

Emerging phylogeographic perspective on the toxigenic diatom genus *Pseudo-nitzschia* in coastal northern European waters and gateways to eastern Arctic seas: Causes, ecological consequences and socio-economic impacts

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ABSTRACT

The diatom *Pseudo-nitzschia* H. Peragallo is perhaps the most intensively researched genus of marine pennate diatoms, with respect to species diversity, life history strategies, toxigenicity, and biogeographical distribution. The global magnitude and consequences of harmful algal blooms (HABs) of *Pseudo-nitzschia* are particularly significant because of the high socioeconomic impacts and environmental and human health risks associated with the production of the neurotoxin domoic acid (DA) among populations of many (although not all) species. This has led to enhanced monitoring and mitigation strategies for toxigenic *Pseudo-nitzschia* blooms and their toxins in recent years. Nevertheless, human adaptive actions based on future scenarios of bloom dynamics and proposed shifts in biogeographical distribution under climate-change regimes have not been implemented on a regional scale. In the CoCliME (Co-development of climate services for adaptation to changing marine ecosystems) program these issues were addressed with respect to past, current and anticipated future status of key HAB genera such as *Pseudo-nitzschia* and expected benefits of enhanced monitoring. Data on the distribution and frequency of *Pseudo-nitzschia* blooms in relation to DA occurrence and associated amnesic shellfish toxin (AST) events were evaluated in a contemporary and historical context over the past several decades from key northern CoCliME Case Study areas. The regional studies comprised the greater North Sea and adjacent Kattegat-Skagerrak and Norwegian Sea, eastern North Atlantic marginal seas and Arctic gateways, and the Baltic Sea. The first evidence of possible biogeographical expansion of *Pseudo-nitzschia* taxa into frontier eastern Arctic gateways was provided from DNA barcoding signatures. Key climate change indicators, such as salinity, temperature, and water-column stratification were identified as drivers of upwelling and advection related to the distribution of regional *Pseudo-nitzschia* blooms. 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 indicators may play key roles in selecting for the occurrence and diversity of *Pseudo-nitzschia* species within the broader microeukaryote communities. Shifts to higher temperature and lower salinity regimes predicted for the southern North Sea indicate the potential for high-magnitude *Pseudo-nitzschia* blooms, currently absent from this area. Ecological and socioeconomic impacts of *Pseudo-*

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nitzschia blooms are evaluated with reference to effects on fisheries and mariculture resources and coastal ecosystem function. Where feasible, effective adaptation strategies are proposed herein as emerging climate services for the northern CoCLiME region.

1. Introduction

Pseudo-nitzschia is a globally distributed genus of pennate diatoms, found in both neritic and oceanic waters, typically forming blooms in coastal zones during spring and occasionally during other periods of phytoplankton succession when diatoms are dominant (Trainer et al., 2012; Lelong et al., 2012). For short periods (weeks), and particularly when present in extensive high cell density thin-layers in seasonally stratified waters, this genus may comprise a major fraction of carbon (C) supply and primary production for local marine food webs, but is not considered a major contributor to global annual C-flux and stoichiometry. The reasons for the genus notoriety and its high profile in marine research and plankton monitoring over the last three decades have little to do with the relative contribution to ocean C-flux and stoichiometry. The main focus of interest on genus *Pseudo-nitzschia* is primarily due to production of the neurotoxin responsible for amnesic shellfish poisoning (ASP) in human consumers of shellfish and associated effects on marine fauna (Bates et al., 2018).

The first confirmed association of a marine diatom with severe human illness was recorded in November 1987 after a major toxicity event arising from consumption of blue mussels (*Mytilus edulis*) originating at aquaculture sites in eastern Prince Edward Island in Atlantic Canada (Bates et al., 1989). In this incident, at least three deaths and >100 illnesses were attributed to ASP caused by consumption of mussels containing high levels of the neurotoxin domoic acid (DA), also known as amnesic shellfish toxin (AST). Identification of the culprit species *P. multiseriata* (originally as *Nitzschia pungens*, Bates et al., 1989; 2002) and the association with production of DA, led to a rapid global expansion of research on the potential toxigenicity and environmental consequences of *Pseudo-nitzschia* blooms.

Within a few years of establishing the initial linkage between *Pseudo-nitzschia* blooms, ASP events, and associated environmental impacts, several reviews were published on human health implications and the toxigenicity of some species (Perl et al., 1990; Todd, 1993). From the perspective of seafood safety and environmental toxicity effects on marine ecosystems, AST derived from *Pseudo-nitzschia* populations has proven to be an ongoing global health risk for human consumers, primarily of bivalve shellfish. Transfer and bioaccumulation of DA through the marine food web can have devastating consequences on marine fish, seabirds and marine mammals (Bates et al., 2018; Smith et al., 2018), as well as unknown effects on other organisms in benthic and pelagic food webs.

Recent excellent global syntheses on genus *Pseudo-nitzschia* have included research highlights based on a comprehensive literature review of taxonomy, biogeographical distribution, bloom dynamics, food web effects, molecular genetics, toxin biosynthesis, and future perspectives (Lelong et al., 2012; Trainer et al., 2012; Bates et al., 2018). Knowledge gaps remain, however, including the eco-evolutionary context for the production of DA and its isomers and the global distribution of potentially toxigenic *Nitzschia* species linked to specific AST events on decadal time scales.

The confusing (for the non-taxonomist) nomenclatural and taxonomic issues are being resolved and revised on a semi-continuous basis, e.g. in databases such as AlgaeBase (<https://www.algaebase.org/browse/taxonomy/#77753>, Guiry and Guiry, 2023), WoRMS (<https://www.marinespecies.org/aphia.php?p=taxdetails&id=291409>, Kocielek et al., 2023) and the IOC Taxonomical List of Harmful Micro Algae (<https://www.marinespecies.org/hab/>, Lundholm et al., 2009 onwards). There remains, however, a critical lack of validated molecular markers to discriminate between toxigenic and non-toxigenic

populations of *Pseudo-nitzschia* species and to identify sexual reproductive stages in the field.

In Europe, human health is protected from the effects of shellfish toxins, including DA, under the current EU regulation 2019/627. This regulation requires member states to monitor shellfish for the presence of ASTs and their growing waters for the presence of causative phytoplankton. When *Pseudo-nitzschia* blooms or ASTs cause a harmful algal event (e.g. closure of shellfish harvesting area, human illness, marine faunal mortalities), the relevant data associated with these events are summarized and entered into the Harmful Algal Event Database (HAE-DAT) (<http://haedat.iode.org/index.php>). Data on the distribution and blooms of *Pseudo-nitzschia* species are currently entered into the UNESCO Ocean Information Biodiversity System (<https://obis.org/>) database including biogeographical descriptors.

As part of the 1st IOC Global HAB Status Report (Hallegraeff et al., 2021), these databases were accessed to describe the regional distribution of AST events and *Pseudo-nitzschia* species in northern Europe, including marginal sea coasts of the NE Atlantic (Bresnan et al., 2021; Karlson et al., 2021). These descriptive reviews provide a solid basis for further exploration of scenarios for future consequences of climate change and anthropogenic influences on the distribution, frequency and magnitude of *Pseudo-nitzschia* blooms from a regional perspective.

In the framework of the recent EU Project CoCLiME (Co-development of Climate Services for adaptation to changing Marine Ecosystems; <http://www.coclime.eu/>) case studies on *Pseudo-nitzschia* biogeography and ecological impacts were developed for northern Europe including Arctic gateway waters. A map of the area considered in the defined case studies of the CoCLiME project, indicating where HAB events presumably caused by *Pseudo-nitzschia* blooms is provided for orientation (Fig. 1). This publication integrates basic research highlights on *Pseudo-nitzschia* with modelling scenarios and projected socioeconomic consequences for climate services in the northern CoCLiME region. A brief comparison with other regions such as the NE Pacific and NW Atlantic upwelling systems where harmful *Pseudo-nitzschia* blooms are prominent is provided with conceptual models. The human dimension – effects on coastal society and activities, human and ecosystem health, and socioeconomics of the seafood industries is critical for the development of adaptive responses and strategies for monitoring and mitigation of *Pseudo-nitzschia* blooms and their consequences.

2. *Pseudo-nitzschia* species and toxigenicity

2.1. Species identification and infra-generic diversity

Members of the genus *Pseudo-nitzschia* were first described by H. Peragallo (1897–1908), then later included within the genus *Nitzschia* (Hustedt, 1958), and finally separated again (Hasle, 1994) (reviewed by Bates, 2000). To date over 60 *Pseudo-nitzschia* species have been described (World Register of Marine Species [WoRMS]; <http://www.marinespecies.org/index.php>), many originally on the basis of characteristics observed under light microscopy (LM) but now primarily by detailed examination of morphological features by electron microscopy (EM). *Pseudo-nitzschia* species diversity has been difficult to assess because many morphological features for critical taxonomy lie at or below the limit of resolution by conventional LM (Lundholm et al., 2003, 2006) and several minor features may vary among geographical and seasonal populations (Moschandreu et al., 2012). The application of EM has largely resolved the ambiguity in species boundaries and identification of *Pseudo-nitzschia* from morphological criteria alone.

The resolution of cryptic species, morphological variation within

genetic species, and phylogenetic reconstruction of genus *Pseudo-nitzschia* is dependent on DNA fingerprinting techniques, e.g., sequencing of ribosomal DNA (Moschandreu et al., 2012; Kim et al., 2020), including detailed analysis of the ITS2 rDNA region (Amato and Montresor, 2008; Andree et al., 2011; Lundholm et al., 2012). DNA sequence analysis often reveals possible cryptic or pseudo-cryptic speciation which is not reflected in the morphology (Amato and Montresor 2008; Lundholm et al., 2012; Giuliotti et al., 2021a). Quantitative PCR (qPCR) has allowed reliable identification and quantitative cell estimates of targeted *Pseudo-nitzschia* species in diverse local populations (Andree et al., 2011; Kim et al., 2020).

2.2. Biosynthetic capacity for AST in toxigenic species

Domoic acid (DA) is recognized as the main AST produced by certain *Pseudo-nitzschia* species and accumulated in shellfish (Jeffery et al., 2004; Zabaglo et al., 2016) and as the proximal cause of ASP in humans and marine vertebrates. *Pseudo-nitzschia* species are the principal source of DA, but among the pennate diatoms a few species of *Nitzschia*, and even certain red algae (rhodophytes) share the biosynthetic capacity (Steele et al., 2022). Globally, more than two dozen species of *Pseudo-nitzschia*, as well as *Nitzschia bizertensis* Smida, Lundholm, Hlaili & Mabrouk and *N. navis-varingica* Lundholm & Moestrup, are known AST producers (Lundholm, 2023), and they are well represented in northern European waters (Table 1).

Closures of shellfish harvesting areas due to levels of DA above regulatory thresholds have been enforced in Northern Europe since monitoring for this toxin began in the 1990s (Bresnan et al., 2021). Isomers of the secondary amine DA (e.g., isodomoic acid A, B and C) have also been found in both *Pseudo-nitzschia* and *Nitzschia* species, but in different ratios and highly variable cell content among strains and species (Hansen et al., 2011; Tan et al., 2016). These diverse analogs of DA are typically much less potent on a molar basis and quantitatively less important in *Pseudo-nitzschia* blooms and shellfish in Europe. Such analogs are therefore typically ignored in AST analysis for regulatory purposes within the EU (EFSA, 2009), but this policy is subject to review pending further toxicological data.

3. Biogeography of *Pseudo-nitzschia* and associated AST in northern European case study areas

3.1. Species distribution and prevalence

Members of the genus *Pseudo-nitzschia* have been recorded from the CoCliME regions of northern Europe for more than a century (e.g. Hasle, 1964; 1996, and references therein; Churro et al., 2009; Bresnan et al., 2015). The first overall regional study on the distribution and diversity of *Pseudo-nitzschia* in northern European waters, encompassing the Skagerrak, the North Atlantic, and adjacent coastal and shelf seas, was, however, rather recent (Hasle et al., 1996). *Pseudo-nitzschia* species

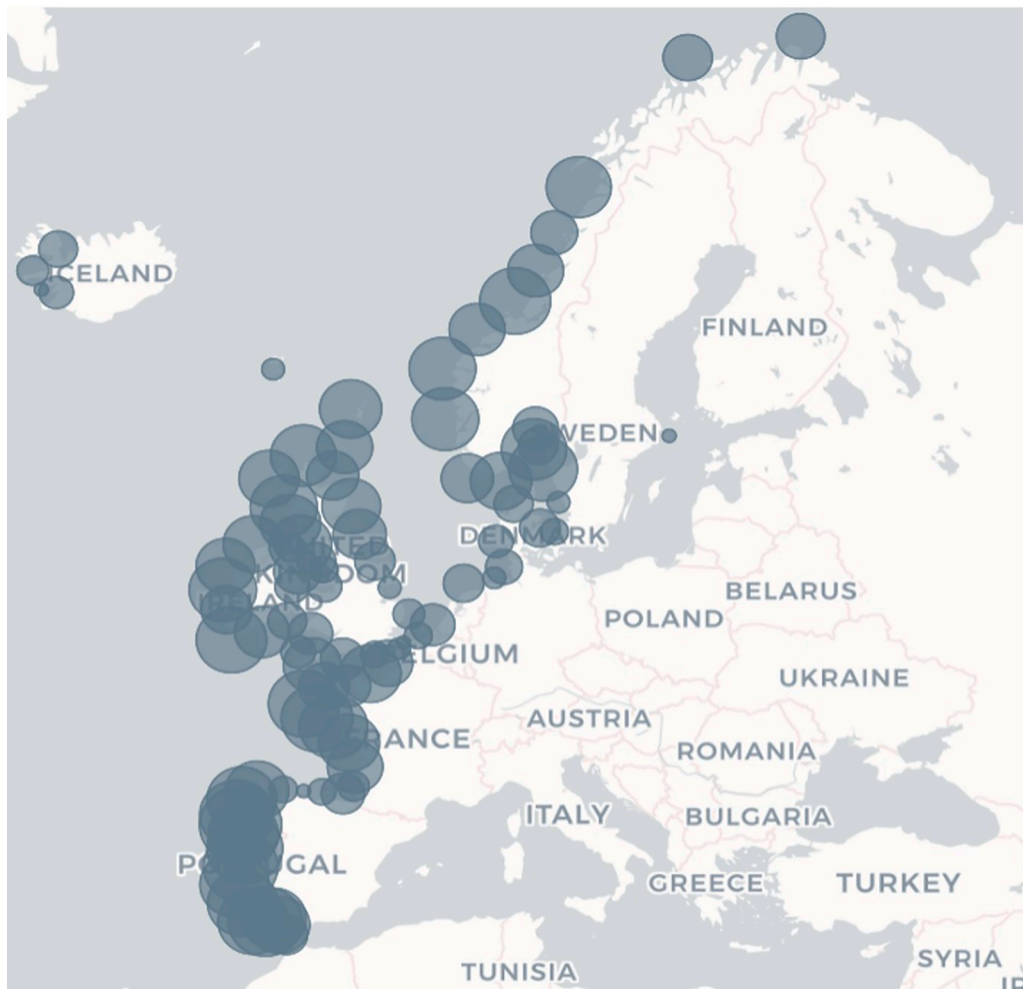


Fig 1. Map of northern European and Arctic gateway waters with northern CoCliME case study region delimited. Circles show where harmful algal events linked to *Pseudo-nitzschia* have been recorded in the IOC-ICES-PICES HAEDAT database. Further details on methods and datasets are described in Bresnan et al. (2021) and Karlson et al. (2021), including Supplementary material.

Table 1

Pseudo-nitzschia species and their known or potential toxigenicity found in northern European and NE Atlantic marginal seas, and Arctic gateway waters. Records are included only where the species has been identified with certainty, i.e. morphological analysis confirmed by electron microscopy and/or gene sequencing (ITS rDNA, rbcL or cox1 genes). Species are indicated as globally toxigenic (+) if at least one isolated strain or natural population from any geographical origin has been confirmed to produce domoic acid (DA) or derivatives (dubbed amnesic shellfish toxin = AST); species confirmed to yield DA from any location in the study region are also marked (+); UNK = toxigenicity unknown, unconfirmed, negative when tested, or unpublished. References for global toxigenicity of *Pseudo-nitzschia* species can be found in [Lundholm \(2023\)](#). Biogeographical location refers to where the species has been found but it not intended to be exclusive or exhaustive for the northern CoCliME region.

