for *Alexandrium* distribution predications are displayed for the recent past and contemporary/future time periods (Fig. 6).

The SW Ireland Ocean model under the RCP 8.5 scenario shows significant warming in the future across the southwest, and a general freshening across the region. This is more evident in surface waters when compared to near-bottom waters (Nagy et al., 2021; this special issue). The PPM predictability was considered "excellent" ($AUC = 0.80$; Hosmer et al., 2013). In the PPM, *Alexandrium* shows a slight increase throughout the year with a larger increase from May to November in the contemporary and future periods (2017 – 2035) when compared to the recent past (1997 – 2016) (Fig. 6).

The PPM shows an increased *Alexandrium* bloom period starting earlier and finishing later in the year in future (2017 – 2035). Geographically, the future estimates show a slight increase in the probability of presence in sheltered coastal areas with a decrease in more exposed oceanic areas. The PPM model shows similar relative influence of all three variables (SST, salinity and stratification index) for

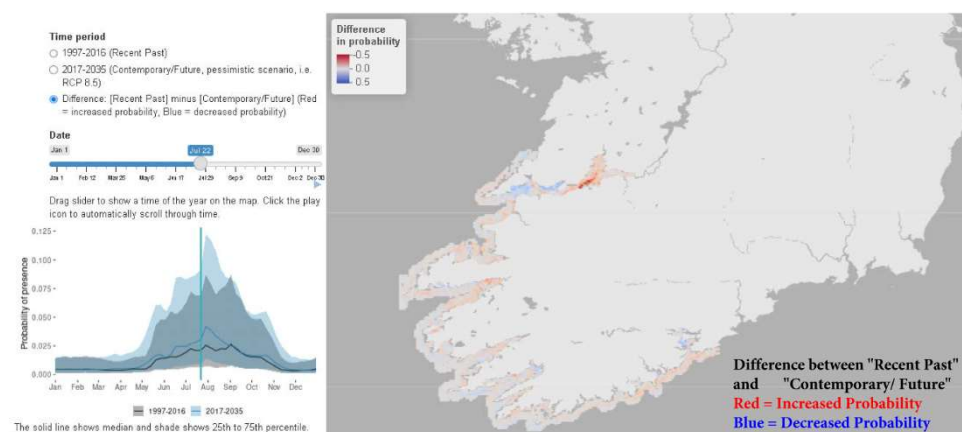


Fig. 6. Prototype climate service model application to estimate the presence/absence probability of occurrence of *Alexandrium* species for SW Ireland. The map here shows estimated PPM (presence/absence probability model) outputs for the difference between the probability presence of *Alexandrium* between 1997 – 2016 (recent past) and 2017 – 2035 (contemporary/future) under an RCP 8.5 scenario. The lower left panel shows the median (solid line) and the 25–75 percentiles with gray shade for recent past and blue shade for contemporary/future projected ocean conditions. The CoCLiME climate service PPM data (presence/absence probability) for *Alexandrium* is available online (https://marine-institute-ireland.shinyapps.io/Alexandrium_probability/).

estimating *Alexandrium* occurrence (32%, 34% and 34% respectively).

The PPM predicts that *Alexandrium* species should prefer a warmer, less salty and stratified environment (Yamanaka et al., in this special issue). The decadal pattern in *Alexandrium* bloom magnitude displays no apparent trend since 2000 on a latitudinal basis for the Irish coasts (Fig. 7). There is a noteworthy increase for SW and SE Ireland within the past two years, but this may be only an anomaly.

3.3. *Alexandrium* in a future climate - key indicators

Recent findings of high cell abundances of *A. ostenfeldii* and *A. pseudogonyaulax* at low salinities in high temperature waters off the Kattegat-Skagerrak coast and during summer in the Baltic Sea suggests that some *Alexandrium* species may already be responding to induced climatic changes in ocean regimes, but perhaps differentially. High *A. ostenfeldii* and *A. pseudogonyaulax* cell densities (“blooms”) along the Kattegat-Skagerrak coast and inner fjords of Denmark and in the inland embayments in the Netherlands at brackish salinities and during periods of high water-temperature are increasingly frequent within the past decade (Kremp et al., 2019). This suggests that the composition of *Alexandrium* species and relative dominance within the post-spring bloom phytoplankton community may be changing with climatic conditions in the local area. Recent findings show that *A. pseudogonyaulax* has become more prominent in recent years, whereas *A. ostenfeldii* has declined in relative abundance in the Skagerrak-Kattegat (Karlsen et al., 2021). Long-term decadal monitoring data from the Limfjord, Denmark confirms a recent shift from *A. ostenfeldii* to *A. pseudogonyaulax* dominance. Furthermore, cyst and PST records of *Alexandrium* species including *A. pseudogonyaulax*, in Kiel Bight suggest a spreading potential into the brackish Baltic Sea, expanding the future distributional range

and forming more frequent blooms as global warming of coastal marginal and shelf seas continues (Kremp et al., 2019).

Environmental climate change indicators for northern Europe were identified and defined herein as those most likely to influence the frequency, expansion and intensity of *Alexandrium* blooms and associated toxicity events in the future. Analysis of time-series information on biogeographical distribution of *Alexandrium* and associated PST events (Figs. 1a and b; 4, 5, and 7) indicate that primary climate indicators identified as salinity, temperature and ocean stratification can be modelled in physical simulations (Fig. 3a and b; Fig. 6) but as yet cannot be directly coupled to bloom dynamics on a regional basis. These key factors are considered separately below for autecological purposes, but of course they are influenced by and partially dependent on other climate-linked variables, such as wind velocity, magnitude and frequency of extreme storm events, and changes in residual current patterns.

Salinity: Freshening of coastal surface waters as a result of increased river run-off is one consequence of climate change. Many *Alexandrium* blooms occur in estuaries or coastal areas influenced by river plumes with lower surface salinity due to the freshwater influence. *Alexandrium* species are rather halotolerant and can in fact grow over a wide range of salinities (Yang et al., 2011; Suikkanen et al., 2013). Salinity tolerance in *Alexandrium* varies widely among species and among strains. Nevertheless, results from culture studies and field observations indicate that *Alexandrium* populations do not survive in pure freshwater (salinity 0). The optimum salinity for many *Alexandrium* species is between 25 and 30 – a typical range in coastal environments with freshwater influence where *Alexandrium* blooms thrive in northern Europe (Laabir et al., 2013). In any case, many *Alexandrium* species or genotypes are readily acclimated or became adapted to lower salinities and even flourish at

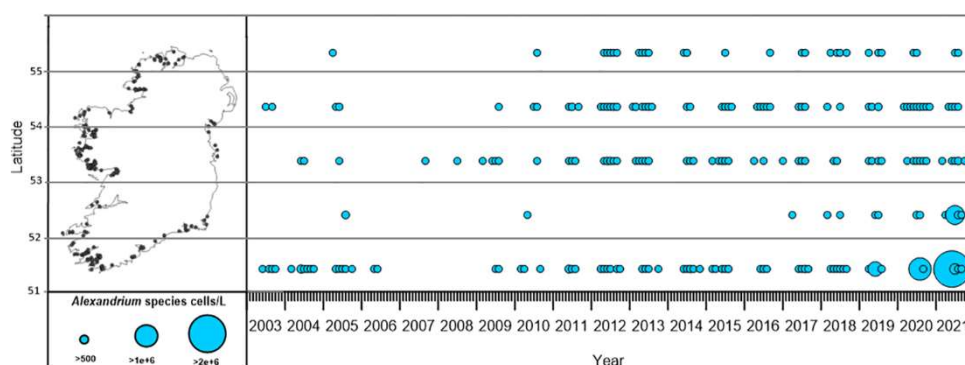


Fig. 7. Temporal and spatial distribution of maximum *Alexandrium* cell abundances displayed on a latitudinal gradient (per °N). Bubble plots represent the highest maximum cell densities (>500 cells L⁻¹) recorded per month in aquaculture production areas monitored in the Irish National Monitoring Program from 2003 to 2021.

low salinities (<15) (Suikkanen et al., 2013). In the brackish Baltic Sea, *A. ostenfeldii* shows tolerance salinity <15, but grows well at an optimum salinity of 10 (Suikkanen et al., 2013). In the north Baltic proper *A. ostenfeldii* thrives at salinities around 6 (Kremp et al., 2009; Martens et al., 2016). This broad halotolerance implies that it is unlikely that any future freshening of coastal waters would *per se* limit the growth or selectively favor most *Alexandrium* species in NE Atlantic marginal seas or northern European coastal regions. Climate-related salinity shifts could, however, influence *Alexandrium* blooms in particular low salinity habitats, such as the Baltic Sea (Suikkanen et al., 2013) and inner coastal waters of the Netherlands (Van de Waal et al., 2015). Projected salinity reductions in the Baltic Sea, e.g., from 6 to 4, could exceed the lower salinity tolerance limit for *A. ostenfeldii* (Kremp et al., 2009).

Temperature: Climate-change driven ocean temperature rises may intensify the geographical dispersion and frequency of *Alexandrium* blooms by earlier and enhanced resting cyst formation and higher cyst deposition rates with temperature-regulated life cycle transformations (Richlen et al., 2016). As described in models (Thomas et al., 2012; Martin et al., 2021; Cael et al., 2021) and pointed out in the IPCC special report (Bindoff et al., 2019), increasing ocean temperatures generally lead to species appearance in higher latitudes when habitats become more suitable for growth. It is worth noting, however, that the global HAB status report (Hallegraeff et al., 2021) did not confirm global increases in HAB frequency and abundance linked to climate changes. No clear links to increasing water temperature are evident from the decadal pattern of PST events (mostly due to *Alexandrium* blooms) in northern Europe (HAEDAT; Bresnan et al., 2021; Karlson et al., 2021). Nevertheless, warmer water extensions to high-latitudes and into the Arctic would facilitate range extension of temperate *Alexandrium* species (or adapted ecotypes) into these areas. Most climate changes projections focus on warming of surface waters, but establishment of resident populations of *Alexandrium* species in new frontier areas may depend on bottom waters temperatures as well (Anderson et al., 2021). Down to 200 m is a plausible depth for viable cysts beds that can seed recurrent annual blooms in surface waters.