<i>Pseudo-nitzschia</i> species	Synonyms	Global toxigenicity (AST)	DA toxigenicity in study area	Biogeographical location	References
<i>P. abrensis</i> Pérez-Aicua & Orive 2013		⁺¹	UNK	Bilbao estuary; Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2013)
<i>P. americana</i> (Hasle) G.A. Fryxell in Hasle 1993	<i>Nitzschia americana</i> (Hasle, 1964)	UNK	UNK	Helgoland, southern German Bight, North Sea	(Bresnan et al., 2015)
			UNK	Aveiro lagoon, NE Atlantic, Portugal	(Churro et al., 2009)
			UNK	Schelde, North Sea, Belgium	(Lundholm et al., 2002)
			UNK	Mariager Fjord, inland from Kattegat, Denmark	(Lundholm et al., 2010)
			UNK	Firth of Lorne, Loch Ewe, west coast; Scapa Bay, Orkney Islands, Scotland	(Fehling et al., 2006; Bresnan et al., 2017)
<i>P. arctica</i> Percopo & Sarno 2016		UNK	UNK	Baie de Vays, Normandy, France	(Nezan et al., 2007; Klein et al., 2010)
			UNK	Beaufort Sea; Baffin Bay, eastern Arctic Sea margin; Disko Bay, west Greenland	(Percopo et al., 2016; Lundholm et al., 2018)
<i>P. arenysensis</i> Quijano-Scheggia, Garcés, Lundholm 2009		UNK	UNK	Nervion River/Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2010; 2013)
<i>P. australis</i> Frenguelli 1939	<i>Nitzschia australis</i> Frenguelli, <i>Nitzschia pseudoseriata</i> Hasle	+	UNK+	Baie de Veys, NE Atlantic, Normandy, France	(Klein et al., 2010; Martin-Jézéquel et al., 2015; Ayache et al., 2020)
			UNK	Celtic Sea; south and SW Ireland	(Cusack, 2002; Cusack et al., 2004)
			UNK	Aveiro coastal lagoon, NE Atlantic, Portugal	(Churro et al., 2009)
			UNK	Loch Ewe and Firth of Lorne, NW and West coast; Scapa Bay, Orkney Islands; Shetland Islands, Scotland	(Gallacher et al., 2001; Brown and Bresnan, 2008; Bresnan et al., 2015; Fehling et al., 2006)
			UNK	NE Atlantic, Iberia coast, Spain and Portugal	(Míguez et al., 1996; Lundholm et al., 2003; Velo-Suárez et al., 2008; Orive et al., 2010; Zapata et al., 2011; Lundholm et al., 1997; 2003; Lundholm et al., 2010, 2012)
<i>P. calliantha</i> Lundholm, Moestrup & Hasle 2003		⁺²	+ UNK	Limfjord, and other inner waters, Denmark	(Hasle, 1996)
			UNK	Western Baltic, e.g., Kiel Bay, Germany	(Hasle et al., 1996; Lundholm et al., 2003; Hostyeva et al., 2012)
			UNK	North Atlantic; Norwegian Sea; outer Oslofjord, Skagerrak, Norway	(Churro et al., 2009)
			UNK	Aveiro coastal lagoon, NE Atlantic, Portugal; NE Atlantic, Spain	(Orive et al., 2010; Penna et al., 2007; Lundholm et al., 2012)
<i>P. cuspidata</i> (Hasle) Hasle 1993	<i>Nitzschia cuspidata</i> (Hasle, 1965)	⁺³	UNK	Las Palmas, Grand Canaria, Canary Islands, NE Atlantic	(Lundholm et al., 2003)
			UNK	Aveiro coastal lagoon, NE Atlantic, Portugal	(Churro et al., 2009)
<i>P. delicatissima sensu lato</i>	<i>Nitzschia delicatissima</i> Cleve, 1897 <i>Nitzschia actyodrophila</i> Hasle	+	UNK	Mariager Fjord, inner waters, Denmark	(Skov et al., 1999; Lundholm et al., 2010)
			UNK	Baie de Veys, Normandy, NE Atlantic, France	(Klein et al., 2010; Delegrange et al., 2018)
			UNK	Celtic Sea, south and SE Ireland; Inner Hebrides, West coast, Scotland	(Cusack, 2002; Cusack et al., 2004; (Brown and Bresnan, 2008)
			UNK	Helgoland, southern German Bight; Stonehaven, North Sea, Scotland	(Bresnan et al., 2015)
			UNK	Barents Sea; Norwegian Sea; outer Oslofjord, Skagerrak, Norway	(Hasle 1965; Hasle et al., 1996; Quillfeldt 1996; Hostyeva et al., 2012)
			UNK	Loch Ewe and Firth of Lorne, NW and West coast; Scapa Bay, Orkney Islands; Shetland Islands, Scotland	(Fehling et al., 2006; Bresnan et al., 2017)

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Table 1 (continued)

<i>Pseudo-nitzschia</i> species	Synonyms	Global toxigenicity (AST)	DA toxigenicity in study area	Biogeographical location	References
			UNK	NE Atlantic, Iberia coast, Spain and Portugal	(Fraga et al., 1998; Moita and Vilarinho 1999; Zapata et al., 2011)
			UNK	Baffin Bay, Davis Strait, eastern Arctic Sea margin	(Quillfeldt 1996; Skov et al., 1999)
<i>P. delicatissima sensu stricto</i> (Cleve) Heiden in Heiden & Kolbe 1928 emend (Lundholm et al., 2006)	<i>Nitzschia delicatissima</i> Cleve 1897 <i>Nitzschia actyrophila</i> Hasle	+4	UNK	Inner waters, Denmark	(Lundholm et al., 2006)
			UNK	Aveiro coastal lagoon, NE Atlantic, Portugal	(Lundholm et al., 2006; Churro et al., 2009)
			UNK	Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2013)
			UNK	Baffin Bay, eastern Arctic Sea margin	(Tammilehto et al., 2012)
<i>P. dolorosa</i> Lundholm and Moestrup 2006		UNK	UNK	Aveiro coastal lagoon, NE Atlantic, Portugal	(Churro et al., 2009; Lundholm et al., 2006)
<i>P. fraudulenta</i> (Cleve) Hasle 1993	<i>P. seriata</i> var. <i>fraudulenta</i> (Cleve) Peragallo, <i>Nitzschia fraudulenta</i> Cleve, 1897	+5	UNK	Stonehaven, North Sea, Scotland	(Bresnan et al., 2015; Cook et al., 2022)
			UNK	Inner waters, Denmark	(Lundholm et al., 1994, 2010)
			UNK	Baie de Veys, NE Atlantic, Normandy, France	(Klein et al., 2010; Delegrange et al., 2018)
				English Channel; Helgoland, southern German Bight, North Sea; Skagerrak	(Hasle, 2002)
			UNK	Denmark Strait, Norwegian Sea, Iceland Sea, Skagerrak	(Hasle et al., 1996)
			UNK	Celtic Sea, south and SE Ireland	(Cusack, 2002; Cusack et al., 2004)
				Oslofjord, Skagerrak; Norwegian Sea; Denmark Strait between Iceland and Greenland	(Hasle 1965; Hasle et al., 1996; 2002; Hostyeva et al., 2012)
				Aveiro coastal lagoon, NE Atlantic, Portugal	(Moita and Vilarinho, 1999; Hasle 2002; Churro et al., 2009)
			UNK	Loch Ewe and Firth of Lorne, NW and West coast; Scapa Bay, Orkney Islands; Shetland Islands, Scotland	((Brown and Bresnan, 2008); Fehling et al., 2006; Bresnan et al., 2017)
			UNK	NE Atlantic, Iberia coast, Spain and Portugal	((Brown and Bresnan, 2008)(Míguez et al., 1996); Moita and Vilarinho, 1999; Lundholm et al., 2003; Orive et al., 2010, (Orive et al., 2013); Zapata et al., 2011)
<i>P. galaxiae</i> (Lundholm and Moestrup, 2002)		+6	UNK	Nervion River/Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2010)
<i>P. granii</i> (Hasle) Hasle 1974	<i>Nitzschia granii</i> Hasle	+7	UNK	Norwegian Sea, North Atlantic, Svalbard	(Hasle, 1965; Balzano et al., 2017; Šupraha et al., 2022)
<i>P. hasleana</i> Lundholm 2012		+8	UNK	Nervion River/ Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2010, 2013); Lundholm et al., 2012)
<i>P. heimii</i> Manguin 1957	<i>Nitzschia heimii</i> Manguin	UNK	UNK	Skagerrak	(Hasle et al., 1996)
<i>P. lineola</i> (Cleve) Hasle 1993	<i>Nitzschia lineola</i> Cleve	UNK	UNK	Inner waters, Denmark	(Lundholm et al., 2010)
				Faeroe-Shetland channel, NE Atlantic)	(Hasle, 1965)
<i>P. multiseriata</i> (Hasle) Hasle 1995	<i>P. pungens</i> f. <i>multiseriata</i> (Hasle) Hasle 1993, <i>Nitzschia pungens</i> f. <i>multiseriata</i> (Hasle, 1965)	+9	+	Inner waters, Denmark	(Skov et al., 1999; Lundholm et al., 2010)
			UNK		
			+	Wadden Sea, Netherlands and Germany	(Vrieling et al., 1996; Evans et al., 2004)
			UNK	NE Atlantic, France	(Amzil et al., 2001)
			UNK	Irish Sea; Celtic Sea - East, South and SW Ireland	(Hasle, 2002; Cusack et al., 2004)
			UNK	Helgoland, southern German Bight, North Sea	(Hasle, 2002; Bresnan et al., 2015)
			UNK	Oslofjord, Skagerrak; Norwegian Sea	(Hasle, 1965, 2002; Lange et al., 1992; Hostyeva et al., 2012)
			+	NE Atlantic, Portugal	(Godinho et al., 2018)
			UNK	Scottish waters	(Brown and Bresnan, 2008)
			UNK	NE Atlantic, Spain	Hasle et al., 1996; Hasle, 2002)
			+	Thames estuary, England, UK	(Martin-Jézéquel et al., 2015)
			UNK	Kiel Bight, west Baltic, Germany; English Channel	(Hasle, 2002)

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Table 1 (continued)

<i>Pseudo-nitzschia</i> species	Synonyms	Global toxigenicity (AST)	DA toxigenicity in study area	Biogeographical location	References
			UNK +	English channel	(Hasle, 2002; Percy et al., 2006)
<i>P. multistriata</i> (Takano) Takano 1995	<i>Nitzschia multistriata</i> Takano 1993	+10	UNK	Nervion River/Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2010)
			UNK	Aveiro coastal lagoon, NE Atlantic, Portugal	(Churro et al., 2009)
			UNK	Baie de Veys, NE Atlantic, Normandy, France	(Klein et al., 2010)
<i>P. obtusa</i> (Hasle) (Hasle and Lundholm, 2005)	<i>P. seriata</i> f. <i>obtusa</i> Hasle, <i>Nitzschia seriata</i> f. <i>obtusa</i> (Hasle, 1965)	+11	UNK	Inner waters, Denmark	(Hasle, 1965)
			UNK	Celtic Sea, South and SW Ireland	(Cusack, 2002)
			UNK	Barents Sea (incl. fjord at Svalbard), Norwegian Sea	(Hasle et al., 1996; Hasle and Lundholm, 2005; Quillfeldt, 1996)
			+	Baffin Bay, eastern Arctic Sea margin	(Harðardóttir et al., 2015)
<i>P. plurisecta</i> Orive & Pérez-Aicua 2013		+12	UNK	Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2013)
			+	Ouest Loscolo, Vilaine Estuary, Atlantic France	(Caruana et al., 2019)
			UNK	Stonehaven, North Sea, Scotland	(Cook et al., 2022)
<i>P. pseudodelicatissima</i> (Hasle) Hasle 1993	<i>Nitzschia pseudodelicatissima</i> Hasle 1976, <i>N. delicatula</i> Hasle	+13	UNK	NE Atlantic, Portugal	(Lundholm et al., 2003)
			UNK	Oslofjord, northern Skagerrak	(Hostyeva et al., 2012)
			UNK	Iceland Sea	(Lundholm et al., 2003)
			UNK	NE Atlantic, Spain	(Orive et al., 2010)
			UNK	Celtic Sea, South and SW Ireland	(Cusack et al., 2004)
			UNK	Denmark Strait between Iceland and Greenland	(Hasle 1965; Lundholm et al., 2003)
			UNK	NE Atlantic, Iberia coast, Spain and Portugal	(Moita and Vilarinho 1999; Velo-Suárez et al., 2008)
			UNK	Loch Ewe, NW coast; Scapa Bay, Orkney Islands, Scotland	(Bresnan et al., 2017)
			UNK	Stonehaven, North Sea, Scotland	(Bresnan et al., 2015)
<i>P. pungens</i> (Grunow ex Cleve) Hasle 1993	<i>Nitzschia pungens</i> Grunow ex Cleve 1897	+14	UNK	Inner waters, Denmark	(Lundholm et al., 1994; Lundholm et al., 2003, 2010)
			UNK	Baie de Veys, NE Atlantic, Normandy, France	(Klein et al., 2010; Delegrange et al., 2018)
			UNK	Celtic Sea, South and SW Ireland	(Cusack et al., 2004)
			UNK	English Channel; Dutch coastal waters; Helgoland, southern German Bight, North Sea; Kiel Bight, SW Baltic Sea, Germany	(Hasle, 1972, (Hasle et al., 1996)
			UNK	Stonehaven, North Sea, Scotland; Helgoland; Helgoland, southern German Bight	(Bresnan et al., 2015; Cook et al., 2022)
			UNK	Oslofjord, northern Skagerrak, Norwegian Sea	(Hasle et al., 1996; Hostyeva et al., 2012)
			UNK	NE Atlantic, Portugal	(Hasle, 1995; Moita and Vilarinho, 1999; Lundholm et al., 2006)
			UNK	Inner Hebrides, Forth of Lorne and Loch Ewe, NW and West oas; Scapa Bay, Orkney Islands; Shetland Islands, Scotland	(Brown and Bresnan, 2008; Fehling et al., 2006; Bresnan et al., 2015, 2017)
			UNK	NE Atlantic, Iberia coast, Spain and Portugal	(Hasle, 1995; Moita and Vilarinho 1999; Orive et al., 2010; Zapata et al., 2011; Torres Palenzuela et al., 2019)
			UNK	Skagerrak	(Lange et al., 1992; Hasle 1995; Hasle et al., 1996; Lundholm et al., 2010)
<i>P. pungens</i> var. <i>pungens</i> (Grunow ex Cleve) Hasle 1993		+	UNK	Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2013)
			UNK	Inner waters, Denmark; North Sea; Celtic Sea; NE Atlantic, Spain and France	(Casteleyn et al., 2008, 2010)

(continued on next page)

Table 1 (continued)

<i>Pseudo-nitzschia</i> species	Synonyms	Global toxigenicity (AST)	DA toxigenicity in study area	Biogeographical location	References
<i>P. pungens</i> var. <i>aveirensis</i> Lundholm, Churro, Carreira & Calado 2009		+	UNK	Aveiro coastal lagoon, NE Atlantic, Portugal	(Churro et al., 2009)
			UNK	Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Orive et al., 2013)
<i>P. seriata</i> (Cleve) H. Peragallo in H. & M. Peragallo 1900	<i>Nitzschia seriata</i> Cleve 1883	+ ¹⁵	UNK	Inner waters, Denmark	(Lundholm et al., 1994; Lundholm et al., 2003, 2010)
			UNK	Iceland Sea	(Hasle, 2002)
			UNK	Celtic Sea, South and SW Ireland	(Cusack, 2002)
			UNK	English Channel; Skagerrak; North Sea; Kiel Bight, SW Baltic Sea, Germany	(Hasle et al., 1996)
			UNK	Oslofjord, northern Skagerrak, Norwegian Sea, Barents Sea ¹	(Hasle, 1965, 1972; Hasle et al., 1996; Quillfeldt 1996; Hostyeva et al., 2012)
			+ UNK	Firth of Lorne, Loch Ewe and Inner Hebrides, NW and West coast; Shetland Islands, Scotland	(Fehling et al., 2004, Fehling et al., 2006; Brown & Bresnan 2006; Bresnan et al., 2017)
			UNK	Stonehaven, North Sea, Scotland	(Bresnan et al., 2015; Cook et al., 2002)
			+	Baffin Bay, Davis Strait, eastern Arctic margin	(Quillfeldt, 1996; Hansen et al., 2011)
<i>P. subfraudulenta</i> (Hasle) Hasle 1993	<i>Nitzschia subfraudulenta</i> Hasle 1974	+ ¹⁶	UNK	NE Atlantic, Portugal	(Hasle, 1972)
<i>P. subpacific</i> (Hasle) Hasle 1993	<i>Nitzschia subpacific</i> Hasle 1965	+ ¹⁷	UNK	Aveiro coastal lagoon, NE Atlantic, Portugal	(Lundholm et al., 2003; Churro et al., 2009)
			UNK	Firth of Lorne, west coast, Scotland	(Fehling et al., 2006)
			UNK	Stonehaven, North Sea, Scotland	(Bresnan et al., 2015)
			UNK	Celtic Sea, south and SE Ireland	(Cusack et al., 2004)
			UNK	Nervion River/ Bilbao estuary, Bay of Biscay, NE Atlantic, Spain	(Lundholm et al., 2003; Orive et al., 2010, 2013; Zapata et al., 2011)
<i>P. cf. turgidula</i> (Hustedt) Hasle 1993	<i>Nitzschia turgidula</i> Hustedt 1958	+ ¹⁸	UNK	off Shetland islands, NE Atlantic margin, Scotland	(Hasle, 1965)
				Barents Sea, Davis Strait, eastern Arctic Sea margin, Svalbard	(Quillfeldt, 1996; Šupraha et al., 2022)

¹ Borneo (Teng et al., 2016)² Tunisia and Chile (Alvarez et al., 2009; Sahraoui et al., 2011)³ Washington State, USA (Kociolek et al., 2021; Trainer et al., 2012)⁴ Canada and New Zealand⁵ Gulf of Maine, USA; North West Atlantic, and California, USA (Sison-Mangus et al., 2014)⁶ Gulf of Naples, Italy (cited in Lundholm et al., 2009 onwards; Kociolek et al., 2021; Trainer et al., 2012)⁷ Ocean Station PAPA (OSP; 50° N, 145° W) in eastern subarctic Pacific (Trick et al., 2010)⁸ Tunisia (Haili et al., 2016)⁹ eastern Canada (Bates et al., 1989; Evans et al., 2004); France (Amzil et al., 2001); Japan (Kotaki et al. 1999)¹⁰ Mediterranean Sea, Italy (Amato et al., 2010); Peru (Tenorio et al., 2021)¹¹ Greenland (Hardardottir et al., 2015)¹² Gulf of Maine, USA (Fernandes et al., 2014)¹³ Greece (Moschandreou et al., 2010)¹⁴ New Zealand (MacKenzie et al., 1993; Rhodes et al., 1996); west coast USA (Bates et al., 1998)¹⁵ northern Europe (Denmark, Ireland, Scotland); Canada (Couture et al., 2001; Gallacher et al., 2001; Bates et al., 2002; Lundholm et al., 2005; Hansen et al., 2011)¹⁶ Borneo (Teng et al., 2016)¹⁷ Gulf of Maine, NW Atlantic (Fernandes et al., 2014); Pacific coast, Mexico (Lim et al., 2020; Quijano-Scheggia et al., 2020)¹⁸ New Zealand (Rhodes et al., 1998)

The morphological characteristics were collated from original descriptions (e.g. Hasle, 1965, as well as those in DiatomBase (Kociolek et al., 2023).

Accessed at: <https://www.marinespecies.org/hab/aphia.php?p=taxdetails&id=832130> on 2022-11-26Kociolek, J.P.; Blanco, S.; Coste, M.; Ector, L.; Liu, Y.; Karthick, B.; Kulikovskiy, M.; Lundholm, N.; Ludwig, T.; Potapova, M.; Rimet, F.; Sabbe, K.; Sala, S.; Sar, E., Taylor, J., Van de Vijver, B., Wetzel, C.E., Williams, D.M., Witkowski, A., Witkowski, J., 2022. DiatomBase. Accessed through: World Register of Marine Species at: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=246609> on 2022-11-26Taxonomic citation on HABs taxon details were collated from Kociolek et al., 2021 (Kociolek, J.P., Blanco, S., Coste, M., Ector, L., Liu, Y., Karthick, B., Kulikovskiy, M., Lundholm, N., Ludwig, T., Potapova, M., Rimet, F., Sabbe, K., Sala, S., Sar, E., Taylor, J., Van de Vijver, B., Wetzel, C.E., Williams, D.M., Witkowski, A., Witkowski, J., 2021. DiatomBase. Accessed through: Moestrup, Ø., Akselmann-Cardella, R., Churro, C., Fraga, S., Hoppenrath, M., Iwataki, M., Larsen, J., Lundholm, N., Zingone, A. (Eds.), 2009 onwards. IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae at: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=246605> on 2022-11-26 Kudela, R. M., 2013. Host-specific adaptation governs the interaction of the marine diatom, *Pseudo-nitzschia* and their microbiota. The ISME Journal 8(1): 63–76.

The thematic species database citation were collated from Lundholm et al. (2009) and onwards (Lundholm, N., Churro, C., Fraga, S., Hoppenrath, M., Iwataki, M., Larsen, J., Mertens, K., Moestrup, Ø., Zingone, A. (Eds.), 2009 onwards. IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. *Pseudo-nitzschia australis* Frenguelli, 1939. Accessed at: <https://www.marinespecies.org/hab/aphia.php?p=taxdetails&id=246604> on 2022-11-27)

identified based on light- (LM) and electron microscopy (EM) included *P. delicatissima*, *P. fraudulenta*, *P. heimii*, *P. multiseriata*, *P. pseudodelicatissima*, *P. pungens*, and *P. seriata* (Hasle et al., 1996), although *P. pseudodelicatissima* and *P. delicatissima* have been subsequently divided into several species (e.g. Lundholm et al., 2003; 2006, Quijano-Scheggia et al., 2009, Orive et al., 2013).

Taxonomic identifications in the historical literature for northern Europe are often difficult to interpret in a modern context because of frequent nomenclatural revisions at the genus and species level, inconsistencies in the morphological characteristics used for identification, and ongoing reclassification based upon molecular criteria in northern European and NE Atlantic waters (Bresnan et al., 2021; Karlsson et al., 2021). In northern Europe and adjacent Atlantic marginal seas, *P. australis*, *P. calliantha*, *P. delicatissima*, *P. fraudulenta*, *P. heimii*, *P. multiseriata*, *P. pseudodelicatissima*, *P. pungens* and *P. seriata* are the most common and abundant species as currently defined from morphological and molecular criteria (details shown in Table 1). A few examples of the

morphological diversity found among *Pseudo-nitzschia* specimens by LM and EM are illustrated (Fig. 2). Molecular phylogenetic analysis of selected *Pseudo-nitzschia* species found in northern European and eastern Arctic gateway waters (Table 1) was conducted to infer evolutionary history of these species. This phylogeny is presented in a Maximum Likelihood tree based on the General Time Reversible model (Nei and Kumar, 2000), following evolutionary analyses conducted in MEGA7 (Kumar et al., 2016) (Fig. 3).

Although there is some biogeographical pattern in the distribution of certain *Pseudo-nitzschia* species, perhaps related to temperature regime, the distinctions are not always clear cut and are subject to taxonomic inconsistencies. Some species, such as *P. arctica* Percopo & Sarno 2016, *P. granii* (Hasle) Hasle 1974, *P. heimii* Manguin 1957, *P. obtusa* (Hasle), Lundholm 2005, and *P. seriata* (Cleve) H. Peragallo in H. & M. Peragallo 1900, are found mainly in the colder waters of the region (Table 1), whereas others, including *P. galaxiae* Lundholm and Moestrup 2002 and *P. multistriata* (Takano) Takano 1995, are confined mainly to southern

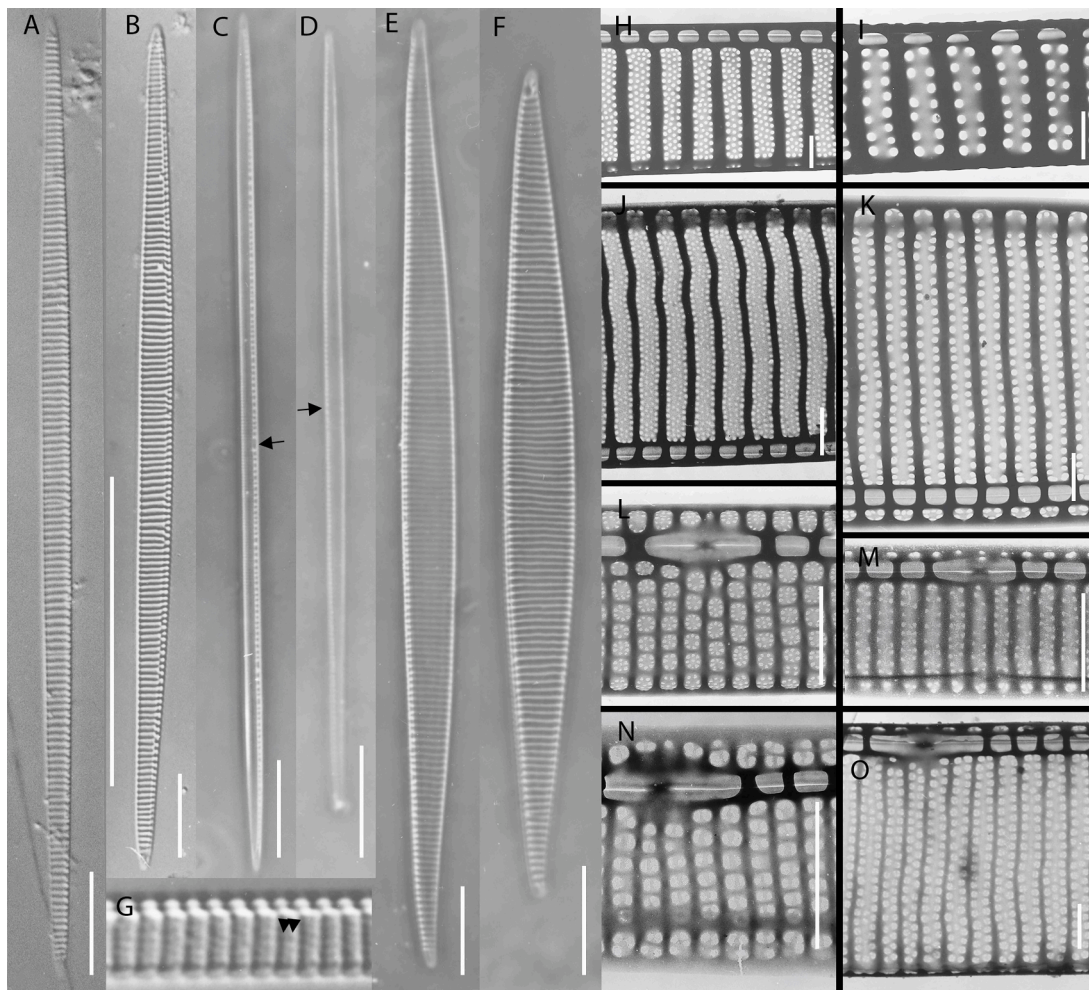


Fig. 2. Light micrographs (LM) (A–G) and electron micrographs (EM) of the most common *Pseudo-nitzschia* species from the northern European study region. Two species similar in LM: (A) *P. pungens*, (B) *P. multiseriata*. Note presence of larger central interspace (arrows), and more or less easily seen fibulae in: (C) *P. calliantha*, (D) *P. delicatissima*. Two species similar in LM: (E) *P. seriata*, (F) *P. australis*. (G) Details of *P. pungens* valve showing the presence of two rows of poroids between the interstriae (arrowheads) – only discernable in some specimens. EM differences of the two species: (H) *P. multiseriata*, (I) *P. pungens*. EM differences of the two species: (J) *P. seriata*, (K) *P. australis*. Species with 1 row of complex poroids: (L): *P. calliantha*. (M) Species with two rows of simple poroids: *P. delicatissima*, (N) Species similar to *P. calliantha* except having poroids divided in only two sectors: *P. pseudodelicatissima*, (O) Species with two-three rows of complex poroids: *P. fraudulenta*. The specimens originate from: Danish waters (A–E, G–J, L, M, O); Western Pacific (F, K); N: Denmark Strait.

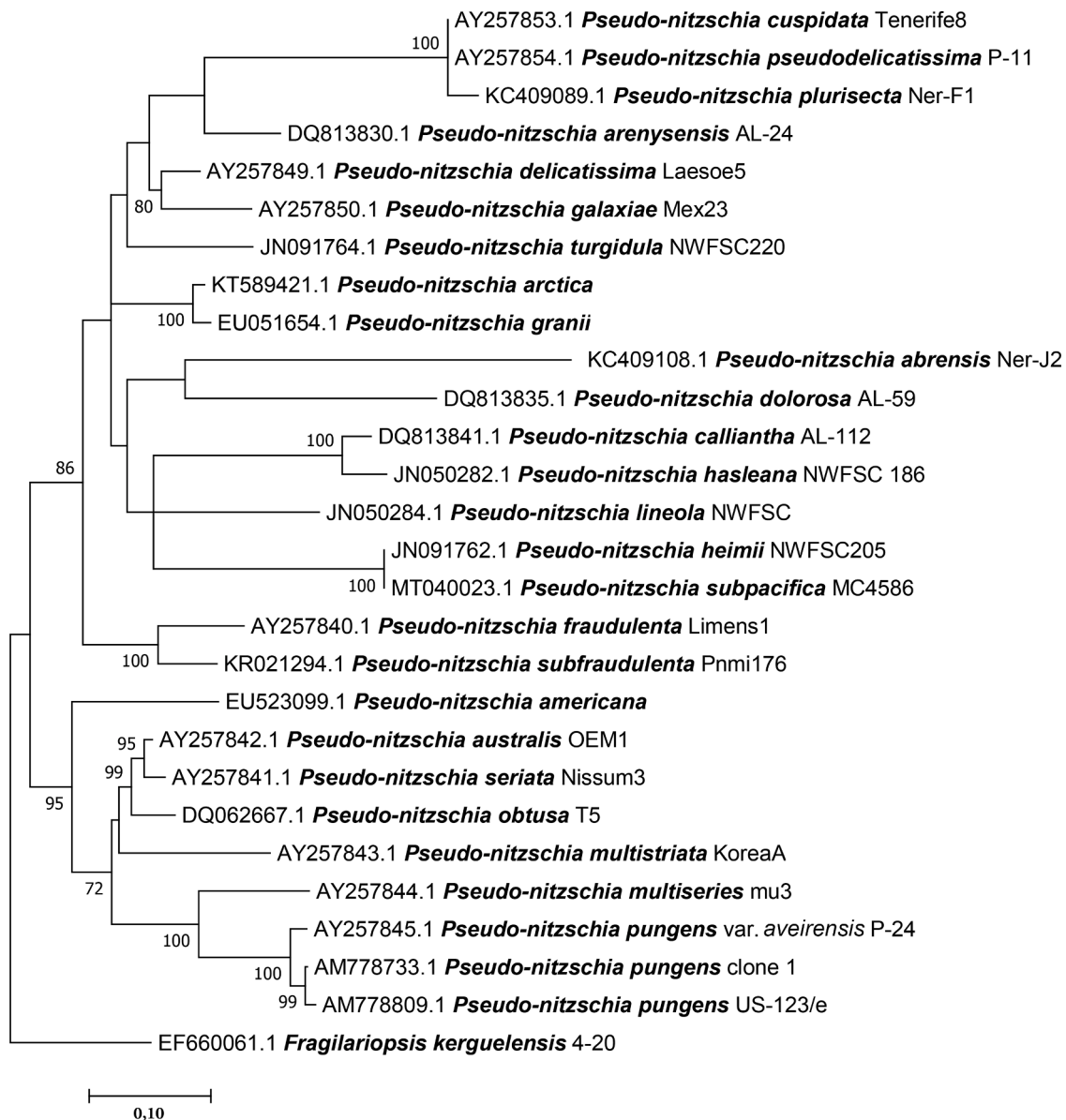


Fig. 3. Molecular phylogenetic analysis of selected *Pseudo-nitzschia* species found in northern European and eastern Arctic gateway waters. The selected 23 reliable internal transcript spacer (ITS) nucleotide sequences of the rRNA operon of all known *Pseudo-nitzschia* species from the study region, with the pennate diatom *Fragilariopsis kerguelensis* as outgroup, were aligned with MAFFT applying the L-INSI settings. The corresponding tree with the highest log likelihood (−5006.83) is shown with the percentage of 1000 bootstrap replicates next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites for 5 categories (+G, parameter = 0.6918). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

European waters, including the Mediterranean Sea. Within the region, the recently described *P. arctica* has only been recorded from Greenlandic waters (Percopo et al., 2016). Some species apparently favoring cold water regimes, such as *P. granii* (Hasle 1974 (Hasle, 1964) and *P. obtusa*, have been found in Norwegian coastal waters. The species *P. americana* has been seen at several locations of northern Europe, ranging from Oslofjorden (Hostyeva et al., 2012) to Helgoland in the southern North Sea (Bresnan et al., 2015). In Danish waters thus far it has been found only in the sediment from Mariager Fjord along the Skagerrak (Lundholm et al., 2010).

Metabarcoding data from CoLiME oceanographic cruises around the Svalbard archipelago have confirmed the presence of apparently cold-water populations of several *Pseudo-nitzschia* taxa, including potentially toxigenic species: *P. seriata*, *P. calliantha*, *P. delicatissima* and *P. multiseries* (Fig. 4). This represents the first reports of these species in

this Arctic gateway archipelago confirmed with molecular signatures. Whether these populations are endemic or reflect recent introduction and range expansion by advection, e.g., from the northern coast of Norway, remains to be established. Prevailing current patterns along the Norwegian coast (shown in Fig. 8, John et al., 2022) indicate that the advective transport hypothesis is plausible, but the distance (>950 km) is rather daunting.