Other temperature-related but poorly studied factors that may influence future *Alexandrium* blooms include mismatch effects of top-down control mechanisms on bloom dynamics, such as shifts in timing and abundance of grazers, pathogens and parasites. *Alexandrium* populations could hatch from cysts, then grow and form blooms earlier in the year because of higher water temperatures. If these blooms were initiated earlier and before the grazer and parasite communities were established, stronger protracted *Alexandrium* blooms could occur (Chambouvet et al., 2008; Alacid et al., 2017).

Stratification: Ocean stratification indicates low levels of turbulence and vertical mixing. Climate-dependent factors such as heat exchange with the atmosphere and rainfall can generate temperature and salinity differences that enhance stratification, whereas tides and winds generate turbulence that can reduce stratification (Condie et al., 2019). Water column stratification in coastal waters is determined primarily by salinity and temperature gradients, but is highly susceptible to disruption by wind-driven upwelling and extreme storm events. The strength and stability of this vertical stratification strongly influences the composition of phytoplankton assemblages and shifts in cell abundances. Many *Alexandrium* blooms are successfully developed and grow well to high cell densities in stratified waters, but are inhibited in highly turbulent regimes (Murray et al., 2021 and references therein). Highly stratified waters, especially when accompanied by high solar radiance in summer, might favor development of *Alexandrium* blooms in fjords and coastal embayments in northern Europe. Projected increased freshening and thermal stratification of surface waters may enhance *Alexandrium* blooms and PST outbreaks by modifying hydrodynamics to select for such vertically migrating opportunists with mixotrophic capacity.

4. Socioeconomic impacts and consequences of *Alexandrium* blooms

Temporal and spatial expansion of *Alexandrium* blooms due to climate change would have substantial negative consequences on the future harvesting and consumption of seafood, particularly shellfish, from northern European waters. A shorter seasonal harvest, extended and more frequent harvesting bans, and/or protracted commercial shellfish closures all create direct negative socioeconomic impacts, particularly for coastal communities. There is also indirect influence on socioeconomic consequences related to reduced long term employment opportunities in shellfisheries and aquaculture due to reduced risk capital investments, increased regulatory monitoring and health compliance burdens, and increased premium cost (or unavailability) of insurance for aquaculture operations and fishing vessels. Loss of economic opportunities in PST-affected areas can lead to further high psychological stress. Of course, the degree to which *Alexandrium* blooms affect human wellbeing strongly depends on the extent and frequency that coastal populations are exposed to such toxic events, and their individual and collective capacity to adapt to the consequences.

Analysis of PST event data from northern European monitoring programs over the past three decades (from HAEDAT and cited in Bresnan et al., 2021; Karlson et al., 2021) provides few clear trends that toxigenic *Alexandrium* blooms have expanded to create more frequent or severe PST events at the regional scale. Relative to other global “hot spots” for PST events linked to *Alexandrium* blooms, the socioeconomic consequences are rather modest and localized for harvest sites in northern Europe. In comparison to other regions, in 2016, the mussel industry in Chile experienced a massive bloom of *A. catenella* which affected 200 shellfish farms, resulting in four-month long site closures, estimated economic losses of US\$2 million, and caused severe unemployment leading to disruptive social protests. In Japan, near annual large scale *Alexandrium* blooms have occurred since 2013, with site closures typically lasting for more than 200 days. In 2005, in New England, USA, a bloom of *A. catenella* (reported as *A. fundyense*) negatively impacted commercial shellfish fisheries with an estimated loss of over \$US 20 million. No *Alexandrium*-linked PST events comparable in magnitude, geographical extent and socioeconomic consequences have ever been recorded from the CoCliME Case Study regions of northern Europe.

The fact that PSP cases in humans are extremely rare (almost non-existent) from shellfish harvested from northern Europe indicates that national PST monitoring in shellfish and HAB surveillance programs have been effective for public health protection and maintaining aquaculture and shellfisheries at current production levels and harvest sites. There is, however, little scope for expansion or intensification for at-risk shellfish harvest within the boundaries of existing national monitoring programs. In fact, in certain northern European countries, e.g., Norway, with only small-scale or artisanal shellfish harvest at multiple sites and with a long coastline to monitor, the number of shellfish farms is actually decreasing. Cost-benefit considerations often reflect that PST- and *Alexandrium* monitoring at the national level may approach or exceed the commercial value of the harvested resource. Under these circumstance, massive expansion of shellfisheries and aquacultures is clearly not viable. Any future climate-driven increase in *Alexandrium* bloom frequency, cell abundance or biogeographical species invasions would only exacerbate the sustainability problem.

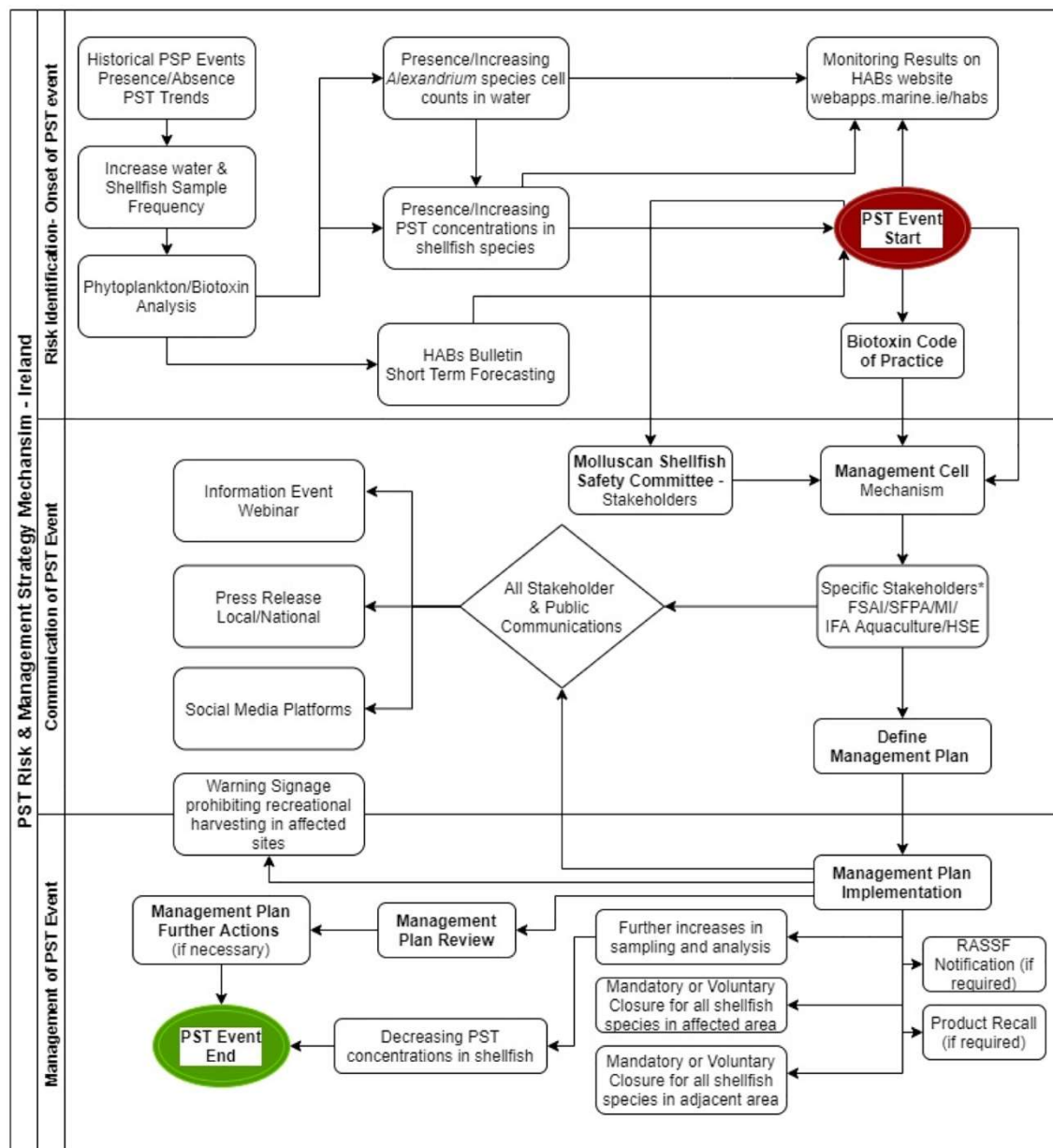
4.1. Stakeholder engagement in PST and *Alexandrium* bloom monitoring

Given these socioeconomic constraints on shellfish aquaculture and shellfisheries sustainability and future development, there is an increasing need for reliable mechanisms and networks for integrating concerns of the primary stakeholders, i.e., production industry, marketing, seafood consumers, public health community, and monitoring and regulatory agencies. Coordination can ensure that legislation is

adhered to and that stakeholder engagement is facilitated with regards to risk management decisions during PST events. Finally, communication with the general public and environmental interest groups on management decisions and their implementation is essential to sustain social support for the seafood industry.