Pseudo-nitzschia species are distributed widely in the Celtic Sea and along the SW coast. In coastal waters of Ireland, *P. pungens*, *P. multiseries*, *P. fraudulenta*, *P. australis*, *P. delicatissima* and *P. pseudodelicatissima* are frequently present in the SW region and the Celtic Sea. Two other *Pseudo-nitzschia* species, provisionally identified as *P. seriata* cf. *seriata* and *P. seriata* cf. *obtusa* and later confirmed as *P. seriata* or *P. obtusa* by molecular methods, as well as *P. subpacific*, have also been observed at sites in SW Ireland though less often (Cusack et al., 2004). The presence

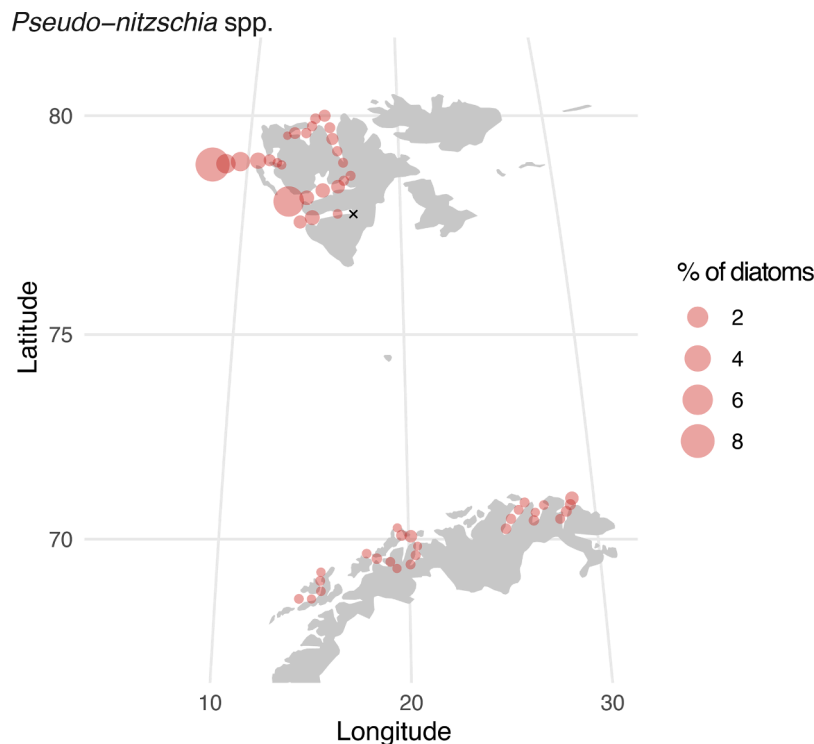


Fig. 4. Relative abundance (% total diatom reads) of *Pseudo-nitzschia* spp. molecular signatures as amplified sequence variants (ASVs) from plankton sampled during oceanographic expeditions aboard *RV Heincke* in the Svalbard archipelago in 2017 (HE492: [10.1594/PANGAEA.881306](https://doi.org/10.1594/PANGAEA.881306); GF BIO: PRJEB49358; [Supraha et al., 2022](#)) and northern Norway in 2019 (HE533: [10.1594/PANGAEA.903511](https://doi.org/10.1594/PANGAEA.903511); GF BIO: PRJEB50059). Metabarcoding data were derived from Illumina sequencing of V4 region of the 18S rRNA gene. The number of *Pseudo-nitzschia* ASVs was normalized against the total number of diatom ASVs. Red dots indicate relative ASV abundance at stations sampled and the black “x” symbol denotes stations where *Pseudo-nitzschia* ASVs were not found.

of *P. fraudulenta*, *P. delicatissima* and *P. pseudodelicatissima* has been frequently noted in shelf waters off the west coast. Indeed, more recently, *P. australis* has also been detected in these waters by a qPCR method applied in the national monitoring program (NMP).

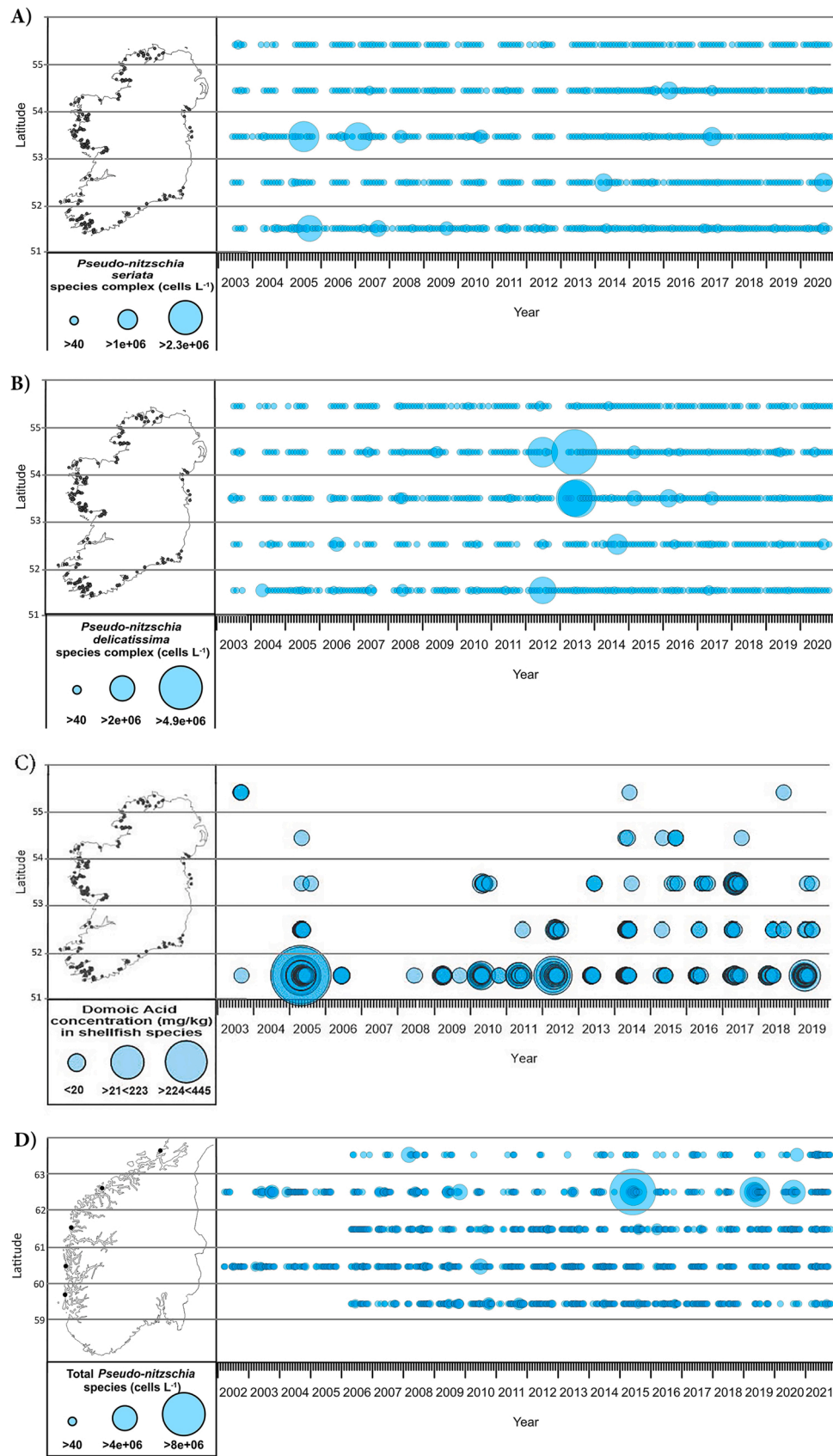
The Irish coastal current is a recognized transport pathway for phytoplankton populations from the south and SW coasts, and it is therefore reasonable to assume transport along the west coast of Ireland. Based on multidecadal observations from the NMP and oceanographic studies, the *P. delicatissima* species complex (size group of cells with a valve width $<3\ \mu\text{m}$) tends to comprise mainly inshore blooms, whereas members of the *P. seriata* complex (size group of cells with a valve width $>3\ \mu\text{m}$) may form high cell density thin-layers found in offshore waters. The *P. delicatissima* species complex is more prevalent inshore in tidally mixed waters and sometimes confined to a specific depth, e.g., *P. pseudodelicatissima sensu lato* has been observed to form a thin-layer in Galway Bay rather than in the open ocean. In shelf waters further offshore, the *P. delicatissima* species complex has been mostly recorded as a minor component of the *Pseudo-nitzschia* assemblage in summer ([Cusack, 2002](#)). However, in one oceanographic transect, when crossing the frontal zone from the Irish Sea into the Celtic Sea, the *Pseudo-nitzschia* population completely shifted from the *P. delicatissima* complex in the tidally mixed waters of the Irish Sea to *P. seriata sensu lato* in thermally well stratified waters of the Celtic Sea in summer.

A multi-decadal time-series plot (2003–2020) of the spatial distribution and cell abundance of *P. seriata* species complex along a latitudinal gradient (51–55.5 °N) of inshore stations along the coast of Ireland ([Fig. 5A](#)) displays no obvious trend. The massive *Pseudo-nitzschia* bloom in 2005 is reflected on both West and SW coast, but the similarly large bloom from the West coast (53–54 °N) in mid-2006 is shifted later in 2007 in the southern region. Similarly, the time-series for the *P. delicatissima* species complex ([Fig. 5B](#)) also shows no apparent latitudinal trend within inshore coastal zones of Ireland, including for mean annual cell abundances. In fact, the measured DA levels in

shellfish (mg kg^{-1}) over the time-series ([Fig. 5C](#)) are poorly correlated with the *Pseudo-nitzschia* cell abundances, with exception of the high DA levels in 2005 and 2012 along the SW coast and Celtic Sea. In these two cases, DA levels in shellfish match the high magnitude of the bloom of *P. seriata* species complex for 2005 and *P. delicatissima* species complex for 2012, respectively. This is not to imply that the typical lack of correlation between specific *Pseudo-nitzschia* blooms and AST events reflects a cryptic DA source, but rather that the sampling regime fails to reflect the coupling of toxin and bloom dynamics.

Pseudo-nitzschia australis is the species most often associated with AST events along the northeastern Atlantic and marginal seas (cited in [Bresnan et al., 2021](#); [Karlson et al., 2021](#)). This species is a confirmed DA producer associated with closures of shellfish harvesting in coastal waters of Atlantic France, Iberia, Scotland, and Ireland, including offshore scallops in the Celtic Sea. *Pseudo-nitzschia seriata* has also caused confirmed AST events in overlapping but slightly colder waters, including the Scottish west coast ([Fehling et al., 2006](#); [Bresnan et al., 2017](#)) and eastern Jutland of Denmark ([Lundholm et al. 2005](#); [Olesen et al., 2020](#)).

Pseudo-nitzschia species are in common occurrence along the Norwegian Sea coast, often in high cell abundance, but are only infrequently associated with AST events. This may be partly due to the fact that *Pseudo-nitzschia* monitoring is conducted at only a few sites at broad geographical distances, and rather infrequently (often only monthly), and shellfish harvest and production is confined a few local operations. With noticeable time gaps, the long-term time series data on *Pseudo-nitzschia* cell abundance along the Norwegian coast ([Fig. 5D](#)) fails to display any significant trends from 2003 to 2020; anomalously high cell abundances were recorded in 2015, 2019, 2020 (at 62–63 °N) but this cannot be interpreted to represent a latitudinal gradient shift. The pattern of *Pseudo-nitzschia* cell abundance in coastal waters of Sweden over the past two decades (2000–2021) does not show any significant trends ([Fig. 6A and B](#)); divided regionally into the Baltic Proper and



(caption on next page)

Fig. 5. Temporal and spatial distribution of maximum *Pseudo-nitzschia* cell abundances displayed on a latitudinal gradient (per °N). Bubble plots represent the highest maximum cell densities (>40 cells L^{-1}) for A) *P. seriata* and B) *P. delicatissima* species complexes, and C) DA levels ($mg\ kg^{-1}$) in shellfish species recorded per month in aquaculture production areas monitored in the Irish National Monitoring Program from 2003 to 2021; D) Temporal and spatial distribution of maximum *Pseudo-nitzschia* cell abundances displayed on a latitudinal gradient (per °N) along the Norwegian coast. Bubble plots represent the highest maximum cell densities (>40 cells L^{-1}) for combined *Pseudo-nitzschia* species recorded per month in aquaculture production areas monitored in the Norwegian National Monitoring Program from 2003 to 2021.

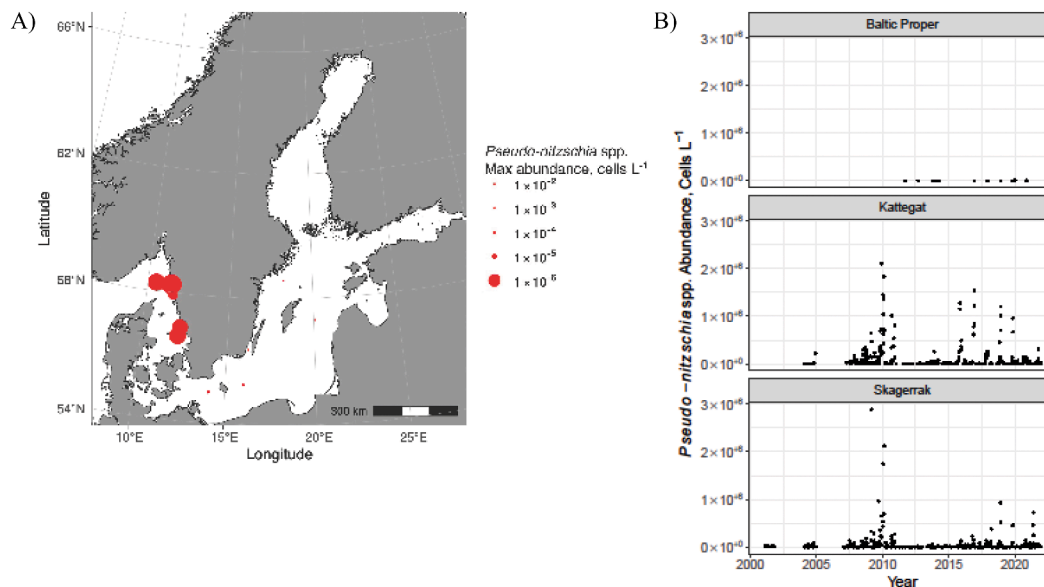


Fig. 6. A) general map and B) sub-regional time-series for the Baltic Sea, Kattegat and Skagerrak of observations of *Pseudo-nitzschia* spp. identified and counted by the Utermöhl microscopic method in the Swedish national marine monitoring program during 2001–2021. Data points representing zeroes, i.e., sample collected but no *Pseudo-nitzschia* observed were omitted in the graphs.

Kattegat/Skagerrak, the data indicate consistently low cell abundances in the Baltic Proper but with frequent presence and occasional high cell density blooms (e.g., in 2010) in the Kattegat/Skagerrak.

The findings of *P. pseudodelicatissima* in the northwestern European region before 2003 are now mainly assigned to *P. calliantha* (Lundholm et al., 2003), but *P. abrensis*, *P. cf. cuspidata*, *P. hasleana*, *P. lineola*, *P. plurisecta* and *P. cf. plurisecta* have also been reported (Lundholm et al., 2010; 2012; Hostyeva et al., 2012, Cook et al., 2022). Confirmed findings of *P. delicatissima*-like species are challenging to identify and require molecular verification, because of the existence of several morphologically similar species, as well as the cryptic *P. arenysensis* (Lundholm et al., 2006; Quijano-Scheggia et al., 2009). In northern and western European waters, these *P. delicatissima*-like taxa have, apart from *P. delicatissima*, also been identified as *P. arenysensis* or *P. dolorosa* (Orive et al., 2013).

3.2. Biogenic origin of AST and associated *Pseudo-nitzschia* toxigenicity

Pseudo-nitzschia species occur frequently and persistently in the microplankton, often forming toxigenic blooms that may cause closures of shellfish harvesting due to AST levels above the regulatory limit (20 mg DA kg⁻¹ shellfish flesh). Nevertheless, confirmed linkages between blooms of particular *Pseudo-nitzschia* species and AST events in northern Europe have proven difficult to establish and resist pattern generalization because of often non-comparable spatio-temporal sampling and incomplete decadal time-series data. It is highly misleading, therefore, to divide *Pseudo-nitzschia* blooms or species into strict “toxic” vs “non-toxic” categories for toxin risk assessment, based on evidence from autecological studies conducted on a few cultured strains or extrapolated from cellular DA measurements from natural populations found elsewhere. A further caveat is the potential effect of extrinsic (biological, chemical and physical) factors for DA production by local populations.

Nevertheless, experience with local and regional strains and blooms can provide a rough guide to expected toxigenicity under prevailing environmental conditions. Of the approximately two dozen *Pseudo-nitzschia* species recorded from NW European and eastern Arctic waters, 20 have been recorded as toxigenic somewhere in the world (Table 1, and cited in Lundholm, 2023), but certain strains of only five species, *P. australis*, *P. calliantha*, *P. multiseriata*, *P. pungens* and *P. seriata*, have been proven toxigenic from the region. Potentially toxigenic *Nitzschia* species have also been reported from the North Atlantic, but compared to planktonic *Pseudo-nitzschia*, they are likely a negligible contributor of DA to AST events.

Again the caveat that not all species identified from field studies have been assayed or analyzed for DA production must be considered. Strains of about a dozen species, including *P. australis*, *P. calliantha*, and *P. pungens* have been found to be non-toxicogenic, or below detection level for cellular DA, illustrating the presence of both nominally non-toxic and toxic strains in the region. The reported DA toxin content of *Pseudonitzschia* cells collected from natural bloom populations or isolated into clonal culture from northern Europe varies dramatically from undetectable to global comparatively high levels (Lelong et al., 2012; Trainer et al., 2012; Bates et al., 2018). Toxin levels of *P. seriata* blooms are generally high in Danish waters (Lundholm et al., 1994), but species such as *P. calliantha* (cited as *P. pseudodelicatissima*; Lundholm et al., 2003; Lundholm et al., 2006) and *P. delicatissima* are non-toxicogenic or only slightly toxic (Lundholm et al., 1997). In any case, the DA toxin content variation among species and infra-specific strains of *Pseudonitzschia* in this region cannot be readily distinguished from populations elsewhere in the world.

Pseudo-nitzschia australis is the species most often associated with AST events along the northeastern Atlantic and marginal sea coasts, as a confirmed DA producer associated with closures of shellfish harvesting in the Galician rias of Iberia, the west coast of Scotland, the NE Atlantic

margin of France and the Celtic Sea in SW Ireland (cited in Bresnan et al., 2021). In Ireland, *P. australis* is the main culprit for AST events in long-line cultured blue mussels and is strongly suspected but not proven as the cause of chronically high DA levels in scallops (Clarke and Gil-martin, 2020). The offshore shellfish harvest in the Celtic Sea, the southern Irish Sea, and the western approaches to England and Wales is primarily of king scallops (*Pecten maximus*) collected by dredging (Tully et al., 2006). The source of DA in these offshore populations has not been confirmed because of complex field sampling logistics and lack of plankton monitoring in the offshore area. A confirmed DA-producing species, *P. seriata*, is mainly responsible for AST events in overlapping but slightly colder waters, such as the west coast of Scotland (Gallacher et al., 2001) and the eastern Jutland coast of Denmark (Olesen et al., 2020).