At present there is no strategy at the EU level to embrace all the above issues within a comprehensive framework. Mandated EU regulatory limits for seafood toxins, including PST, are a notable exception to this lack of international coordination. The stakeholder engagement system developed in Ireland is detailed herein as an appropriate national model to follow, but specific applications and priorities vary widely among countries. The Molluscan Shellfish Safety Committee (MSSC) was established in Ireland in the late 1990s as a national forum comprising representatives of all parties involved in the production and placement of bivalve molluscs in the marketplace. Representatives from government departments, national agencies and the Irish shellfish industry that

are active in production, harvest, aquaculture and fisheries development, and toxin analyses and regulation are members of the MSSC, but anyone with a relevant matter to discuss may participate. The forum allows members to express their views on seafood safety, risk management and consumer protection perspectives as well as economic development. This provides collective learning and yields consensus decisions to improve the system and conduct risk management. The MSSC also acts as a consultative body from which the Official Agencies take advice on their statutory roles. The MSSC operates a risk management tool called the Management Cell (MC) to proactively estimate the public health risk presented by shellfish from aquaculture production, particularly during HAB events. The MC facilitates rapid decisions on shellfish safety in non-routine situations. For example, in recent years such decisions have involved accumulation and depuration of PSTs in shellfish flesh above regulatory levels, often at sites in Ireland with no history of such events. Evolving over the last 20 years, the MSSC has delivered



* Stakeholders FSAI (Foods Safety Authority of Ireland), SFPA (Sea Fisheries Protection Authority), MI (Marine Institute), IFA (Irish Farmers Association), HSE (Health Services Executive)

Fig. 8. Flow diagram summarizing the risk identification, communication and management actions and processes from the onset and throughout the duration of PST events in Ireland.

improved decision-making processes in support of the Irish shellfish industry by ensuring seafood safety and the promotion of safe seafood from aquaculture. This activity has been extremely beneficial in facilitating a stakeholder consultation and clear guidance. The terms of reference of both the MSSC and MC are described in the document ‘Code of Practice for the Irish Shellfish Monitoring Programme (Biotoxins)’ (Vr 8.0, FSAI 2018).

The ‘ladder of stakeholder engagement’ (summarised in Fig. 8) provides an overview of the pathway toward co-creation and co-development with stakeholders. The MSSC processes for Ireland represent how a wide range of partners can work together to make shellfish safety management decisions and co-produce new knowledge to ensure seafood safety throughout the onset and duration of PST events.

5. Identified gaps and solutions

5.1. *Alexandrium* detection and identification in field populations

Rapid and reliable detection and discrimination of *Alexandrium* species are critical for routine monitoring and field ecological studies. The resolution of morphological features observed by light microscopy is often insufficient to distinguish clearly between *Alexandrium* species, especially in preserved samples. An alternative and/or complementary approach is to use molecular methods targeting nucleic acid sequences for species detection and discrimination within the genus *Alexandrium*. DNA metabarcoding (e.g., Elferink et al., 2020; Bruhn et al., 2021), quantitative real time PCR (qPCR) (e.g., Hatfield et al., 2019) and fluorescence *in situ* hybridization (FISH) (e.g., John et al., 2003, 2005; Toebe et al., 2013), based on probes to selected sequences of 18S, 28S and/or ITS rRNA regions, have been extensively applied for species discrimination and phylogenetic reconstruction within this genus. As yet none of these methods are fully integrated within the structure of national monitoring programs for PST-producing *Alexandrium* blooms in northern Europe.

Molecular genetic approaches for *Alexandrium* species identification can be loosely grouped into whole-cell based approaches, such as fluorescence *in situ* hybridization (FISH), or cell homogenate-based methods, such as quantitative PCR. The whole-cell approach offers the advantage that the cells stay intact and this permits microscopic visualization for identification. Visual ready discrimination of false positive or negative fluorescence signals permits probe validation and accurate cell enumeration of the target *Alexandrium* taxa (Murray et al., 2019). The disadvantages of microscopic visualization methods such as FISH for *Alexandrium* species detection are that only small sample volumes can be analyzed efficiently and that cells may emit confusing auto-fluorescence from photosynthetic pigments, food vacuoles, lipid inclusions, or aggregated surface detritus. The *Alexandrium* cell-labeling preparation for epifluorescence microscopy is time-consuming; automated high-throughput formats for fluorescent cell counting are available (Toebe et al., 2006). Extracted DNA-based methods, such as qPCR are highly sensitive and given the appropriate primer sequences enable detection of even rare *Alexandrium* species (Vandersea et al., 2017). Such extractive methods can be semi-automated for high-throughput analysis. In some cases, PCR-inhibiting substances, particularly in cell-dense environmental samples, or poor assay probe design can lead to under- or overestimations of target species cell abundance. Such nucleic acid-based methods have to be carefully calibrated before applying them in monitoring programs, especially if estimates of taxon-specific cell abundance are required. Most inter-calibration exercises with *Alexandrium* from northern Europe (e.g., John et al., 2005; Godhe et al., 2007; Taylor et al., 2013; Toebe et al., 2013) have been developed from a few strains of laboratory cultures and tested only against selective few populations of natural blooms. Similarly, in metabarcoding analyses of *Alexandrium* species, the PCR can also be a source of bias, such as over-amplification of certain taxa sequences. Again, inaccuracies can result from application of inappropriate primers or

poor sampling design, which are not effective in capturing the full taxonomic diversity (Zinger et al., 2019).

Ideally, for toxin risk-assessment it is desirable to deploy not only “taxon-specific” but also “toxin (gene)”-specific probes for *Alexandrium* blooms because of the high variability in cell toxin content and composition among and within strains, population and morphospecies. Direct harvest of cells from field bloom populations followed by analysis by chemical analytical methods, i.e., LC-fluorescence or mass spectrometric detection, can resolve the PST-toxin content and composition of the bloom, but are not practical for near-real time routine plankton monitoring. With recently acquired knowledge of the STX biosynthetic genes, toxigenic and non-toxigenic strains of the same species can be discriminated by targeting key biosynthetic genes (e.g., the STX A4 gene) (Murray et al., 2011) even in kit format.

5.2. Future integration of *Alexandrium* bloom detection methods as climate services

Development of Climate Services, as a strategy for dealing with expected enhancement in intensity and distribution of *Alexandrium* blooms, is essential for future monitoring programs. The incorporation of molecular detection methods into comprehensive monitoring systems is an example of adaptive Climate Services. Many global gene probes for *Alexandrium* are available and the necessary platform technologies and bioinformatic programs already exist. Lack of *Alexandrium*-specific protocols for trouble-shooting and validation of probe methods remains a weak link for general incorporation into routine monitoring programs and should be established across the different monitoring programs.

Even at the current developmental stage, methods such as qPCR and metabarcoding could be readily incorporated into plankton monitoring programs. Methods can be automated for high-throughput gene screening like e.g., DNA microarrays (Taylor et al., 2013; Taylor et al., 2014; McCoy et al., 2014) and the potential to analyze many samples simultaneously renders them efficient with respect to capital investment in platform instrumentation, operational costs and dedicated labor required in the laboratory.

There remain major knowledge gaps regarding the function of PST-toxin production in *Alexandrium* species, i.e., why some species or strains are toxigenic and others not. To some extent these gaps continue to hinder the implementation of direct toxin detection in the plankton as a risk-assessment strategy for regional monitoring. Quantitative declaration of *Alexandrium* blooms as either “toxigenic” or “non-toxigenic” within a region, when based on one or a few historical measurements of PST content in natural blooms, is not a reliable indicator for risk assessment. Details of the molecular mechanisms regulating PST-toxin biosynthesis also remain unclear with respect to “bottom up” processes (light, temperature, salinity, nutrients) affecting bloom dynamics. In a few cases, “top down” influences of zooplankton grazers may also shift up PST toxin production in toxigenic *Alexandrium*. The presence of certain copepods has been shown to enhance the production of PSTs in some *Alexandrium* strains (Selander et al., 2006; Wohlrab et al., 2010, 2017; Yang et al., 2011). In these cases, the copepods release an array of fatty acid derivatives known as copepodamides that are sensed by *Alexandrium* cells that respond by upregulation of biosynthesis and increased cell PST content, presumably as anti-grazer defense (Selander et al., 2015). The abundance of such copepods and release of these chemical cues in proximity to *Alexandrium* blooms may therefore indirectly increase the risk for PST contamination of bivalve mollusks. These PST-enhancement phenomena either biotic or abiotic are rapid responses and difficult to monitor in the plankton, but quantitative analysis of chemical induction cues such as copepodamides might prove useful for forecasting toxigenicity risk in HAB species assemblages (Trapp et al., 2021).

A major goal of advanced monitoring strategies for *Alexandrium* blooms is the integrated *in situ* deployment of taxon- and toxin-specific operational systems with capability of providing near-real time

observations over extended periods (e.g., bloom cycles). The ultimate objective, the development and deployment of operational early warning systems (EWS) with forecasting capabilities for *Alexandrium* blooms has thus far lagged far behind the current application of molecular probes in the laboratory. Nevertheless, from a global perspective, a few molecular probes targeting rRNA of *Alexandrium* species, and even immunodiagnostic detection of PSTs in natural blooms, have been incorporated into near-real time field monitoring devices such as the Environmental Sample Processor (ESP, Scholin et al., 2009). Potential EWS based on *in situ* bloom detection technology such as ESP and imaging Flow Cytobot (IFCB) have been deployed in research or β -testing mode (Campbell et al., 2013) at a few locations, but have not been implemented into *Alexandrium* bloom monitoring programs as the strategic national level. Within the northern CoCliME region, there remain large gaps between method development and acceptance by monitoring agencies. To some extent this lag period is due to the high capital and maintenance costs of equipment, operational complexity, and technical deficiencies in providing near-real time *in situ* observations on the appropriate spatio-temporal scale. Other gaps and limitations on molecular probes implementation and deployment of EWS within *Alexandrium* bloom monitoring programs are related to lack of trained personnel and standardization protocols.

The key to a successful risk-assessment monitoring program for *Alexandrium* blooms is to ensure adequately trained monitoring personnel in each of the appropriate methods. Such training must be addressed to technical personnel actively participating in or planning to participate in HAB monitoring in the field and laboratory, and to include the target species and molecular probes corresponding to the region, while ensuring the availability of hardware and bioinformatic platforms. In the framework of the CoCliME project, a training workshop on selected molecular methods to monitor harmful algae was convened, based on practical laboratory sessions for detection and quantification of key HAB species (CoCliME North Sea services: A transnational training service, <https://docs.google.com/document/d/1T9FqBlnRN0i9Q3s-ZG1Pu2lEyK-imjps>). This Climate Services training workshop focused on molecular detection and cell quantitation methods applicable to *Alexandrium* species (including *A. minutum*) and selected other HAB taxa of highest relevance to monitoring of harmful species in northern European waters. After providing the necessary theoretical background for species-specific molecular detection, culture and field samples were directly assayed by qPCR and FISH-probes in the laboratory, followed by bioinformatic analysis. The workshop was designed to train the participants on how to apply these molecular tools autonomously in direct context to their respective national programs dealing with key HAB taxa. Quasi-standardized methods were applied to yield comparative results in current operational formats, rather than to explore advanced prototypes and frontier methods. In other words, to simulate the operation of an actual molecular taxon monitoring program as a partial replacement or supplement to classical microscopic identification and cell enumeration. The success of this workshop format indicated the need for series of basic workshops on application of molecular tools, perhaps with other key species of regional relevance, into monitoring programs. This series should not preclude advanced user workshops aimed at participants who have years of practical experience or deep theoretical knowledge in molecular biological or bioinformatic methods on aquatic species. Future training workshops and intercalibration exercises on application of molecular methods as a climate service would assist in the operational deployment of DNA barcoding and metabarcoding methods for *Alexandrium* blooms, beyond identification of known species and semi-quantitative analysis of cell densities. As a climate service tool these methods would help to detect shifts in microbial communities due to climate change at a very early stage. In addition, barcoding surveys of the entire eukaryotic microalgal assemblages with probes of different levels of taxonomic resolution offer the possibility of detecting previously undocumented *Alexandrium* taxa and cryptic species within a designated geographical area. Such high sensitivity resolution, initiated

as part of a time-series EWS would assist in future risk assessment in response to climate changes and in developing mitigation and adaptation strategies in the affected coastal area.

6. Conclusion

Climate based modeling projections for *Alexandrium* blooms developed in CoCliME case study areas of northern Europe and eastern Arctic gateway waters remain highly uncertain. Current technological developments for reliable detection, identification and cell enumeration of *Alexandrium* species have advanced to the point that they are ready for deployment beyond the laboratory and can be incorporated into operational EWS. *Alexandrium* bloom prediction from niche modeling in the face of global climate change will require further integration of biological properties (life history strategies, growth rate, grazing and parasitism, interspecific competition, buoyancy, gene regulation, etc.) at the infraspecific or even population level over regional spatial scales.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This study is a part of the EU project CoCliME, a component of ERA4CS, an ERA-NET initiated by JPI Climate. Funding was obtained via EPA (IE), ANR (FR), BMBF (DE), UEFISCDI (RO), RCN (NO) and FORMAS (SE), with co-funding by the European Union (Grant 690462) and was financially supported under the POF IV Topic 6 ST 6.2 of the Alfred Wegener Institute, Helmholtz center for Polar and Marine Research, Germany and the Helmholtz Institute for Functional Marine Biodiversity. We acknowledge and appreciate the data access (HAIS (<https://data.hais.ioc-unesco.org>) via HAEDAT/OBIS and interpretative support of colleagues affiliated with the IOC-ICES HABD Working Group and IOC-IPHAB from northern Europe.

References

- Aasen, J., Mackinnon, S.L., Leblanc, P., Walter, J.A., Hovgaard, P., Aune, T., Quilliam, M. A., 2005. Detection and identification of spiroides in Norwegian shellfish and plankton. *Chem. Res. Toxicol.* 18, 509–515.
- Alacid, E., Reñé, A., Camp, J., Garcés, E., 2017. *In situ* occurrence, prevalence and dynamics of *parvilucifera* parasitoids during recurrent blooms of the toxic dinoflagellate *Alexandrium minutum*. *Front. Microbiol.* 8, 1624.
- Alpermann, T.J., Beszteri, B., John, U., Tillmann, U., Cembella, A.D., 2009. Implications of life history transitions on the population genetic structure of the toxigenic marine dinoflagellate *Alexandrium tamarense*. *Mol. Ecol.* 18, 2122–2133.
- Andersen, P., 2010. Filtering–calcofluor staining–quantitative epifluorescence microscopy for phytoplankton analysis. In: Karlson, B., Cusack, C., Bresnan, E. (Eds.), *Microscopic and molecular methods for quantitative phytoplankton analysis*. UNESCO, Paris, pp. 31–35.
- Anderson, D.M., Wall, D., 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. *J. Phycol.* 13, 224–234.
- Anderson, D.M., 1997. Bloom dynamics of toxic *Alexandrium* species in the northeastern U.S. *Limnol. Oceanogr.* 42 (5 II), 1009–1022.
- Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25, 704–726.
- Anderson, D.M., Townsend, D., McGillicuddy, D., Turner, J., 2005. The ecology and oceanography of toxic *Alexandrium fundyense* blooms in the Gulf of Maine. *Deep Sea Res. Part II: Topical Stud. Oceanography* 52, 2365–2876.
- Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012. The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* 14, 10–35.