Multidecadal databases containing records of *Pseudo-nitzschia* occurrence and AST events (e.g., HAEDAT <http://hae.dat.iode.org/>) and maps derived from these data (Fig. 7A and B) apparently show high incidence of AST events along the eastern North Atlantic margin (Bresnan et al., 2021), i.e. west coasts of Ireland and Scotland, NE Scotland and the NE Atlantic coast of France, with fewer toxic events in the Skagerrak-Kattegat and on the Norwegian Sea coast (Karlson et al., 2021). This impression is biased, however, by the fact that the events plots do not represent the magnitude or frequency of *Pseudo-nitzschia* blooms, but rather events defined as “harmful”, comprising diverse factors such as shellfish toxin closures, human illnesses, marine faunal mortalities and even water discoloration. The events data in HAEDAT are heavily weighted (99.9% of total) towards areas with aquaculture activities with reports of closures of shellfish harvesting or offshore benthic populations (typically of scallops) due to high DA levels; there is only one recorded event of human illness from ASTs in shellfish from Europe. In most cases, the HAEDAT AST events are recorded for AST

levels above regulatory limits and should not be interpreted to infer *Pseudo-nitzschia* cell abundance or bloom distribution patterns. There are also inconsistencies in AST events reported by national monitoring programs for different target species. Regional distribution frequencies of AST events caused by *Pseudo-nitzschia* derived from HAEDAT do not reflect the substantial differences in reporting events among countries and regional seas. For example, in Norway, AST levels in shellfish determined directly for the aquaculture industry tend to indicate much higher levels in scallops (*Pecten maximus*) than mussels, but these data are not contributed to HAEDAT, which is dominated by reports from monitoring AST events in mussels. Changes in the monitoring of AST levels in scallops from the Celtic Sea and North Sea initiated in 2005, argues for caution in comparing regional toxicity data sets even within Europe. The long retention period for DA in *P. maximus* (at least several months) particularly in cold deep benthos (Blanco et al., 2002; Bresnan et al., 2017) constitutes an enhanced DA risk for exploitation of offshore bivalve populations as seafood, and complicates DA toxin monitoring and *Pseudo-nitzschia* bloom surveys. Furthermore, the inclusion of multiple reports of different shellfish species affected by the same *Pseudo-nitzschia* blooms in the Bay of Biscay, and the fact that in France scallop harvesting areas may be closed if AST levels in gonadal tissues are above the regulatory limit may contribute to an apparent inflation of AST events. In contrast, in many other European countries, scallops are shucked and only the low-DA-retaining adductor muscle is marketed, thereby creating less reportable AST events.

The first documented toxigenic bloom of *Pseudo-nitzschia* in Scandinavia linked to DA levels above the regulatory level in shellfish happened in 2005, in the inner coastal waters of Denmark and the Kattegat (Lundholm et al., 2005). In Ireland, the first report of DA levels exceeding the regulatory limit in blue mussels grown on longlines was not until spring 2005 off SW Ireland, whereas high DA levels have been

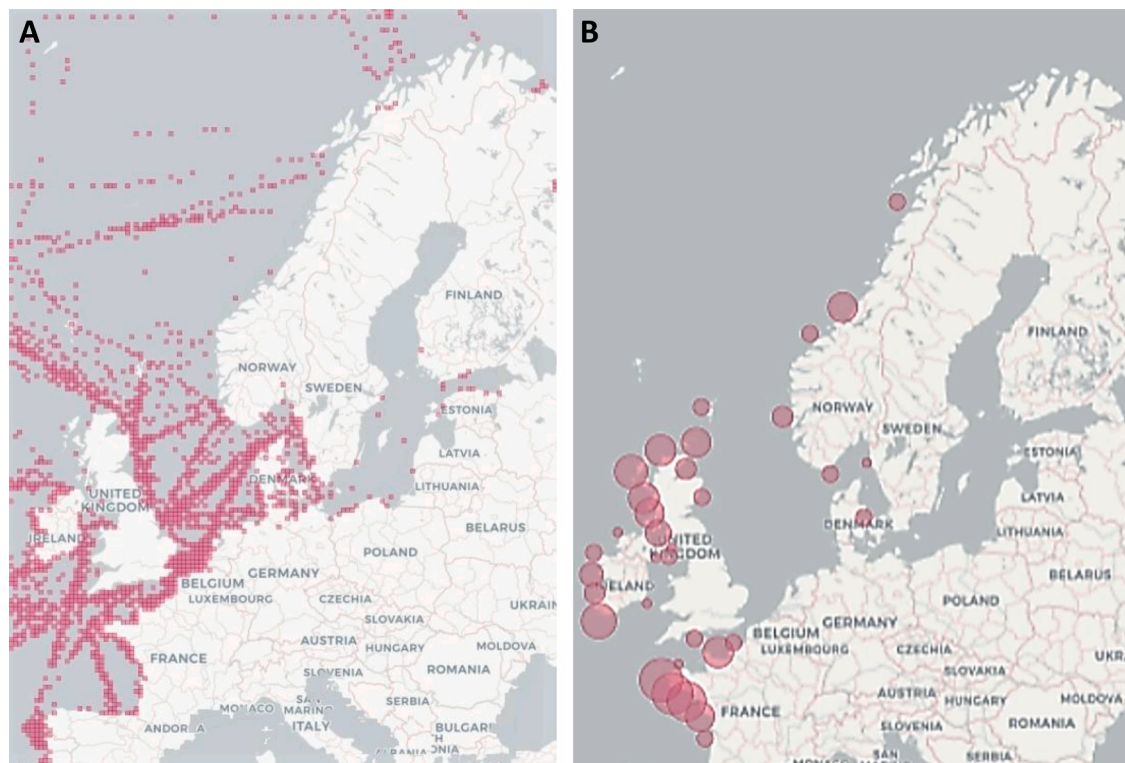


Fig. 7. A,B Geographical distribution of A) all *Pseudo-nitzschia* species (presence) and B) amnesic shellfish toxin (AST) events recorded in the northeastern Atlantic region up to 2020. The *Pseudo-nitzschia* map is derived from HAIS and includes data sets from survey, national monitoring, research programs and HAEDAT stored and retrievable from OBIS (<https://obis.org>) of IOC-UNESCO. *Pseudo-nitzschia* species records outside of the northern CoCLIME Study Area (e.g., Mediterranean region and Black Sea) are not displayed. AST events distribution was extracted from the Harmful Algal Event Database (HAEDAT) accessed via <http://ipt.iobis.org/haab/resource?r=haedat>. Absence of data points for the Baltic, Mediterranean and Black Seas indicates that no AST events were recorded in HAEDAT from these areas. HAEDAT cannot account for lack of monitoring effort or unreported events.

recorded in offshore scallop fisheries since late 1999. In SW Ireland, AST events have occurred nearly annually in spring when *P. australis* is present, but only a small number of production area closures have occurred on the west coast, affecting nearshore blue mussels (*Mytilus edulis*), Pacific oysters (*Magallana* (= *Crassostrea*) *gigas*) and king scallops (*Pecten maximus*) (Clarke and Gilmartin, 2020).

After correction for the above mentioned bias in the collective AST databases the lack of tight correlation between *Pseudo-nitzschia* blooms and AST events could be explained by several but not mutually exclusive hypotheses: i) the blooms are usually non-toxicogenic, yield extremely low DA cell content and/or are only conditionally toxicogenic (and the induction mechanisms are not often triggered); ii) the uptake of DA from toxicogenic blooms is closely matched by high detoxification rates; or iii) the spatio-temporal regime for sampling of phytoplankton and toxin monitoring in shellfish are not appropriately coordinated or monitored for toxin content simultaneously in different ecological compartments. With notable regional exceptions, such as in Brittany, France and in Galicia where shellfish aquaculture is widely practiced, reported AST events are rather infrequent in northern Europe, despite extensive EU-mandated monitoring of DA levels in shellfish. These AST events (cited in Bresnan et al., 2021; Karlson et al., 2021), remain uncommon and often short-lived events.

High AST levels in shellfish cannot simply serve as a qualitative or quantitative index of prior *Pseudo-nitzschia* prevalence or cell abundance, but rough hindcasting of the cell density and DA toxicity of the causative *Pseudo-nitzschia* blooms is theoretically possible from shellfish toxin data (Silvert and Subba Rao, 1992). Reverse modelling of toxin dynamics, however, requires detailed knowledge of the specific uptake and retention characteristics. These can vary greatly among species, e.g. of mussels versus scallops (Scarratt et al., 1991; Blanco et al., 2006), and perhaps also biogeographically, depending upon genetic factors and environmental conditions. Since DA depurates from mussels relatively quickly compared with scallops, it is very likely that past AST events were simply overlooked. Persistent AST contamination of the king scallop (*Pecten maximus*) has frequently been recorded in northern European harvest areas (Gallacher et al., 2001; Bogan et al., 2007; Husson et al., 2016; Rowland-Pilgrim et al., 2019) because this species retains DA for an extraordinarily long time (at least several months), particularly in cold deep sediments (Blanco et al., 2002; Bresnan et al., 2017). Further bias in attempting to infer toxin time-series or bloom occurrences is reflected in the changes to sampling frequency and monitoring strategies for *Pseudo-nitzschia* blooms and accumulated DA levels in shellfish in various European countries, particularly within the last decade (Bresnan et al., 2021). Although *Pseudo-nitzschia* blooms at high cell densities are the only plausible source of DA contamination in shellfish in northern Europe, AST event databases such as HAEDAT or maps of event frequencies (e.g., Fig. 7B) cannot be simply extrapolated to infer the magnitude, persistence, frequency or toxigenicity of *Pseudo-nitzschia* blooms.

4. Environmental factors and climate-change as drivers of *Pseudo-nitzschia* bloom dynamics

The genus *Pseudo-nitzschia* is globally distributed from polar latitudes to the tropics, with some species appearing cosmopolitan whereas others are restricted to defined temperature regimes. It is tempting, but misleading, to define species-specific ecological niches based on changing ocean temperature scenarios or on the basis of autecological culture experiments on a few strains of selected species. Global ocean models parameterized for temperature or salinity changes alone will not yield reliable forecasting scenarios for climate change effects at the genus level, much less for individual species or ecotypes. In fact, other than DA production by a few species, there is not much ecophysiological basis for discriminating the *Pseudo-nitzschia* ecological niche from that of other chain-forming pennate diatoms. Yet such attempts are still reasonable at the species and population level and have achieved some

success at the appropriate spatio-temporal scale.

Oceanographic and meteorological conditions primarily related to stratification often determine the initiation and development of *Pseudo-nitzschia* blooms, whereas distribution from offshore to inshore locations and longshore transport of the bloom generally depends upon advective processes. Globally, different regional circulation patterns and cross shelf transport pathways determine how *Pseudo-nitzschia* blooms arrive from shelf waters to inshore coastal sites. Classic seasonal upwelling systems include the Iberian coast in the North Atlantic, the Benguela off SW Africa and the Pacific coast of North America from California to Alaska. The basic mechanisms of *Pseudo-nitzschia* bloom formation offshore, i.e. upwelling of nutrient-rich cold bottom water followed by stratification, are similar to those found in the Benguela and off the NE Pacific coast; in northern Europe, the generally weaker and inconsistent upwelling/relaxation tends to yield more diffuse and lower magnitude blooms.

In SW Ireland, *Pseudo-nitzschia* blooms are also associated with nutrient-rich cold-water upwelling events but the delivery into coastal embayments is somewhat different; in this case, a wind-driven two-way oscillatory flow advects shelf water at depth into the bays (Cusack et al., 2016), which then serve as bloom incubator basins for further growth.

Pseudo-nitzschia bloom retention and transport are dependent on complex physical ocean dynamics (e.g., density driven currents, upwelling/downwelling events driven by local wind conditions, etc.). An example of the dynamics arising from coastal upwelling and leading to onshore transport of *Pseudo-nitzschia* blooms in Ireland is illustrated in Fig. 8A with comparison for dynamics in other global regions (Fig. 8B). Changes in wind direction drive the flow of water into and out of the coastal embayment, such as Bantry Bay in the SW. Shelf-residing *Pseudo-nitzschia* blooms are advected shoreward when embayment water leaves at the surface and shelf water enters the bay at depth. Wind driven two-layered exchange events are responsible for the sudden appearance of *Pseudo-nitzschia* blooms in coastal embayments such as the bays of SW Ireland (Cusack et al., 2015; 2016). During spring, environmental conditions reflect the interlude between the well mixed waters associated with the spring bloom and the deep seasonal stratification that materializes in summer (Cusack, 2002; Cusack et al., 2016). This stratification pattern is partially reflected in the multi-year seasonal distribution of predominant *Pseudo-nitzschia* species (some of which are non-toxicogenic) identified from vertical phytoplankton net hauls from stations off the south, west and SW coasts of Ireland (Fig. 9). This flags the fact that the ecology and dynamics of *Pseudo-nitzschia* blooms in northern Europe is not exclusively determined by classic “bottom up” hydrodynamic processes following clearly defined seasonal patterns.

Strong upwelling conditions created by surface wind stress, e.g., along the Iberian Peninsula, bring nutrient-rich waters from the deep and have the potential to seed populations with vegetative cells or resting stages, as well as drive growth and bloom expansion (Smayda and Trainer, 2010). Under upwelling circumstances along the North Atlantic coast of Portugal, increases in *Pseudo-nitzschia* cell densities within the diatom assemblage have been observed less than a week after an upwelling pulse (Palma et al., 2010). Similarly, in Galicia, Spain, upwelling events can promote the formation of thin layers of *Pseudo-nitzschia* cells when the pycnocline shoals, providing an environment with appropriate nutrient concentrations and light intensity for rapid cell growth (Velo-Suárez et al., 2008). These thin layers can be also be displaced downwards during downwelling thereby introducing toxicogenic cells to the benthos of rias (Velo-Suárez et al., 2008). The appropriate regime of nutrients and light availability favors development of *Pseudo-nitzschia* blooms, which are then transported via diverse physical transport mechanisms to aquaculture areas along the coastline, causing AST events.

Advection of *Pseudo-nitzschia* blooms into coastal embayments and fjords provides a semi-enclosed system within which the fate of these blooms is at least partially determined by biological (“top down”) processes and expression of genetic mechanisms. Many and perhaps all

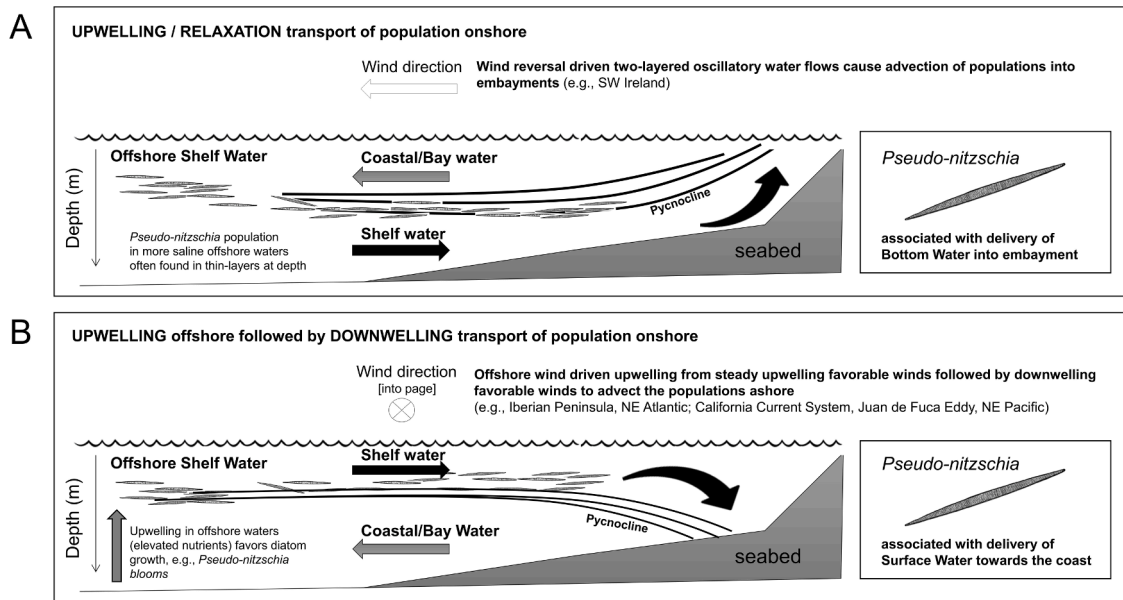


Fig. 8. A,B Simple conceptual model of the offshore–onshore wind-driven advection of *Pseudo-nitzschia* blooms (modified version of Cusack et al., 2016 for SW Ireland embayments): A) changes in wind direction drive water flows into and out of the embayments, e.g., in SW Ireland. The advection of shelf residing *Pseudo-nitzschia* blooms shoreward when bay water leaves on the surface and shelf water enters at depth (referred to as a “local upwelling event”); B) *Pseudo-nitzschia* populations develop in nutrient-rich upwelled ocean water and are delivered via surface waters to the coast during periods of relaxed upwelling or downwelling-favorable winds, e.g. in the NE Pacific.