- Anderson, D.M., Couture, D.A., Kleindinst, J.L., Keafer, B.A., McGillicuddy Jr, D.J., Martin, J.L., Richlen, M.L., Hickey, J.M., Solow, A.R., 2014. Understanding interannual, decadal level variability in paralytic shellfish poisoning toxicity in the Gulf of Maine: the HAB Index. Deep Sea Res. Part II: Topical Stud. Oceanography 103, 264–276.
- Anderson, D.M., Fachon, E., Pickart, R.S., Lin, P., Fischer, A.D., Richlen, M.L., Uva, V., Brosnahan, M.L., McRaven, L., Bahr, F., Lefebvre, K., Grebmeier, J.M., Danielson, S. L., Lyu, Y., Fukai, Y., 2021. Evidence for massive and recurrent toxic blooms of *Alexandrium catenella* in the Alaskan Arctic. Proc. Natl. Acad. Sci. USA 118, e2107387118.
- Balech, E., 1967. Dinoflagelados nuevos o interesantes del Golfo de México y Caribe. Revista museo argentino ciencias naturales "B. Rivadavia". Hydrobiologia 2, 77–144.
- Balech, E., 1971. Microplancton de la Campaña Oceanográfica Productividad III. Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" e instituto nacional de investigación de las ciencias naturales (Argentina). Hydrobiologia 3, 1–202 pls 1–12.
- Balech, E., de Mendiola, B.R., 1977. Un nuevo *Gonyaulax* productor de hemotolasia en Peru. Neotropica 23, 49–54.
- Balech, E., 1985. The genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group. In: Anderson, D.M., White, A.W., Baden, D.G. (Eds.), Toxic Dinoflagellates. Elsevier, Amsterdam, pp. 33–38.
- Balech, E., Tange, K., 1985. Morphology and taxonomy of toxic species in the *tamarensis* group (Dinophyceae): *alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen) comb. nov. Sarsia 70, 333–343.
- Balech, E., 1995. The genus *Alexandrium* halim (Dinoflagellata). Sherkin Island Marine Station. Sherkin Island, Co. Cork, Ireland, p. 151.
- Band-Schmidt, C.J., Lilly, E.L., Anderson, D.M., 2003. Identification of *Alexandrium affine* and *A. margalefi* (Dinophyceae) using DNA sequencing and LSU rDNA- based RFLP-PCR assays. Phycologia 42, 261–268.
- Biecheler, B., 1952. Recherches Sur Les Peridiniens, 36. Suppléments au Bulletin biologique de la France et de la Belgique, pp. 1–149.
- Bindoff, N.L., Cheung, W.W.L., Kairo, J.G., et al., 2019. Changing ocean, marine ecosystems, and dependent communities (Eds.). In: Pörtner, H.O., Roberts, D.C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., et al. (Eds.), IPCC Special Report on the Ocean and Cryosphere in a Changing Climate.
- Branco, S., Oliveira, M.M.M., Salgueiro, F., Vilar, M.C.P., Azevedo, S.M.F.O., Menezes, M., 2020. Morphology and molecular phylogeny of a new PST-producing dinoflagellate species: *Alexandrium fragae* sp. nov. (Gonyaulacales, dinophyceae). Harmful Algae 95, 101793.
- Bravo, I., Garcés, E., Diogene, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. Eur. J. Phycol. 41, 293–302.
- Bresnan, E., Arévalo, F., Belin, C., Branco, M.A.C., Cembella, A.D., Clarke, D., Correa, J., Davidson, K., Dhanji-Rapkova, M., Lozano, R.F., Fernández-Tejedor, M., Guðfinsson, H., Carbonell, D.J., Laza-Martínez, A., Lemoine, M., Lewis, A.M., Menéndez, L.M., Maskrey, B.H., McKinney, A., Pazos, Y., Revilla, M., Siano, R., Silva, A., Swan, S., Turner, A.D., Schweibold, L., Provoost, P., Enevoldsen, H., 2021. Diversity and regional distribution of harmful algal events along the Atlantic margin of Europe. Harmful Algae 102, 101976.
- Brosnahan, M.L., Kulis, D.M., Solow, A.R., Erdner, D.L., Percy, L., Lewis, J., Anderson, D. M., 2010. Outbreeding lethality between toxic Group I and nontoxic Group III *Alexandrium tamarens* spp. isolates: predominance of heterotypic encystment and implications for mating interactions and biogeography. Deep Sea Research Part II: Topical Stud. Oceanography 57, 175–189.
- Brosnahan, M.L., Fischer, A.D., Lopez, C.B., Moore, S.K., Anderson, D.M., 2020. Cyst-forming dinoflagellates in a warming climate. Harmful Algae 91, 101728.
- Bruhn, C.S., Wohlrab, S., Krock, B., Lundholm, N., John, U., 2021. Seasonal plankton succession is in accordance with phycotoxin occurrence in Disko Bay, West Greenland. Harmful Algae 103.
- Bucci, A.F., Thomas, A.C., Cetinić, I., 2020. Interannual variability in the thermal habitat of *Alexandrium catenella* in the bay of fundy and the implications of climate change. Front. Mar. Sci. 7, 1060.
- Cael, B.B., Bisson, K., Conte, M., Duret, M.T., Follett, C.L., Henson, S.A., Honda, M.C., Iversen, M.H., Karl, D.M., Lampitt, R.S., Mouw, C.B., Muller-Karger, F., Pebody, Corinne A., Smith, K.L., Talmay, D., 2021. Open ocean particle flux variability from surface to seafloor. Geophys. Res. Lett. 48, e2021GL092895.
- Campbell, L., Henrichs, D.W., Olson, R.J., Sosik, H.M., 2013. Continuous automated imaging-in-flow cytometry for detection and early warning of *Karenia brevis* blooms in the Gulf of Mexico. Environ. Sci. Pollut. Res. 20, 6896–6902. <https://doi.org/10.1007/s11356-012-1437-4>.
- Castell Perez, C., Roy, S., Levassur, M., Anderson, D.M., 1998. Control of germination of *Alexandrium tamarens* (Dinophyceae) cysts from the lower St. Lawrence Estuary (Canada). J. Phycol. 34, 242–249.
- Cembella, A.D., Sullivan, J.J., Boyer, G.L., Taylor, F.J.R., Andersen, R.J., 1987. Variation in paralytic shellfish toxin composition within the *Protogonyaulax tamarens*/*catenella* species complex, red tide dinoflagellates. Biochem. Syst. Ecol. 15, 171–186.
- Cembella, A.D., 1998. Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Heidelberg, pp. 381–404.
- Cembella, A.D., 2018. Chapter 16: harmful Algal Species Fact Sheets. In: Shumway, S., Burkholder, J., Morton, S. (Eds.), *Alexandrium* /Harmful Algal Blooms: A Compendium Desk Reference. Wiley-Blackwell, p. 12. ISBN: 978-1-118-99465-8.
- Cembella, A.D., Durán-Riveroll, L.M., 2021. Marine guanidinium neurotoxins: biogenic origins and interactions, biosynthesis and pharmacology. In: Novelli, A., Fernández-Sánchez, M.T., Aschner, M., Costa, L.G. (Eds.), Advances in Neurotoxicology, 6. Academic Press, pp. 1–47.
- Chambouvet, A., Morin, P., Marie, D., Guillou, L., 2008. Control of toxic marine dinoflagellate blooms by serial parasitic killers. Science 322, 1254–1257.
- Chapelle, A., Le Gac, M., Labry, C., Siano, R., Quere, J., Caradec, F., et al., 2015. The Bay of Brest (France), a new risky site for toxic *Alexandrium minutum* blooms and PSP shellfish contamination. Harmful Algal News 51, 4–5.
- Ciminiello, P., Fattorusso, E., Forino, M., Montresor, M., 2000. Saxitoxin and neosaxitoxin as toxic principles of *Alexandrium andersoni* (Dinophyceae) from the Gulf of Naples, Italy. Toxicon 38, 1871–1877.
- Condie, S.A., Oliver, E.C.J., Hallegraeff, G.M., 2019. Environmental drivers of unprecedented *Alexandrium catenella* dinoflagellate blooms off eastern Tasmania, 2012–2018. Harmful Algae 87, 101628.
- Davidson, K., Gowen, R.J., Harrison, P.J., Fleming, L.E., Hoagland, P., Moschonas, G., 2014. Anthropogenic nutrients and harmful algae in coastal water. J. Environ. Manag. 146, 206–216.
- Delebecq, G., Schmidt, S., Erhold, A., Latimier, M., Raffaele, S., 2020. Revival of ancient marine dinoflagellates using molecular biostimulation. J. Phycol. 56, 1077–1089.
- Dodge, J.D., 1981. Three new generic names in the Dinophyceae: *herdmania*, *sclerodinium* and *triadinium* to replace *heteraulacus* and *goniodoma*. Br. Phycol. J. 16, 273–280.
- Durán-Riveroll, L.M., Cembella, A.D., Correa-Basurto, J., 2018. Guanidinium toxins: natural biogenic origin, chemistry, biosynthesis, and biotechnological applications. In: La Barre, S., Bates, S.S. (Eds.), Blue Biotechnology: Production and Use of Marine Molecules. John Wiley & Sons, Inc, USA, Hoboken, NJ, pp. 323–370.
- Elferink, S., Wohlrab, S., Neuhaus, S., Cembella, A., Harms, L., John, U., 2020. Comparative metabarcoding and metatranscriptomic analysis of microeukaryotes within coastal surface waters of West Greenland and Northwest Iceland. Front. Mar. Sci. 7, 1–20.
- Ellegaard, M., Ribeiro, S., 2018. The long-term persistence of phytoplankton resting stages in aquatic seed banks. Biol. Rev. 93, 166–183.
- Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142.
- Fistariol, G.O., Legrand, C., Selander, E., Hummert, C., Stolte, W., Granélie, E., 2004. Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures. Aquatic Microbial Ecol. 35, 45–56. <https://doi.org/10.3354/ame035045>.
- Flynn, K.J., Mitra, A., Anestis, K., Anschütz, A.A., Calbet, A., Duarte Ferreira, G., Gypens, N., Hansen, P.J., John, U., Lapeyre Martin, J., Mansour, Maselli, M., Medic, N., Norlin, A., Not, F., Pitta, P., Romano, F., Saiz, E., Schneider, L.K., Stolte, W., Traboni, C., 2019. Mixotrophic protists and a new paradigm for marine ecology: where does plankton research go now? J. Plankton Res. 41, 375–391.
- Fraga, S., Sánchez, F., 1985. Toxic and potentially toxic dinoflagellates found in Galician Rias (NW Spain). In: Anderson, D.M., White, A., Baden, D. (Eds.), Toxic Dinoflagellates. Elsevier Science Publishing Co., Inc., New York, pp. 51–54.
- Frangopoulos, M., Guisande, C., deBlas, E., Maneiro, I., 2004. Toxin production and competitive abilities under phosphorus limitation of *Alexandrium* species. Harmful Algae 3, 131–139.
- Franks, P.J.S., Anderson, D.M., 1992. Alongshore transport of a toxic phytoplankton bloom in a buoyancy current: *Alexandrium tamarens* in the Gulf of Maine. Mar. Biol. 112, 153–164.
- Fukuyo, Y., Yoshida, K., Inoue, H., 1985. *Protogonyaulax* in Japanese coastal waters (Eds.). In: Anderson, D.M., White, A.W., Baden, D.G. (Eds.), Toxic Dinoflagellates. Elsevier, Amsterdam, pp. 27–32.
- Gaarder, K.R., 1954. Dinoflagellatae from the "Micheal Sars" North Atlantic deep-sea expedition 1910. Rep. Scient. Res. 'Michael Sars' N. Atl. Deep-Sea Exped. 1910 (2), 1–62.
- Geffroy, S., Lechat, M.M., Gac, M., Rovillon, G.A., Marie, D., Bigeard, E., Malo, F., Amzil, Z., Guillou, L., Amandine, C., 2021. From the sxtA4 Gene to Saxitoxin production: what controls the variability among *Alexandrium minutum* and *Alexandrium pacificum* strains? Front. Microbiol. 12 <https://doi.org/10.3389/fmicb.2021.613199>.
- Girault, M., Siano, R., Labry, C., Latimier, M., Jauzein, C., Beneyton, T., Buisson, L., Del Amo, Y., Baret, J.C., 2021. Variable inter and intraspecies alkaline phosphatase activity within single cells of revived dinoflagellates. ISME J. 15, 2057–2069.
- Godhe, A., Cusack, C., Pedersen, J., Andersen, P., Anderson, D.M., Bresnan, E., Cembella, A., Dahl, E., Diercks, S., Elbrächter, M., Edler, L., Gescher, C., Gladstone, M., Karlson, B., Kulis, D., LeGresley, M., Lindahl, O., Marin, R., McDermott, G., Medlin, L.K., Naustvoll, L.J., Penna, A., Toebe, K., 2007. Intercomparison of classical and molecular techniques for identification of *Alexandrium fundyense* (Dinophyceae) and estimation of cell densities. Harmful Algae 6, 56–72.
- Gómez, F., Artigas, L.F., 2019. Redefinition of the dinoflagellate genus *Alexandrium* based on *Centrodinium*: reinstatement of *Gessnerium* and *Protogonyaulax*, and *Episemicolon* gen. nov. (Gonyaulacales, Dinophyceae). J. Marine Sci., 1284104.
- Gröger, M., Arneborg, L., Dieterich, C., Höglund, A., Meier, H.E.M., 2019. Summer hydrographic changes in the Baltic Sea, Kattegat and Skagerrak projected in an ensemble of climate scenarios downscaled with a coupled regional ocean–sea ice–atmosphere model. Clim. Dyn. 53, 5945–5966.
- Grzebyk, D., Bechemin, C., Ward, C.J., Verite, C., Codd, G.A., Maestrini, S.Y., 2003. Effects of salinity and two coastal waters on the growth and toxin content of the dinoflagellate *Alexandrium minutum*. J. Plankton Res. 25, 1185–1199.
- Guallar, C., Chapelle, A., Bacher, C., 2021. Realised niche and suitability index highlight spatial and temporal distribution of toxic phytoplankton species. Mar. Ecol. Prog. Ser. 662, 15–34.