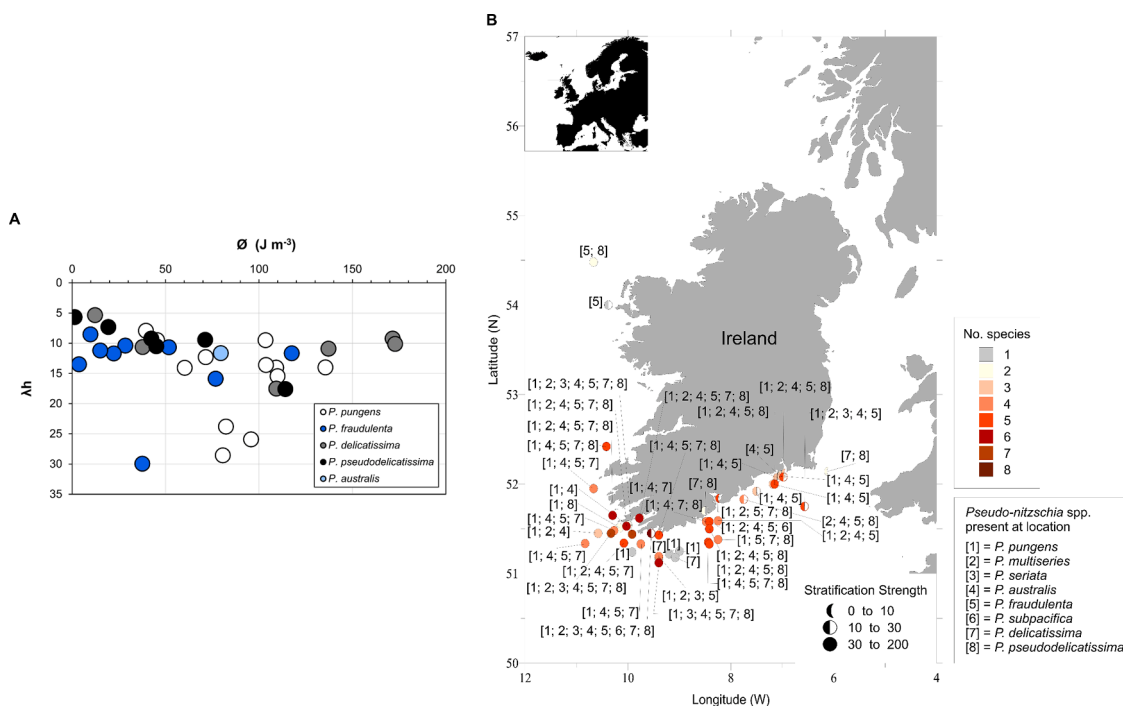


Fig. 9. *Pseudo-nitzschia* species identified by electron microscopy from vertical phytoplankton net hauls (Fig. 9A = predominant species at 34 stations; Fig. 9B = species presence at 46 stations) plotted against water column stratification strength ($\Phi J m^{-3}$; Fig. 9A and Fig. 9B) and the dimensionless optical depth (λh ; Fig. 9A) for stations off the south, southwest and west coasts of Ireland. This data was collected on several research cruises between 1993 and 1997 in Celtic summer (May, June, July) and autumn (August, September, October). Where: λ is the diffuse attenuation coefficient for downwelling irradiance (i.e. $1.7/\text{Secchi depth}$), h is the water column depth (m), λh is water clarity scaled with depth derived from Secchi depths recorded during daylight and the sounding at each station (100 m used when the sea bed was at >100 m), and Φ is the potential energy anomaly which as an index of bulk water column stratification calculated using the equation in Simpson et al. (1982). Values of $\Phi < 10 J m^{-3}$ indicate that the water column is well mixed, $\Phi = 10\text{--}30 J m^{-3}$ indicate that the water column is transitionally (weakly-moderately) stratified and $\Phi > 30 J m^{-3}$ indicate that the water column is well stratified.

Pseudo-nitzschia species are subject to viral, bacterial and/or fungal parasite attack (reviewed by Bates et al., 2018). In addition, grazing by metazoan and microplanktonic grazers may contribute to growth rate

limitation, restricted biomass production and termination of *Pseudo-nitzschia* blooms.

Physical factors and hydrodynamics may affect the partitioning of

alternative life history stages of *Pseudo-nitzschia* in terms of settling rate, initiation of transitions and transport within the water column and eventually to the sediments. Many *Pseudo-nitzschia* species do exhibit a known sexual stage ('auxospore') in strains in culture (Bates et al., 2018). Sexual life-history transitional stages including auxospore formation have also been (rarely) observed in natural populations of *Pseudo-nitzschia* (Holtermann et al., 2010), but are likely underreported because of lack of careful analysis in routine monitoring.

Dormant resting spores are known for a very few pennate diatoms, but do not include *Pseudo-nitzschia*. In any case, auxospores do not seem to represent a close analogy to flagellate resting stages ('cysts') forming seed beds for bloom initiation, and dynamics of alternative life cycle forms are clearly different from flagellates. Cells of *P. pungens* with intact chloroplasts have been observed from 25-year-old sediments from Mariager Fjord, Denmark, however, indicating that they may serve as a viable dormant stage (Lundholm et al., 2010). Bloom initiation may otherwise proceed from low densities of overwintering vegetative cells already within the upper water column or resuspended to the euphotic zone from deeper water or shallow benthos. In contrast to the strong upwelling along the northeastern Atlantic margin, *Pseudo-nitzschia* populations in the North Sea, Skagerrak-Kattegat and the Baltic Sea are typically (but with exceptions) at lower cell densities and more diffuse in vertical distribution.

Nutrient supply and ratios play a major role in *Pseudo-nitzschia* bloom dynamics and biomass production. *Pseudo-nitzschia* cells require silicate (SiO_4) for the cell wall (frustule), and other macronutrients (nitrate, NO_3 , phosphate, PO_4) and micronutrients (e.g., vitamins, trace metals) for growth. Despite an extensive literature on nutrient-dependent growth of *Pseudo-nitzschia* in culture experiments (reviewed in Bates et al., 1998; Pan et al., 1998; Trainer et al., 2012), there are no clearly defined exceptional requirements that would selectively favor this genus over other diatoms under specific nutrient-limitation.

The role of macronutrients (N, P, Si) in driving *Pseudo-nitzschia* bloom dynamics and regulating DA toxigenicity is frequently invoked but definitive data sets for field populations from northern European waters remain scarce. Comparisons of nutrient-associated cell abundance of members of the *P. seriata* versus *P. delicatissima* species complex may, however, be instructive at the sub-generic level (Cusack, 2002). In a case study in Ireland, when *Pseudo-nitzschia* cell densities off the south and SW coasts were plotted directly against nutrient concentrations, there was no obvious relationship between cell numbers and N-levels, but cells of the *P. seriata* complex were more abundant at low ($<0.2 \mu\text{M}$) P-levels.

Most diatoms are thought to grow best at Si concentrations $>2 \mu\text{M}$ in large enclosure experiments (Egge and Aksnes, 1992). Not surprisingly, cell numbers of both species complexes in the case study from south and SW Ireland (Cusack, 2002) tended to be maximal when SiO_4 was in the range of $1\text{--}4 \mu\text{M}$.

Fragile or broken frustules are frequently observed in LM- or EM-preparations of *Pseudo-nitzschia* specimens, particularly from senescent blooms under low ambient Si concentrations. Persistent Si limitation of *Pseudo-nitzschia* growth rate if not cell biomass production is likely rare in North European waters, and cannot account for the rapid termination of many blooms. In the Celtic Sea and off SW Ireland the *P. seriata* complex was most abundant in warmer waters during July, August and October when Si levels varied from 0.2 to $8.8 \mu\text{M}$ (Cusack, 2002). The presence of high cell densities of *Pseudo-nitzschia* at SiO_4 levels of between 1 and $2 \mu\text{M}$ reflects a variety of depths within the photic zone.

Nutrient and/or light limitation of growth could arise within high cell density thin-layers under extreme stratified conditions following relaxation of upwelling but these are transient events. Upwelling of deep water to the surface and Ekman transport advecting surface water away from the coast, and vertical mixing caused by storm events leads to deep nutrient enrichment of surface waters. Mass advective displacement may thereby create spatial differences in the occurrence of AST events. Surface nutrient enrichment in coastal waters also includes land runoff

which can favor *Pseudo-nitzschia* population development at the pycnocline from increased freshwater inflow. This effect may have occurred in embayments along the English Channel and Atlantic coast of France, where an anomalous *Pseudo-nitzschia* bloom linked to AST events impacting the scallop *Pecten maximus* fisheries in 2010 followed storm 'Xynthia' (Husson et al., 2016).

In an unintended massive nutrient-enrichment experiment, an anomalous toxic bloom dominated by *Pseudo-nitzschia* was noted after two silos containing N-fertilizer collapsed in Fredericia Harbor, Denmark, releasing >2750 metric tons of fertilizer into inner coastal Danish waters (Olesen et al., 2020). This massive local N-fertilization event at least demonstrates the capacity to enhance diatom blooms via eutrophication of the coastal zones but provides little evidence for species selection.

Increasing local pH due to high biomass production, or conversely ocean acidification, could conceivably play a role in *Pseudo-nitzschia* growth and bloom termination, but it is difficult to extrapolate from small-scale laboratory studies to field populations without detailed knowledge of the carbonate chemistry. In unialgal batch culture experiments, high pH between 8.7 and 9.1 inhibits growth of several *Pseudo-nitzschia* species, even at pH 8.4 growth may be reduced, depending on the species (Lundholm et al., 2004). *Pseudo-nitzschia australis* strains from France and Namibia varied from positive, no response, to negative growth at pH 7.8 compared to pH $8.0\text{--}8.1$, whereas *P. fraudulenta* strains from France were not affected by similar pH changes (Ayache et al., 2021). *Pseudo-nitzschia australis* isolated from the California Current exhibited lower growth at pH 7.8 than at $7.9\text{--}8.1$ in batch cultures (Wingert, 2017), whereas in *P. subcurvata* from the Antarctic, growth rate increased with pCO_2 , accompanied by an increase in C:Si ratio at higher pCO_2 but no change in C:N, N:P, C:P ratios (Zhu et al., 2017).

In *P. pseudodelicatissima* an increase in pCO_2 leads to an increase in C:P and decrease in Si:C and Si:N ratios (Sugie and Yoshimura, 2013), but these experiments invoke the additional complexity of Fe availability. The confusing and often contradictory inferences on the relationship between ocean acidification and the specific growth rate of *Pseudo-nitzschia* species as determined from different culture studies have been noted by Wingert and Cochlan (2021), whereby elevated pCO_2 can yield an increase, decrease or no change in growth rate depending on the *Pseudo-nitzschia* species and experimental conditions.

Simple conclusions on pH effects on *Pseudo-nitzschia* cannot be extrapolated to populations from northern Europe based on autecological experiments on single strains from diverse environments, even from within the same species. Presumably the pH effects are related to differences and difficulties of C-acquisition and primary metabolism, but the mechanisms have not been demonstrated for field populations. There is no clear evidence of genus-specific growth regulation in *Pseudo-nitzschia* due to ocean acidification in nature within the CoCLiME region. Further research is required to establish the impacts of oceanic pH shifts on natural populations of *Pseudo-nitzschia* as they interact with other phytoplankton species in the community.

5. Ecophysiological factors and environmental effects on *Pseudo-nitzschia* AST production

Chemical, physical and biological factors interact with genetic capacity to affect the production of DA in *Pseudo-nitzschia* (see reviews by Lelong et al., 2012; Trainer et al., 2012). Unlike most of the dinoflagellate-associated neurotoxins, DA production in *Pseudo-nitzschia* is highly inducible/repressible under various environmental stress regimes in culture (Bates et al., 1998; Pan et al., 1998), provided the necessary functional genetic machinery is in place. In general, Si and/or P-depletion, and increasing concentrations of N, high irradiance and the presence of bacteria all tend to enhance DA production. Changes in pH, pCO_2 , salinity and temperature can affect DA production, but presently no clear general picture has emerged for *Pseudo-nitzschia* that is not

species- or even population-dependent. For example, in cultures of *P. australis* from the California Current System, DA production was roughly stable in exponential growth but increased in stationary phase as pH decreased (Wingert, 2017). Other studies reflect an increase in pH in *Pseudo-nitzschia* cultures with increasing biomass and C-demand for growth, as indicated in a summary of factors affecting the production of DA or cell physiology (updated in Bates et al., 2018 from previous reviews).

Most laboratory ecophysiological studies in batch- or semi-continuous cultures have demonstrated that macronutrient limitation, particularly depletion of Si or P, and Fe limitation stress, can trigger DA production and yield increased cellular DA content as growth ceases (Bates et al., 1998; Pan et al., 1998; Trainer et al., 2012). These nutrient-dependent induction responses are sometimes invoked to explain high DA production in field populations but conclusive evidence of this coupling is generally lacking. Some field studies would support the statement that depletion of Si and P are related with higher DA cell content (if not biosynthesis) in *Pseudo-nitzschia* blooms (Anderson et al., 2006; Ryan et al., 2017; Thorel et al., 2017), whereas others do not (Marchetti et al., 2004; Trick et al., 2018).

Biological inductive mechanisms for DA production by zooplankton grazers have now been demonstrated in both laboratory experiments and field populations. In some circumstances the presence of certain copepods can induce DA production, and act as a grazing deterrent (Lundholm et al., 2018). Proximity of copepods and exposure to their waterborne chemical cues known as copepodamides can enhance DA content in *Pseudo-nitzschia* cells and even induce DA production in typically non-toxicogenic species (Tammilehto et al., 2015; Harðardóttir et al., 2015; 2019a; 2019b; Selander et al., 2019). Only herbivorous copepods induce such an effect, indicating that DA production is part of a defense mechanism for these diatoms (Lundholm et al., 2018). Recently, *Pseudo-nitzschia* cells cultured together with certain bivalve shellfish also exhibited an increase in DA content (Sauvey et al., 2021), but it remains to be shown whether the effect is due to the presence of the shellfish as inducers and not other co-culturing factors, e.g., increased N levels.

6. Current status and knowledge gaps on *Pseudo-nitzschia* and AST distribution and consequences

6.1. Environmental impacts and ecological consequences

Amnesic shellfish poisoning has caused numerous illness and deaths of marine mammals and seabirds via food chain transfer of AST on a global scale. Cases of ASP responsible for marine faunal morbidity and mortalities are particularly well known along the west coast of North America (McCabe et al., 2016; Trainer et al., 2017; Scholin et al., 2000) and more recently from Alaskan mammals (Lefebvre et al., 2016). Apart from acute poisoning of marine mammals due to ingestion of DA-contaminated prey, there are reports of a chronic poisoning syndrome linked to AST ((Brodie et al., 2006); (Goldstein et al., 2008)). Domoic acid is relatively stable in seawater and can potentially remain in the food chain for weeks or months (Johannessen, 2000); in the sediments DA may persist for years (Sekula-Wood et al., 2011). This AST accumulates in a wide array of marine species and many can act as toxin vectors, including transfer from copepods and krill, marine snails, cephalopods, and polychaetes to seabirds, fish and marine mammals (Bates et al., 2018). Domoic acid has also been found at high levels in pelagic fish like anchovies (Lefebvre et al., 2002), indicating that although most records of DA above regulatory levels are from bivalve shellfish, the global consequences of toxic *Pseudo-nitzschia* blooms on marine food chains and seafood safety are potentially severe (Lefebvre and Robertson, 2010).

In northern Europe, the paucity of reports of mass mortalities of marine fauna at higher trophic levels linked to ASTs has led to the assumption that this may reflect the low magnitude, frequency or

toxicogenicity of *Pseudo-nitzschia* blooms from the region. But this conclusion would be premature; almost nothing is known about DA in marine megafauna in Scandinavia or northeastern Atlantic waters, and AST is rarely surveyed much less monitored within northern Europe in species not considered as seafood. A small suite of studies showed that DA may be found in copepods (Cook et al., 2022) and in a number of fish species from Scottish waters (Kershaw et al., 2021). DA has also found in the amniotic fluid, urine and feces of harbor seals on the coast of Scotland (Hall and Frame, 2010; Jensen et al., 2015), suggesting that DA is readily passed along the marine food chain to top predators. In one report, DA found in harbor seals was shown to affect their immune system (Jensen et al., 2015). Exposure of these seals to DA, perhaps acting synergistically with Paralytic Shellfish Toxins (PSTs), through feeding on toxic prey has been suggested as key factor driving harbor seal decline in Scotland. As well as impacting the trophic balance within marine ecosystems, and hence fisheries resources, this decline may negatively impact statutory national and international assessments of diversity, such as the Scotland Marine Assessment (<https://marine.gov.scot/sma/>), the Marine Strategy Framework Directive, Descriptor 1, Diversity, and the Oslo Paris Commission (OSPAR) North East Atlantic Environment Strategy 2030 (<https://www.ospar.org/convention/strategy>). This implicates follow up responsibilities for European governments, monitoring and regulatory agencies if 'Good Environmental Status' for all trophic levels is not achieved in their waters.

6.2. Socioeconomic aspects of *Pseudo-nitzschia* blooms and current adaptive responses

The economic value of the aquaculture industry in Europe has dramatically increased recent decades, but particularly with respect to finfish species (EUROSTAT; <https://ec.europa.eu/eurostat/databrowser/explore/all/agric?lang=en&subtheme=fish&display=list&sort=category>). Aquaculture value of marine species in the EU-27 and other northern European countries (e.g., Norway, UK) has been driven mainly by the farming of Atlantic salmon (*Salmo salar*) and other salmonids. In contrast, the shellfish aquaculture industry in northern Europe has been declining in absolute terms within the past decade, with cheaper imports from outside Europe also reducing the economic value of shellfish as seafood. Shellfisheries and shellfish aquaculture operations are typically small scale and locally owned enterprises, and have less consolidated industry capital invested in stock, equipment and logistics than for the farmed fish industry. Nevertheless, shellfish aquaculture continues to play a significant economic role, particularly to provide employment and generate revenue in coastal communities. Thriving shellfish aquaculture can provide social cohesion in remote rural locations, and can impart cultural values and contribute to local traditional appreciation of seafood resources (Krause et al., 2020).

Socioeconomic and ecological impacts of *Pseudo-nitzschia* in the northern CoCLiME regions are most keenly expressed after advection of blooms into coastal embayments and fjords, where coastal populations are concentrated, and where many shellfisheries and aquaculture activities are conducted in the vicinity. HABs are considered amongst current major environmental threats to the shellfish aquaculture industry in the EU (Avdelas et al., 2020). In contrast to the situation with finfish aquaculture, the risks of AST accumulation in shellfish from *Pseudo-nitzschia* blooms are a significant component of the risk portfolio. *Pseudo-nitzschia* blooms and associated AST events have had relatively few known impacts on expansion of finfish aquaculture in northern Europe.

Detailed studies of the societal and economic impacts of AST events on aquaculture development in northern Europe are generally lacking; data and statistics are usually compiled on a national level and are difficult to compare across national boundaries. Even the cost/benefit analysis of HAB toxins and bloom monitoring compared to the value of the shellfish industry are difficult to estimate and compare. For example, direct and indirect costs of the Irish HAB toxin monitoring program

(about 1.5 million euros per annum) to run do not include the in-kind contribution of sampling by shellfish farmers. There is no simple way to estimate the monitoring costs for *Pseudo-nitzschia* blooms and AST as separate from the species and toxins responsible for other HAB toxin syndromes.

The rare confirmed human ASP cases (only one) in Europe, uncertain environmental impacts of *Pseudo-nitzschia* blooms on marine fauna, and lower frequency of AST events in major shellfish growing areas compared to PST and Diarrhetic Shellfish Toxin (DST) events (reviewed in (Bresnan et al., 2021); Karlson et al., 2021) tends to assign AST as a lower risk priority in northern Europe. For the northeast Atlantic area, the impacts of ASTs on shellfish harvesting (e.g., of mussels *M. edulis*, *M. galloprovincialis*) are mostly sporadic, short term and seasonally limited to peak periods of *Pseudo-nitzschia* cell abundance, e.g. in late spring and early autumn in northern regions. The majority of closures are enforced in shellfish growing waters with an Atlantic influence and last only a couple of weeks (Bresnan et al., 2021; Karlson et al., 2021). The overall economic and societal impact AST events is therefore usually manageable at the national level in this region (Davidson and Bresnan, 2009).

The exception is the higher impacts of AST events on the king scallop (*Pecten maximus*) fishing industry in the Celtic Sea and North Sea and adjacent waters. The depuration rate of DA from *P. maximus* is slow, taking many months for DA levels, once exceeded, to return to levels beneath regulatory thresholds (Blanco et al., 2002; Bresnan et al., 2017). In Europe, levels of DA exceeding regulatory thresholds in the gonad of *P. maximus* have led to prolonged closures of shellfish fishing areas – in some instances for more than a year, with a knock-on impact on associated industries and rural economies. For example, in 1999 high levels of DA in *P. maximus* gonad tissue from Scottish waters resulted in an area of ~37,000 km² being closed for scallop harvesting for up to a year with an approximate economic impact at the time of £17 million (~\$23 million US) (Gallacher et al., 2001). Shucking and removal of the digestive tissues (primarily hepatopancreas) for larger species and individuals of clams, scallops, cockles, etc. can mitigate the risk of ASP, but adds to the cost of processing and toxin monitoring. These enhanced costs and AST risks already limit the exploitation of natural shellfish populations and may compromise future development of aquaculture (e.g. in Norway and Sweden) of long-DA-retaining species.

6.2.1. Current mitigation and adaptive responses to environmental and climate-driven changes

A recent optimal matching analysis of shellfish closure decrees has highlighted the vulnerability of shellfish farmers to HAB events (Guil-lotreau et al., 2021), while also providing a plausible mitigation strategy. Based on anecdotal reports and consultations with Irish shellfish farmers in the CoCliME project, shellfish farmers within the northern CoCliME region are already adapting to increasing magnitude and severity of winter storms by submerging the longline buoys in winter to avoid damage and to limit loss of product during storms. The shellfish industry has also started to target the winter shellfish market due to increased prolonged summer closures due to HABs in summer. While this allows the shellfish sufficient time to depurate HAB biotoxins after severe HAB events in summer, targeting the winter markets bring other risks. On a socio-economic level, to compensate for the decline in revenue from shellfish aquaculture many farmers are already diversifying their practices and supplementing income with terrestrial farming, tourism-related activities and other part time jobs. Shellfish farmers have also adapted to the impact of HABs in warmer seasons by selling product in the winter markets. In the event that climate change results in an increased number of HABs beyond an undefined tolerance threshold, these small businesses have indicated that they will just give up shellfish farming.

To reduce the socio-economic impact from AST events, scientists, regulators and policy makers came together to mitigate the influence of these toxins on the rural economies and societies impacted. Early studies

showed that the highest levels of DA in *P. maximus* were located in the digestive glands and the lowest in the adductor muscle (Campbell et al., 2001; Blanco et al., 2002). This led to the development of amendments (2002/226/EC, EU854/2004) to the EU Shellfish Hygiene Directive to facilitate end product testing and shucking of scallops, facilitating the sale of adductor muscle if DA levels were less than 4.6 mg kg⁻¹ (Bresnan et al., 2021). These amendments eliminated the requirement for monitoring and potential closures of offshore scallop harvesting areas and ensured that scallop fishermen had a product that they could sell. This approach is now adopted in many countries in the North Atlantic area. In France, however, *P. maximus* harvesting areas remain closed when DA levels in gonad tissue are exceeded, and because of this “roe-on” product, AST events continue to have a higher impact.

Climate-projected HAB estimates could support aquaculture and fisheries manager decisions, assist with implementing mitigation responses and risk assessment, and even influence their long-term strategies, e.g., site selection criteria for shellfish aquaculture expansion and/or relocation. Unfortunately, sophisticated climatological models for *Pseudo-nitzschia* bloom dynamics and biogeographical distribution patterns are not yet available for providing forecasting on the time scales required for short term risk assessment and mitigation actions relevant to shellfish harvesting and production. Short term forecasting and status reports of impending blooms, including of *Pseudo-nitzschia*, already provide basic information for shellfish farmers to manage risk of shellfish harvesting closures in several countries on the NE Atlantic (Fernandes-Salvador et al., 2021). For example, the online HAB reports system (<https://www.habreports.org/>) yields early warning of HAB and biotoxins risk to shellfish farmers in Scotland (details in Davidson et al., 2021). At present these systems have not been implemented at the European level and instead are designed for national or regional requirements.

7. Modelling of *Pseudo-nitzschia* blooms and DA toxin dynamics

Advective transport and the tendency for vertical stratification into thin-layers present a massive challenge for modelling the diversity and growth dynamics of *Pseudo-nitzschia* blooms and their distribution in time and space. A diverse array of alternative modelling strategies have been applied on a local or regional scale, with varying degrees of success in global applications. A selected few of these modelling approaches are presented herein as examples of how models may function to track *Pseudo-nitzschia* blooms and to test alternative scenarios for effects of climate-driven changes in ocean conditions on bloom dynamics and potential range extensions or contractions. The first predictive logistic model for resolution of toxigenic *Pseudo-nitzschia* blooms at annual and seasonal time-scales was developed for Monterey Bay, California from a multi-project dataset representing 8.3 years of observational sampling (Raimondi and Kudela, 2009). A logistic Generalized Linear Model (GLM) approach showed that predictor variables of *Pseudo-nitzschia* blooms (e.g., time window, nutrients, temperature etc.) for Chesapeake Bay in the NW Atlantic were similar for locations on the west and east coasts of North America (Anderson et al., 2010). A process-based ocean numerical model that assimilated observational data based on the work of Ryan et al. (2014) on stratification-enhanced upwelling in Monterey Bay, California indicated the importance of boundary influences on HAB species, including of *Pseudo-nitzschia* spp., in upwelling regions. In combination with *in situ* observations, this approach has proven useful for general hypothesis testing about bloom dynamics of *Pseudo-nitzschia* (Franks, 2018). Coupling a hydrodynamic model with Lagrangian particle tracking can help to direct attentions towards the most likely source of a specific *Pseudo-nitzschia* bloom. The particle tracking modelling (Clark et al., 2021) for the Gulf of Maine, indicated that the Scotian Shelf was the most likely source of *P. australis* which arrived as a massive toxigenic bloom event in 2016. While the modelling exercise failed to identify the ultimate source of *P. australis*, the approach offers a template for modelling introduced *Pseudo-nitzschia* species around the NW

Atlantic marginal seas and extension throughout the subpolar Arctic gateway and connectively to Greenland.

A mechanistic model to explore the factors controlling DA production by *Pseudo-nitzschia* with DA production has been set as secondary metabolism, showed that under growth limitation shared common precursors can accumulate, yielding increased DA production (Terseleer et al., 2013). The general pattern that emerged is that excess light, in combination with Si or P limitation, favors DA production, provided nitrogen (N) is sufficient. This interpretation suggests that sustained DA toxigenicity in natural populations is indeed linked to the excess of primary C that accumulates during photosynthesis under growth limitation by nutrients. If the hypothesis is sustained by further field investigations, increased input of nutrients would amplify bloom toxicity by simultaneous increase in DA production and enhanced *Pseudo-nitzschia* biomass.

7.1. Regional modelling of *Pseudo-nitzschia* bloom dynamics in northern European waters

Recent attempts to model *Pseudo-nitzschia* bloom dynamics and distribution in northern Europe by coupling hydrodynamic parameters with species and cell abundance data have yielded plausible explanations for the driving functions (e.g., Cusack et al., 2016), but as yet are not widely fully operational or provide only weak short-term forecasting capacity, even over weekly time-scales. Predictive-simulation models, however, intended to extend diagnostic modelling to yield temporal predictions beyond existing data have not been extensively developed for *Pseudo-nitzschia* bloom dynamics on a regional scale in the NE Atlantic marginal seas and other northern European waters.

In northern European and NE Atlantic regions, the only statistical modelling approaches to examine and predict *Pseudo-nitzschia* dynamics and distribution for short-term forecasts include the zero-inflated negative binomial regression focused on blooms in SW Ireland (Cusack et al., 2015), a Generalized Poisson Regression Model (GPRM) (Palma et al., 2010) for coastal upwelling in Lisbon Bay, Portugal, and a support vector machine (SVM) model for NW Spain (González Vilas et al., 2014).

While the aforementioned studies focused on short-term bloom predictions, Townhill et al. (2018) considered the effects of climate projections to the end of the 21st century on habitat suitability for various HAB species distributions with a Maximum Entropy (Maxent) bioclimate envelope model. Based on a numerically simulated possible future ocean under a “medium” climate change emissions scenario, they generated a high resolution, downscaled shelf seas climate projections for the NW European shelf. The Maxent projections suggested that the habitat of most HAB species (defined by temperature, salinity, depth, and stratification) will shift north this century. With respect to *Pseudo-nitzschia*, the model implies that NW European shelf waters will remain suitable for a number of *Pseudo-nitzschia* species; in principle these include *P. australis*, *P. delicatissima*, *P. fraudulenta*, *P. seriata* - known DA producers on a global basis. At the regional NW European shelf scale, however, the habitat suitability distribution yielded only weak predictive *P. delicatissima* and *P. seriata* because of their widespread global distributions and they were deleted from further analysis. The distributional projections for the remaining *P. australis* and *P. fraudulenta* were based primarily on the contributions of the hydrodynamic variables of bathymetry and near bed temperature.

The latter Maxent approach was recently improved with machine learning aspects, e.g. Random Forest and AdaBoost, and applied for the shelf sea of NW Europe adjacent to the Galician Rias Baxias (Aláez et al., 2021). An earlier hybrid approach for short term forecasting combined *in situ* observations of *Pseudo-nitzschia* blooms with simulated water current flows visualized with a particle tracking technique assisted in interpreting *Pseudo-nitzschia* bloom patterns in SW Ireland (Cusack et al., 2016). This hybrid hydrodynamic and particle tracking modelling has also recently helped to better understand the relatively new appearance

of *P. australis* blooms in shelf waters of the NW Atlantic (Clark et al., 2021).

These alternative modeling approaches for *Pseudo-nitzschia* blooms have yielded generally complementary interpretations of bloom dynamic drivers, despite large regional differences in species biogeography and hydrodynamic factors. Cusack et al. (2015) noted that *Pseudo-nitzschia* bloom arrival into the bays of SW Ireland was clearly associated with wind driven exchange events. The SVM model developed by González Vilas et al. (2014) was based on several variables (ría identification code, year, day, temperature, salinity) combined with an upwelling index and bloom occurrence during previous weeks. A major finding was that *Pseudo-nitzschia* bloom occurrence in the previous weeks was a key parameter to enable prediction. In comparison, the only hybrid model study in the CoCliME region of northern Europe (Cusack et al., 2016), also supported previous *Pseudo-nitzschia* observational studies in the region that wind induced water mass advection is responsible for the transport of shelf populations into the bays of SW Ireland (Raine, 2014).

CoCliME co-developers (scientists and policy makers) requested a climate service that could yield future weekly *Pseudo-nitzschia* bloom projections at decadal time scales rather than merely at the end of the century (i.e., 2100). The CoCliME modelling efforts towards explaining *Pseudo-nitzschia* bloom distributions and intensities focused on future projections under a high CO₂ emission scenario. Alternative types of models investigated included a Habitat Suitability Model (Maxent) which incorporates species presence and absence data and a Gradient Boosting Machine (GBM) model driven by weekly cell count data. Modelling focused on estimating *Pseudo-nitzschia* distributions and intensities in the future ocean under a high CO₂ emission scenario (Representative Concentration Pathway; RCP 8.5) for the Atlantic coast of Ireland.

In the Maxent model, sea surface temperature and bathymetry were the two variables with the highest percentage contribution to the predicted changes in *Pseudo-nitzschia* distribution. The majority of phytoplankton samples accessed for the Maxent modelling came from coastal sites but the role of bathymetry remained unclear from the model. While the Maxent approach was fully investigated by model developers in CoCliME, the application was not further developed into a climate service as it was limited by over-generalization. This approach was considered more suitable for long time-series datasets (e.g., up to the beginning of the 21st century) because it uses decadal averaged measurements and therefore sacrifices precision and detail.

A Gradient Boosting Machine (GBM) modelling approach driven by weekly coastal cell count data was considered a more suitable model to develop the prototype climate service because it provides both present and future weekly *Pseudo-nitzschia* bloom estimates. The GBM model was developed using *Pseudo-nitzschia* cell presence/absence data, and the essential ocean variables for temperature, salinity and a water column stratification index. This prototype climate services application was based on physical environmental variables from the only available high resolution numerical ocean climate model for the study area (Nagy et al., 2021; this SI); *Pseudo-nitzschia* cell counts from the Irish national monitoring program were accessed weekly to build the GBM model (see Nagy et al. (2021) this SI, for details on model development). The CoCliME climate services for *Pseudo-nitzschia* are available online (https://marine-institute-ireland.shinyapps.io/P_seriata_probability/) and the difference between the recent past and contemporary/future time periods are displayed in Fig. 10. Given this information, the model determined that the relative influence of the key ocean variables for temperature (SST), salinity and water column stratification for estimating probability of presence of *Pseudo-nitzschia* were almost equally weighted and roughly similar (35%, 33% and 32% respectively). The *P. seriata* complex, which includes known DA producers, e.g., *P. australis*, as well as non-toxicogenic taxa, is associated with warm, salty, stratified water in the GBM model simulation – similar to past synecological studies for the Celtic Sea and SE Ireland region (Cusack, 2002;