- Guiry, M.D., Guiry, G.M., 2022. *AlgaeBase*. World-wide electronic Publication. National University of Ireland, Galway. <https://www.algaebase.org>. searched on July 19, 2022.
- Hakanen, P., Suikkanen, S., Franzén, J., Franzén, H., Kankaanpää, H., Kremp, A., 2012. Bloom and toxin dynamics of *Alexandrium ostenfeldii* in a shallow embayment at the SW coast of Finland, northern Baltic Sea. *Harmful Algae* 15, 91–99.
- Halim, Y., 1960. *Alexandrium minutum* n. gen. s. sp., dinoflagellate provocant des eaux rouges. *Vie. et Milieu* 11, 102–105.
- Hallegraeff, G., Bolch, C., Blackburn, S., Oshima, Y., 1991. Species of the toxigenic dinoflagellate genus *Alexandrium* in southeastern Australian waters. *Botanica Marina* 34, 575–588.
- Hallegraeff, G., Anderson, D., Belin, C., Dechraoui Bottein, M.Y., Bresnan, E., Chinnai, M., Enevoldsen, H., Iwataki, M., Karlson, B., McKenzie, C., Sunesen, I., Pitcher, G., Provoost, P., Richardson, A., Schweibold, L., Tester, P., Trainer, V., Yñiguez, A., Zingone, A., 2021. Perceived global increase in algal blooms is attributable to intensified monitoring and emerging bloom impacts. *Commun. Earth & Environ.* 2 <https://doi.org/10.1038/s43247-021-00178-8>.
- Hatfield, R.G., Bean, T., Turner, A.D., Lees, D.N., Lowther, J., Lewis, A., Baker-Austin, C., 2019. Development of a TaqMan qPCR assay for detection of *Alexandrium* spp. and application to harmful algal bloom monitoring. *Toxicon* 2, 100011.
- Hattenrath-Lehmann, T.K., Smith, J.L., Wallace, R.B., Merlo, L.R., Koch, F., Mittelsdorf, H., Góleski, J.A., Anderson, D.M., Gobler, C.J., 2015. The effects of elevated CO₂ on the growth and toxicity of field populations and cultures of the saxitoxin-producing dinoflagellate, *Alexandrium fundyense*. *Limnol. Oceanogr.* 60, 198–214. <https://doi.org/10.1002/lno.10012>.
- Hosmer, D.W., Lemeshow, S., Sturdivant, R.X., 2013. *Applied Logistic Regression*, 3rd ed. Wiley, New York, NY.
- Hsu, B.C.-C., 1967. Study of Paralytic Shellfish Toxicity Causative Organisms in the State of Washington Master of Science Thesis. University of Washington, pp. 1–129.
- IPCC, Core Writing Team, 2014. In: Pachauri, R.K., Meyer, L.A. (Eds.), *Climate Change 2014. Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Geneva, Switzerland, p. 151 (Eds.).
- John, U., Fensome, R.A., Medlin, L.K., 2003. The application of a molecular clock based on molecular sequences and the fossil record to explain biogeographic distributions within the *Alexandrium tamarense* “species complex” (Dinophyceae). *Mol. Biol. Evol.* 20, 1015–1102.
- John, U., Medlin, L.K., Groben, R., 2005. Development of specific rRNA probes to distinguish between geographic clades of the *Alexandrium tamarense* species complex. *J. Plankton Res.* 27, 199–204.
- John, U., Litaker, R.W., Montresor, M., Murray, S., Brosnahan, M.L., Anderson, D.M., 2014. Formal Revision of the *Alexandrium tamarense* Species Complex (Dinophyceae) Taxonomy: the Introduction of Five Species with Emphasis on Molecular-based (rDNA) Classification. *Protist* 165, 779–804.
- John, U., Tillmann, U., Hülkötter, J., Alpermann, T.J., Wohlrab, S., Van de Waal, D.B., 2015. Intraspecific facilitation by allelochemical mediated grazing protection within a toxigenic dinoflagellate population. *Proceed. Royal Soc. B: Biol. Sci.* B 282, 1–9.
- Kao, C.Y., 1993. Paralytic Shellfish Poisoning. In: Falconer, E.R. (Ed.), *Algal Toxins in Seafood and Drinking Water*. Academic Press Ltd., London, pp. 75–86.
- Karlson, B., Anderson, P., Arneborg, L., Cembella, A., Eikrem, W., John, U., West, J.J., Klemm, K., Kobos, J., Lehtinen, S., Lundholm, N., Mazur-Marzec, H., Naustvoll, L.J., Poelman, M., Provoost, P., De Rijcke, M., Suikkanen, S., 2021. Harmful algal blooms and their effects in coastal seas of Northern Europe. *Harmful Algae* 102, 101989.
- Klouch, K.Z., Schmidt, S., Andrieux-Loyer, F., Le Gac, M., Hervio-Heath, D., Qui-Minet, Z. N., Quere, J., Bigeard, E., Guillou, L., Siano, R., 2016a. Historical records from dated sediment cores reveal the multidecadal dynamic of the toxic dinoflagellate *Alexandrium minutum* in the Bay of Brest (France). *FEMS Microbiol. Ecol.* 92, fw101.
- Klouch, K.Z., Caradec, F., Plus, M., Hernandez-Farinas, T., Pineau-Guillou, L., Chapelle, A., Schmitt, S., Quere, J., Pineau-Guillou, L., Siano, R., 2016b. Heterogeneous distribution in sediments and dispersal in waters of *Alexandrium minutum* in a semi-enclosed coastal ecosystem. *Harmful Algae* 60, 81–91.
- Konovalova, G.V., 1993. Toxic and potentially toxic dinoflagellates from the east coast waters of the USSR. In: Smayda, T., Shimizu, Y. (Eds.), *Toxic phytoplankton blooms in the Sea*. Elsevier, New York, pp. 169–174.
- Kremp, A., Lindholm, T., Dreßler, N., Erler, K., Gerdt, G., Eirtovaara, S., Leskinen, E., 2009. Bloom forming *Alexandrium ostenfeldii* (Dinophyceae) in shallow waters of the Åland Archipelago, Northern Baltic Sea. *Harmful Algae* 8, 318–328.
- Kremp, A., Tahvanainen, P., Litaker, W., Krock, B., Suikkanen, S., Leaw, C.P., Tomas, C., 2014. Phylogenetic relationships, morphological variations, and toxin patterns in the *Alexandrium ostenfeldii* (Dinophyceae) complex: implications for species boundaries and identities. *J. Phycol.* 50, 81–100.
- Kremp, A., Hansen, P.J., Tillmann, U., Savel, H., Suikkanen, S., Voß, D., Barrera, F., Jakobsen, H.H., Krock, B., 2019. Distributions of three *Alexandrium* species and their toxins across a salinity gradient suggest an increasing impact of GDA producing *A. pseudogonyaulax* in shallow brackish waters of Northern Europe. *Harmful Algae* 87, 101622.
- Krock, B., Tillmann, U., Wen, Y., Hansen, P.J., Ostenfeld Larsen, T., Andersen, A.J.C., 2018. Development of a LC-MS/MS method for the quantification of goniodomins A and B and its application to *Alexandrium pseudogonyaulax* strains and plankton field samples of Danish coastal waters. *Toxicon* 155, 51–60.
- Laabir, M., Collos, Y., Masseret, E., Grzebyk, D., Abadie, E., Savar, V., Sibet, M., Amzil, Z., 2013. Influence of environmental factors on the paralytic shellfish toxin content and profile of *Alexandrium catenella* (Dinophyceae) isolated from the mediterranean sea. *Mar. Drugs* 11, 1583–1601.
- Labry, C., Erard-Le Denn, E., Chapelle, A., Fauchot, J., Youenou, A., Crassous, M.P., Le Grand, J., Lorgeoux, B., 2008. Competition for phosphorus between two dinoflagellates: a toxic *Alexandrium minutum* and a non-toxic *Heterocapsa triquetra*. *J. Exp. Mar. Biol. Ecol.* 358, 124–135.
- Lambert, L., Penaud, A., Vidal, M., Klouch, K., Gregoire, G., Ehrhold, A., Frédérique Eynaud, F., Schmidt, S., Ragueneau, O., Siano, R., 2018. Human-induced River runoff overlapping natural climate variability over the last 150 years: palynological evidence (Bay of Brest, NW France). *Glob. Planet. Change* 160, 109–122.
- Larsen, J., Nguyen-Ngoc, L., 2004. Potentially toxic microalgae of Vietnamese waters. *Opera Botanica* 140, 5–216.
- Lassus, P., Chomérat, N., Hess, P., Nézan, E., 2016. Toxic and harmful micro algae of the World Ocean/Micro-algues toxiques et nuisibles de l’océan Mondial. Denmark, international society for the study of harmful algae/intergovernmental oceanographic commission of UNESCO. IOC Manuals and Guides 68 (Bilingual English/French).
- Lau, W.L.S., Law, I.K., Liow, G.R., Hii, K.S., Usup, G., Lim, P.T., Leaw, C.P., 2017. Life-history stages of natural bloom populations and the bloom dynamics of a tropical Asian ribotype of *Alexandrium minutum*. *Harmful Algae* 70, 52–63. <https://doi.org/10.1016/j.hal.2017.10.006>.
- Lebour, M.V., 1925. The Dinoflagellates of the Northern Seas. Plymouth Marine Biol. Assoc. 1–250.
- Lewis, A.M., Coates, L.N., Turner, A.D., Percy, L., Lewis, J., 2018. A review of the global distribution of *Alexandrium minutum* (dinophyceae) and comments on ecology and associated paralytic shellfish toxin profiles, with a focus on Northern Europe. *J. Phycol.* 54, 581–598.
- Lilly, E.L., Halanaych, K.M., Anderson, D.M., 2005. Phylogeny, biogeography, and species boundaries within the *Alexandrium minutum* group. *Harmful Algae* 4, 1004–1020.
- Lilly, E.L., Halanaych, K.M., Anderson, D., 2007. Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae) 1. *J. Phycol.* 43, 1329–1338.
- Lim, A.S., Jeong, H.J., Kim, J.H., Lee, S.Y., 2015. Description of the new phototrophic dinoflagellate *Alexandrium pohangense* sp. nov. from Korean coastal waters. *Harmful Algae* 46, 49–61.
- Liow, G.R., Lau, W.L.S., Law, I.K., Gu, H., Leaw, C.P., Lim, P.T., 2021. Sexual processes and life cycle transitions of the tropical Pacific *Alexandrium minutum* (Dinophyceae). *Phycol. Res.* 69, 188–199.
- Litaker, R.W., Fraga, S., Montresor, M., Brosnahan, M., Anderson, D.M., Hoppenrath, M., Murray, S., Wolny, J., John, U., Sampedro, N., Larsen, J., Calado, A.J., 2018. A practical guide to new nomenclature for species within the “*Alexandrium tamarense* species complex”. *Harmful Algae News* 61, 13–15.
- Llewellyn, L., Negri, A., Robertson, A., 2006. Paralytic shellfish toxins in tropical oceans. *Toxin Rev.* 25, 159–196.
- Loeblich III, A.R., 1970. The amphiesma or dinoflagellate cell covering. In: Yochelson, E. L. (Ed.), *Proceedings of the North American Paleontological Convention: Field Museum of Natural History, Chicago, September 5-7, 1969*, 2. Allen Press, Lawrence, Kansas, pp. 867–929.
- Loeblich III, A.R., Loeblich, L.A., 1979. *Gessnerium catenellum* (Whedon & Kofoid). In: Taylor, D.L., Seliger, H.H. (Eds.), *Toxic Dinoflagellate blooms*. Elsevier Science Publishers, New York, pp. 41–46.
- Long, M., Krock, B., Castrec, J., Tillmann, U., 2021. Unknown extracellular and bioactive metabolites of the genus *Alexandrium*: a review of overlooked toxins. *Toxins* (Basel) 13, 905. <https://doi.org/10.3390/toxins13120905>.
- Lundholm, N., Churro, C., Fraga, S., Hoppenrath, M., Iwataki, M., Larsen, J., Mertens, K., Moestrup, Ø., Zingone, A., 2009 onwards. IOC-UNESCO taxonomic reference list of harmful micro algae. accessed at <https://www.marinespecies.org/hab> on 2022-07-07. doi:10.14284/362.
- Mardones, J.L., Holland, D.S., Anderson, L., Le Bihan, V., Gianella, F., Clément, A., Davidson, K., Sakamoto, S., Yoshida, T., Trainer, V.L., 2020. Estimating and mitigating the economic costs of harmful algal blooms on commercial and recreational shellfish harvesters. In: Trainer, V.L. (Ed.), *Global HAB. Evaluating, Reducing and Mitigating the Cost of Harmful Algal Blooms: A Compendium of Case Studies*. PICES Sci. Rep. No. 59, 107, pp. 66–83.
- Martens, H., Van de Waal, D.B., Brandenburg, K.M., Krock, B., Tillmann, U., 2016. Salinity effects on growth and toxin production in an *Alexandrium ostenfeldii* (Dinophyceae) isolate from The Netherlands. *J. Plankton Res.* 38, 1302–1316.
- Martin, J.L., LeGresley, M.M., Hanke, A.R., 2014. Thirty years *Alexandrium fundyense* cyst, bloom dynamics and shellfish toxicity in the Bay of Fundy, eastern Canada. *Deep Sea Res. Part II: Topical Stud. Oceanography* 103, 27–39.
- Martin, K., Schmidt, K., Toseland, A., et al., 2021. The biogeographic differentiation of algal microbiomes in the upper ocean from pole to pole. *Nat. Commun.* 12, 5483.
- Matrai, P., Thompson, B., Keller, M., 2005. Circannual exocystment of resting cysts of *Alexandrium* spp. from eastern Gulf of Maine populations. *Deep Sea Res. Part II: Topical Stud. Oceanography* 52, 2560–2568.
- McCauley, L.A.R., Erdner, D.L., Nagai, S., Richlen, M.L., Anderson, D.M., 2009. Biogeographic analysis of the globally distributed harmful algal bloom species *Alexandrium minutum* (Dinophyceae) based on rRNA gene sequences and microsatellite markers. *J. Phycol.* 45, 454–463.
- McCoy, G.R., McNamee, S., Campbell, K., Elliott, C.T., Fleming, G.T.A., Raine, R., 2014. Monitoring a toxic bloom of *Alexandrium minutum* using novel microarray and multiplex surface plasmon resonance biosensor technology. *Harmful Algae* 32, 40–48. <https://doi.org/10.1016/j.hal.2013.12.003>.
- Montresor, M., John, U., Beran, A., Medlin, L.K., 2004. *Alexandrium tamutum* sp. nov. (Dinophyceae): a new, non-toxic species in the genus *Alexandrium*. *J. Phycol.* 40, 398–411.
- Murray, S.A., Wiese, M., Stüken, A., Brett, S., Kellmann, R., Hallegraeff, G., Neilan, B.A., 2011. sxtA-based quantitative molecular assay to identify saxitoxin-producing harmful algal blooms in marine waters. *Appl. Environ. Microbiol.* 77, 7050–7057.

- Murray, S.A., Hoppenrath, M., Orr, R.J.S., Bolch, C., John, U., Diwan, R., Yauwenas, R., Harwood, T., de Salas, M., Neilan, B., Hallegraeff, G., 2014. *Alexandrium diversaporum* sp. nov., a new non-saxitoxin producing species: phylogeny, morphology and sxtA genes. *Harmful Algae* 31, 54–65.
- Murray, S.A., Diwan, R., Orr, R.J.S., Kohli, G.S., John, U., 2015. Gene duplication, loss and selection in the evolution of saxitoxin biosynthesis in alveolates. *Mol. Phylogenet. Evol.* 92, 165–180.
- Murray, S.A., Ruvindy, R., Kohli, G.S., Anderson, D.M., Brosnahan, M.L., 2019. Evaluation of sxtA and rDNA qPCR assays through monitoring of an inshore bloom of *Alexandrium catenella* Group 1. *Sci. Rep.* 9, 1–12.
- Murray, S., John, U., Savelle, H., Kremp, A., 2021. *Alexandrium* spp.: genetic and ecological factors influencing saxitoxin production and proliferation. In: Botana, L., Louzao, C., Vilarino, N. (Eds.), *Climate Change and Marine and Freshwater Toxins*. De Gruyter, Berlin, pp. 133–166.
- Nagy, H., Pereira, D., Yamanaka, T., Cusack, C., Nolan, G., Tinker, J., Dabrowski, T., 2021. The Irish Atlantic CoCLIME case study configuration, validation and application of a downscaled ROMS ocean climate model off SW Ireland. *Harmful Algae*, 102053.
- Orr, R.J., Stüken, A., Rundberget, T., Eikrem, W., Jakobsen, K.S., 2011. Improved phylogenetic resolution of toxic and non-toxic *Alexandrium* strains using a concatenated rDNA approach. *Harmful Algae* 10, 676–688.
- Parke, M., Dixon, P.S., 1976. Check-list of British marine algae - third revision. *J. Marine Biol. Assoc. United Kingdom* 56, 527–594.
- Paulsen, O., 1904. Plankton investigation in the waters round Iceland in 1903. *Medd. Komm. Havunders. Kopenhagen, Ser. Plankton* 1, 1–40.
- Paulsen, O., 1949. Observations on Dinoflagellates. *Kongelige Danske Videnskabernes Selskabs Skrifter* 6, 1–67.
- Persson, A., Godhe, A., Karlson, B., 2000. Dinoflagellate cysts in recent sediments from the west coast of Sweden. *Botanica Marina* 43, 69–79.
- Persson, M., Karlson, B., Zuberovic Muratovic, A., Simonsson, M., Bergkvist, P., Renborg, E., 2020. L 2020 nr 24: kontrollprogrammet för tvåskaliga blötdjur, Årsrapport 2014–2019. Livsmedelsverkets rapportserie. Livsmedelsverket, Uppsala.
- Prud'homme van Reine, W.F., 2017. Report of the nomenclature committee for algae: 15. *Taxon* 66, 191–192.
- Purz, A., Hodapp, D., Moorthi, S., 2021. Dispersal, location of bloom initiation, and nutrient conditions determine the dominance of the harmful dinoflagellate *Alexandrium catenella*: a meta-ecosystem study. *Limnol. Oceanogr.* 66 <https://doi.org/10.1002/lno.11933>.
- Raine, R., 2014. A review of the biophysical interactions relevant to the promotion of HABs in stratified systems: the case study of Ireland. *Deep Sea Res. Part II: Topical Stud. Oceanography* 101, 21–31.
- Rathaille, A.N., Raine, R., 2011. Seasonality in the excystment of *Alexandrium minutum* and *Alexandrium tamarense* in Irish coastal waters. *Harmful Algae* 10, 629–635.
- Richlen, M.L., Zielinski, O., Hollinde, L., Tillmann, U., Cembella, A., Lyu, Y., Anderson, D.M., 2016. Distribution of *Alexandrium fundyense* (Dinophyceae) cysts in Greenland and Iceland, with an emphasis on viability and growth in the Arctic. *Mar. Ecol. Prog. Ser.* 547, 33–46.
- Ruvindy, R., Bolch, C.J., MacKenzie, L., Smith, K.F., Murray, S.A., 2018. qPCR assays for the detection and quantification of multiple paralytic shellfish toxin-producing species of *Alexandrium*. *Front. Microbiol.* 9, 3153.
- Sampedro, N., Franco, J.M., Zapata, M., Riobó, P., Garcés, E., Penna, A., Caillaud, A., Diogène, J., Cacho, E., Camp, J., 2013. The toxicity and intraspecific variability of *Alexandrium andersonii* Balech. *Harmful Algae* 25, 26–38.
- Scholin, C.A., Doucette, G., Jensen, S., Roman, B., Pargett, D., Marin, R., Preston, C., Jones, W., Feldman, J., Everlove, C., Harris, A., Alvarado, N., Massion, E., Birch, J., Greenfield, D., 2009. Remote sensing of marine microbes, small invertebrates, harmful algae and biotoxins using the environmental sample processor (ESP). *Oceanography* 22, 158–167. <https://doi.org/10.5670/oceanog.2009.46>.
- Schrum, C., Lowe, J., Meier, H.E.M., Grabemann, I., Holt, J., Mathis, M., Pohlmann, T., Skogen, M.D., Sterl, A., Wakelin, S., 2016. Projected change-North Sea. In: Quante, M., Colijn, F. (Eds.), *North Sea Region climate change assessment*. Springer, Berlin, pp. 175–217.
- Selander, E., Thor, P., Toth, G., Pavia, H., 2006. Copepods induce paralytic shellfish toxin production in marine dinoflagellates. *Proceed. Royal Biol. Sci.* 273, 1673–1680.
- Selander, E., Kubanek, J., Hamberg, M., Andersson, M.X., Cervin, G., Pavia, H., 2015. Predator lipids induce paralytic shellfish toxins in bloom-forming algae. *PNAS* 112, 6395–6400.
- Shaw, J.L.A., Weyrich, L.S., Hallegraeff, G., Cooper, A., 2019. Retrospective eDNA assessment of potentially harmful algae in historical ship ballast tank and marine port sediments. *Mol. Ecol.* 28, 2476–2485. <https://doi.org/10.1111/mec.15055>.
- Siano, R., Lassudrie Duchesne, M., Cuzin, P., Briant, N., Loizeau, V., Schmidt, S., Ehrhold, A., Mertens, K., Lambert, C., Quintric, L., Noël, C., Latimier, M., Quéré, J., Durand, P., Penaud, A., 2021. Sediment archives reveal irreversible shifts in plankton communities after World War II and agricultural pollution. *Current Biol.* 31, 2682–2689. e7.
- Stock, C.A., McGillicuddy, D.J., Solow, A.R., Anderson, D.M., 2005. Evaluating hypotheses for the initiation and development of *Alexandrium fundyense* blooms in the western Gulf of Maine using a coupled physical-biological model. *Deep Sea Res. Part II: Topical Stud. Oceanography* 52, 2715–2744.
- Stüken, A., Orr, R.J.S., Kellmann, R., Murray, S.A., Neilan, B.A., et al., 2011. Discovery of nuclear-encoded genes for the neurotoxin Saxitoxin in dinoflagellates. *PLoS ONE* 6, e20096. <https://doi.org/10.1371/journal.pone.0020096>.
- Suikkanen, S., Pulina, S., Engström-Öst, J., Lehtiniemi, M., Lehtinen, S., Brutemark, A., 2013. Climate change and eutrophication induced shifts in Northern summer plankton communities. *PLoS ONE* 8, e66475.
- Tahvanainen, P., Alpermann, T.J., Figueroa, R.I., John, U., Hakanen, P., Nagai, S., Blomster, J., Kremp, A., 2012. Patterns of post-glacial genetic differentiation in marginal populations of a marine micro-alga. *PLoS ONE* 7, e33602.
- Tang, Y.Z., Kong, L., Holmes, M.J., 2007. Dinoflagellate *Alexandrium leei* (Dinophyceae) from Singapore coastal waters produces a water-soluble ichthyotoxin. *Mar. Biol.* 150, 541–549. <https://doi.org/10.1007/s00227-006-0396-z>.
- Taylor, F.J.R., 1976. Dinoflagellates from the International Indian ocean expedition: a report on material collected by the R.V. "Anton Bruun". *Bibliotheca Botanica* 132, 1–234.
- Taylor, F.J.R., 1979. The toxigenic gonyaulacoid dinoflagellates. In: Taylor, D.L., Seliger, H.H. (Eds.), *Toxic dinoflagellate blooms. Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms, Key Biscayne, Florida, October 31–November 5, 1978*. Elsevier/North-Holland, New York, pp. 47–56.
- Taylor, J.D., Berzano, M., Percy, L., Lewis, J., 2013. Evaluation of the MIDTAL microarray chip for monitoring toxic microalgae in the Orkney Islands, UK. *Environ. Sci. Pollut. Res.* 20, 6765–6777.
- Taylor, J.D., Kegel, J.U., Lewis, J.M., Medlin, L.K., 2014. Validation of the detection of *Alexandrium* species using specific RNA probes tested in a microarray format: calibration of signal using variability of RNA content with environmental conditions. *Harmful Algae* 37, 17–27. <https://doi.org/10.1016/j.hal.2014.05.001>.
- Thomas, M.K., Kremer, C.T., Klausmeier, C.A., Litchman, E., 2012. A global pattern of thermal adaptation in marine phytoplankton. *Science* 338, 1085–1088.
- Tillmann, U., John, U., 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defense mechanism independent of PSP-toxin content. *Mar. Ecol. Prog. Ser.* 230, 47–58.
- Tillmann, U., Alpermann, T.L., da Purificação, R.C., Krock, B., Cembella, A., 2009. Intra-population clonal variability in allelochemical potency of the toxigenic dinoflagellate *Alexandrium tamarense*. *Harmful Algae* 8, 759–769.
- Tillmann, U., Hansen, P.J., 2009. Allelopathic effects of *Alexandrium tamarense* on other algae: evidence from mixed growth experiments. *Aquatic Microbial Ecol.* 57, 101–112. <https://doi.org/10.3354/ame01329>.
- Toebe, K., Eller, G., Medlin, L.K., 2006. Automated detection and enumeration for toxic algae by solid-phase cytometry and the introduction of a new probe for *Prymnesium parvum* (Haptophyta: prymnesiophyceae). *J. Plankton Res.* 28, 643–657.
- Toebe, K., Alpermann, T.J., Tillmann, U., Krock, B., Cembella, A., John, U., 2013. Molecular discrimination of toxic and non-toxic *Alexandrium* species (Dinophyta) in natural phytoplankton assemblages from the Scottish coast of the North Sea. *Eur. J. Phycol.* 48, 12–26.
- Touzet, N., Franco, J.M., Raine, R., 2007. Characterization of nontoxic and toxin-producing strains of *Alexandrium minutum* (Dinophyceae) in Irish coastal waters. *Appl. Environ. Microbiol.* 73, 3333–3342.
- Touzet, N., Franco, J.M., Raine, R., 2008. Morphogenetic diversity and biotoxin composition of *Alexandrium* (Dinophyceae) in Irish coastal waters. *Harmful Algae* 7, 782–797.
- Touzet, N., Lacaze, J.P., Maher, M., Turrell, E., Raine, R., 2011. Summer dynamics of *Alexandrium ostenfeldii* (Dinophyceae) and spirolide toxins in Cork Harbour, Ireland. *Mar. Ecol. Prog. Ser.* 425, 21–33.
- Trapp, A., Heuschele, J., Selander, E., 2021. Eavesdropping on plankton—Can zooplankton monitoring improve forecasting of biotoxins from harmful algae blooms? *Limnol. Oceanogr.* 66, 3455–3471. <https://doi.org/10.1002/lno.11891>.
- Weissbach, A., Tillmann, U., Legrand, C., 2010. Allelopathic potential of the dinoflagellate *Alexandrium tamarense* on marine microbial communities. *Harmful Algae* 10, 9–18.
- Whedon, W.F., Kofoed, C.A., 1936. Dinoflagellate of the San Francisco region. I. On the skeletal morphology of two new species, *Gonyaulax catenella* and *G. acatenella*. *Univ. Calif. Publ. Zool.* 41, 25–34.
- Whitehead, P.G., Crossman, J., 2012. Macronutrient cycles and climate change: key science areas and an international perspective. *Sci. Total Environ.* 434, 13–17. <https://doi.org/10.1016/j.scitotenv.2011.08.046>. ISSN 0048-9697.
- Woloszynska, J., Conrad, W., 1939. *Pryodinium phoneus*, n. sp., agent de la toxicité des moules du canal maritime de Bruges à Zeebrugge. *Bullet. du Musée Royal d'Histoire naturelle de Belgique* 15, 1–5.
- Vandersea, M.W., Kibler, S.R., Van Sant, S.B., Tester, P.A., Sullivan, K., Eckert, G., Cammarata, C., Reece, K., Scott, G., Place, A., Holderied, K., Hondolero, D., Litaker, R.W., 2017. qPCR assays for *Alexandrium fundyense* and *A. ostenfeldii* (Dinophyceae) identified from Alaskan waters and a review of species-specific *Alexandrium* molecular assays. *Phycologia* 56, 303–320. <https://doi.org/10.2216/16-41.1>.
- Van Dolah, F.M., 2000. Marine algal toxins: origins, health effects, and their increased occurrence. Environmental health perspectives. public health services, US Dept of health and human services. marine toxins, neurotoxic shellfish poisoning, okadaic acid, paralytic shellfish poisoning. *Environ. Health Perspect.* 108, 133–141.
- Van de Waal, D., Tillmann, U., Martens, H., Krock, B., Van Scheepeningen, Y., John, U., 2015. Characterization of multiple isolates from an *Alexandrium ostenfeldii* bloom in The Netherlands. *Harmful Algae* 49, 94–104.
- Wang, D.Z., Zhang, S.G., Gu, H.F., Chan, L.L., Hong, H.S., 2006. Paralytic shellfish toxin profiles and toxin variability of the genus *Alexandrium* (Dinophyceae) isolated from the Southeast China Sea. *Toxicon* 48, 138–151. <https://doi.org/10.1016/j.toxicon.2006.04.002>.
- Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S.O., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D.M., Cochlan, W.P., 2015. Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae* 49, 68–93. <https://doi.org/10.1016/j.hal.2015.07.009>.
- Wohlrab, S., Iversen, M.H., John, U., 2010. A molecular and co-evolutionary context for grazer induced toxin production in *Alexandrium tamarense*. *PLoS ONE*.

- Wohlrab, S., Selander, E., John, U., 2017. Predator cues reduce intraspecific trait variability in a marine dinoflagellate. *BMC Ecol.* 17 (8) <https://doi.org/10.1186/s12898-017-0119-y>. PMID: 28241803, PMCID: PMC5327569.
- Yang, I., Beszteri, S., Tillmann, U., Cembella, A., John, U., 2011. Growth- and nutrient-dependent gene expression in the toxigenic marine dinoflagellate *Alexandrium minutum*. *Harmful Algae* 12, 55–69. <https://doi.org/10.1016/j.hal.2011.08.012>.
- Zinger, L., Bonin, A., Alsos, I.G., Bálint, M., Bik, H., Boyer, F., Chariton, A.A., Creer, S., Coissac, E., Deagle, B.E., De Barba, M., Dickie, I.A., Dumbrell, A.J., Ficetola, G.F., Fierer, N., Fumagalli, L., Gilbert, M.T.P., Jarman, S., Jumpponen, A., Kauserud, H., Orlando, L., Pansu, J., Pawlowski, J., Tedersoo, L., Thomsen, P.F., Willerslev, E., Taberlet, P., 2019. DNA metabarcoding-need for robust experimental designs to draw sound ecological conclusions. *Mol. Ecol.* 28, 1857–1862.