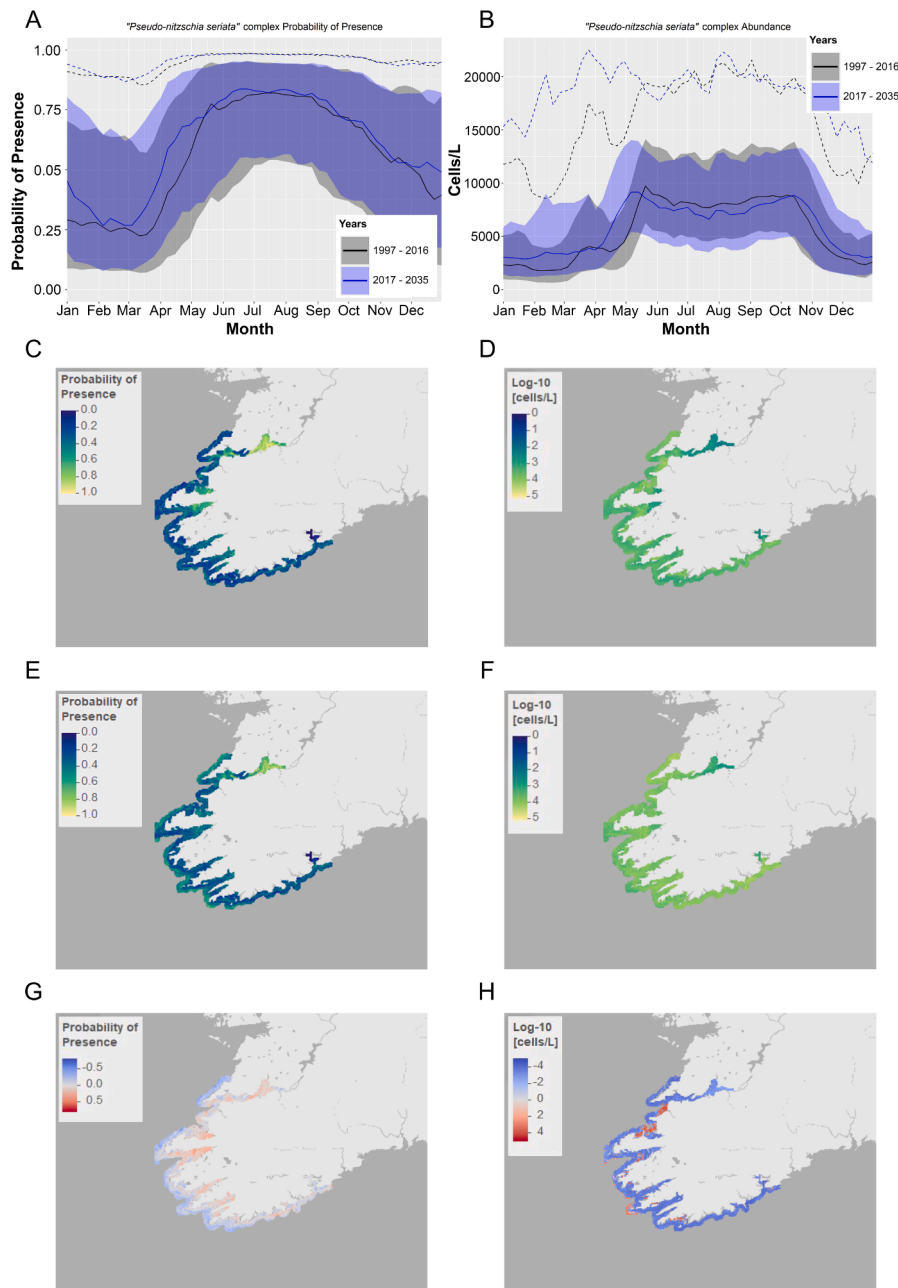


Fig. 10. GBM model Climate Services App applied to the SW Ireland coast for probability estimates of the *Pseudo-nitzschia seriata* species complex. Figs. A and B show the difference between the statistically derived presence probabilities and cell abundances of *Pseudo-nitzschia* for two discrete time periods 1997–2016 (“recent past”) and 2017–2035 (“contemporary/future”) under an RCP 8.5 scenario (adapted and redrawn from Yamanaka et al., this SI). The median (50th percentile) statistic is represented by the solid line, the 25th to 27th percentiles in the shaded area and the 90th percentile as a dashed line, where in A, C, E, and G the presence/absence probabilities are shown, whereas the $\log_{10}$ cells L^{-1} of the *P. seriata* complex are indicated in B, D, F and H. C – H, geographical maps showing *P. seriata* complex for mid-February (week 6); in C, D the recent past, and in E, F the contemporary/future under a pessimistic scenario, i.e. RCP 8.5. In G, H the difference between the recent past and the contemporary/future is shown in each grid, whereas in G and H, the red color indicates an increased probability and the blue color indicates a decreased probability of presence (or cell abundance). Probability of presence varies from 0 to 1, where 0 indicates impossibility of occurrence and 1 indicates certainty. Detailed information on this model application is available online at https://marine-institute-ireland.shinyapps.io/P_seriata_probability/ and https://marine-institute-ireland.shinyapps.io/P_seriata_abundance/ for presence probability and cell abundance of the *P. seriata* complex, respectively.

Yamanaka et al., this SI), where *P. australis* tends to dominate in the spring bloom. In contrast, in the North Sea and NE Atlantic margin north of Scotland, *P. australis* is typically an autumn bloom-forming species (Bresnan et al., 2017). In SW Ireland, the model projects a slightly elevated probability presence of *P. seriata* complex in autumn, winter, spring and early summer (October to May) in the contemporary/future period (2017–2035) compared to the recent past (1997–2016). In the modelled future ocean, *Pseudo-nitzschia* blooms occur earlier in the year.

The future estimates also predict a more widespread geographic distribution of the *P. seriata* complex in the coastal waters off SW Ireland (prototype climate service application: https://marine-institute-ireland.shinyapps.io/P_seriata_probability/). Geographically, the *P. seriata* complex is predicted to occur more commonly throughout SW Ireland by 2035 with a general increase in the probability of both presence and cell abundance in some of the SW Ireland bays (e.g., Dingle Bay, Tralee Bay), but a general decrease of the *P. seriata* complex along the southern coast

of Ireland.

The coupled atmosphere-ocean regional climate model RCA4-NEMO (Gröger et al., 2019) was used within CoCLIME case studies to generate regional projections of future ocean conditions for the North Sea and English Channel, and adjacent waters of the southern Norwegian coast, Skagerrak/Kattegat and Baltic Sea. This model was applied to investigate environmental factors potentially influencing *Pseudo-nitzschia* growth and distribution in a changing climate based on output for salinity, temperature, and mixed layer depth (MLD), indicative of the degree of water column stratification. Five global climate model projections were downscaled to obtain regional ocean conditions under the RCP 4.5 and 8.5 scenarios. The RCP 4.5 scenario assumes a strategy for reduced greenhouse gas emissions to yield a stabilization of the radiative forcing at 4.5 W/m^2 before year 2100, whereas RCP 8.5 assumes increasing greenhouse gas emissions to allow radiative forcing to reach 8.5 W/m^2 year by 2100. Model projections based on RCP 8.5 indicate an increase in yearly mean surface ocean temperatures of about 2°C in the North Sea and 3°C in the Baltic Proper, whereas a decrease in mean salinity of ca. $0.5\text{--}1.5$ is projected for the modelled period 1970–1999 compared with 2070–2099 (Fig. 11 derived from the RCA4 Nemo Model). The reduction of salinity would be highest in the Baltic Sea according to RCP 8.5 model results. The MLD would be reduced in the northern part of the North Sea (ca. $5\text{--}9 \text{ m}$) but increased in the Baltic Proper and the Bothnian Sea (ca. $1\text{--}5 \text{ m}$). MLD would remain stable or marginally increase in the Bothnian Bay. Application of the model to the RCP 4.5 scenario indicates a similar general pattern but with smaller changes (Fig. S1).

The scenarios presented in Fig. 11 and Fig. S1 are based on annual mean variables. In any case, the RCA4-NEMO model projections of differences in ocean conditions based on estimates restricted to the northern spring (March to May, data not shown), when *Pseudo-nitzschia* blooms are often initiated, do not substantially deviate from this pattern. An exception is the MLD which is unchanged in the Baltic Proper and decreases in the Gulf of Bothnia in spring when comparing modelled

period 1970–1999 with 2070–2099. Cautious extrapolation from these hydrodynamic parameters tends to favor a projected annual increase in *Pseudo-nitzschia* abundance and distribution, particularly in sub-regions with an expected decrease in MLD, but without sufficient sensitivity to select among species. On the other hand, a reduction in salinity may cause a shrinking of the area in which *Pseudo-nitzschia* is distributed. At present, *Pseudo-nitzschia* appears to be salinity constrained in the Baltic Sea because it rarely appears at salinities $6\text{--}10$ and is effectively absent below salinity 6 (Fig. 6). Any future reduction in salinity in the Baltic Proper may therefore result in the disappearance of *Pseudo-nitzschia* blooms from this sea.

8. *Pseudo-nitzschia* in a future climate - key indicators and drivers

Climate change will affect the environmental regime of NE Atlantic and Arctic gateway waters with knock on impacts on vulnerable industries and societies that rely on marine resources. Detailed studies of the societal impacts of ASTs on aquaculture are lacking, but environmental scenarios of past and future conditions that may drive *Pseudo-nitzschia* dynamics have emerged in the last decade. Evidence from long time-series from 50-year records (1960–2009) from the continuous plankton recorder (CPR) in an earlier analysis (Hinder et al., 2012) of the combined NE Atlantic and North Sea region demonstrate an overall decline in abundance of dinoflagellates, including HAB taxa, without a general decrease in diatoms. Over this period there has been a relative increase in diatoms versus dinoflagellates, and even an absolute increase in certain common diatoms, including species of *Pseudo-nitzschia*. These apparent shifts were attributed as putatively driven by an interaction of increasing sea surface temperatures coupled with increasingly windy conditions in summer.

A later study incorporating offshore multi-decadal data from the CPR with inshore plankton observations from long-term inshore fixed stations around the UK coastline (Bedford et al., 2020) showed the broad

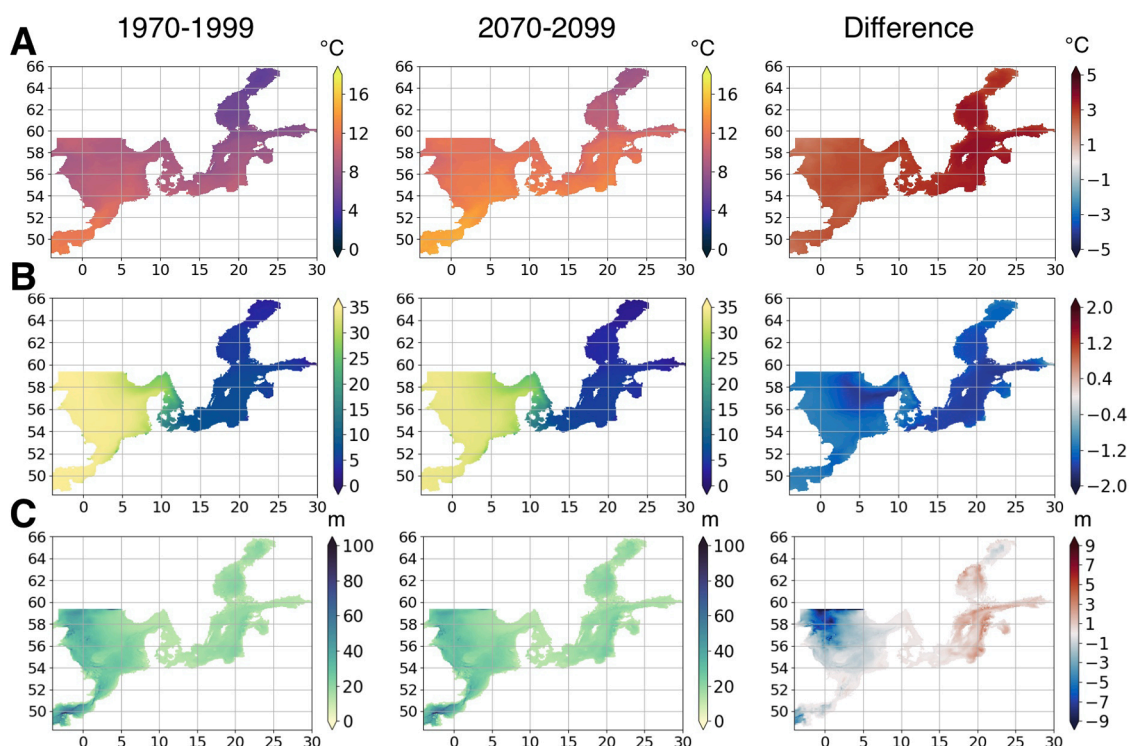


Fig. 11. Ocean climate scenarios for the greater North Sea, Skagerrak–Kattegat and Baltic Sea regions based on the SMHI regional coupled atmosphere-ocean climate model RCA4-NEMO. Ensemble annual means of 5 regionalized global climate model projections for climate scenario RCP8.5. Left panel: 1970–1999, middle panel: 2070–2099 and right panel: difference between the periods 2070–2099 and 1970–1999. A. Temperature (0–10 m) ($^\circ\text{C}$), B. Salinity (0–10 m) (unitless) and C. Mixed layer depth (MLD) (m). Note that Fig. S1 showing scenarios for RCP 4.5 is provided in supplementary materials.

alignment of broad functional groups defined as “plankton life forms”. The lifeform approach correlated to sea surface temperature across the NW European shelf showed sub-regional differences for the Greater North Sea versus the Celtic Sea, with diatoms exhibiting a broad general increase in the North Sea region with corresponding decrease for the Celtic Sea. The inconsistent correlation of diatoms with SST particularly for the inshore time-series stations likely indicates that SST *per se* is not the sole climate variable to be considered for future *Pseudo-nitzschia* dynamics or even at the functional group level (e.g., phototrophic pennate diatoms).

A recent interpretation of climate variability and multi-decadal diatom abundance in the Northeast Atlantic, based on CPR data on diatoms combined with climate parameters (wind intensity and direction, sea surface temperature) in principal components analysis (PCA) (Edwards et al., 2022), confirmed a multidecadal trend of increasing diatom populations in northernly systems but decreasing in southern regions. The PCA indicated a positive correlation between SST and diatom abundance in the northern NE Atlantic, such as the northern North Sea and the Icelandic basin, but negative correlations in the Celtic Sea and Bay of Biscay. The study focused on general diatom abundance for the entire NE Atlantic but also provided data on individual species (or complexes) for the North Sea region. The highest species/taxa correlations with the PC1 axis were for neritic spring diatoms, including *P. delicatissima* and *P. seriata* complexes. Within the past two decades, diatoms in general have apparently increased in abundance and dispersion during seasonal growth, including through the summer, followed by an autumnal growth period. The *Pseudo-nitzschia seriata* complex, in particular, has increased in seasonal dispersion and autumnal bloom formation.

In addition to caveats such as the consistency in capture efficiency of the CPR and taxonomic precision and consistency in identification at the species level throughout the time-series (acknowledged by Edwards et al., 2022), such indications of environmental drivers on species-level biogeography much be interpreted cautiously at the regional and meso-scale for *Pseudo-nitzschia* bloom dynamics and toxin risk assessment. This apparent “diatom trend” (Hinder et al., 2012) applies to both common non-HAB centric diatoms (e.g., *Thalassiosira* spp.) and potentially toxigenic pennate diatoms (e.g., *Pseudo-nitzschia*). On this basis, it is premature to conclude that easy to measure climate-related key indicators – temperature, salinity, wind velocity and associated stratification parameters (e.g., MLD) – will lead to reliable predictors of the dynamics of *Pseudo-nitzschia* as distinct from other pennate diatoms at the genus level, much less to evaluate toxigenic risk among various species or ecotypes.

Climate-driven scenarios for feedback mechanisms of phytoplankton group dynamics have tended to focus on temperature shifts in defining regime changes. Under these scenarios biologically driven carbon pumps should be less efficient with surface ocean warming creating changes in the eukaryotic microplankton assemblages and hence favoring small flagellates over diatoms (Edwards et al., 2022). Regional climate warming in some areas of the North Sea has been linked to an increase in certain diatoms that are associated with Harmful Algal Blooms, in particular the taxa *Pseudo-nitzschia seriata* complex (Edwards et al., 2006; Hinder et al., 2012).

Climate variability in the NE Atlantic as defined by SST and wind velocity and direction affecting stratification and advection is less than homogenous, with distinct spatial and temporal patterns emerging in various sub-systems. This highlights the significance of regional context in defining drivers of *Pseudo-nitzschia* bloom dynamics and argues against simple application of global hydrodynamics models. The cosmopolitan distribution of many *Pseudo-nitzschia* species, and failure to define general species-specific temperature and salinity boundaries in autecological studies, suggests that key indicators remain a challenge to identify on a global scale. The effects of climate driven changes in the environmental conditions are complex since all trophic levels and species would be affected. An increase in temperature may tend to favor

Pseudo-nitzschia, based on evidence from the massive bloom of *Pseudo-nitzschia* extending along the entire NE Pacific coast in 2015 (McCabe et al., 2016; Trainer et al., 2017; (Trainer et al., 2020)). An unusually large warm “blob” essentially defined the boundaries of the bloom. In northern European waters, it remains to be determined whether climate-related temperature increase in surface waters would actually promote growth of *Pseudo-nitzschia* blooms or only serve to define the hydrodynamic regime within which the bloom develops.

In any case, attempts to define key indicators for *Pseudo-nitzschia* on a regional scale remains a worthwhile endeavor. The tendency for *Pseudo-nitzschia* species to form dense thin-layers following relaxation of major upwelling events and wind-driven on-shore transport is rather unique, even among chain-forming diatoms. This suggests potential for development of key indicators based on water column stratification parameters, e.g. maximum buoyancy frequency, MLD, and/or upwelling parameters affecting bloom distribution in horizontal and vertical dimensions. As a key indicator, nutrient status also determines to some extent both the magnitude and toxicity associated with *Pseudo-nitzschia* blooms, as P- and Si-depletion limit growth while inducing toxicity, at least in some populations.

9. Challenges for risk assessment, forecasting and management strategies

A major challenge in designing effective monitoring strategies for *Pseudo-nitzschia* risk assessment and climate-services is the inconsistency in toxigenicity (the associated risk) among strains, species and populations, and extreme dependency upon nutrient status, growth conditions and presence of grazers. Toxigenicity is mostly determined from autecological studies on one or a few cultured strains and may not represent risk under natural bloom conditions. It is therefore misleading to refer to “toxic” versus “non-toxic” *Pseudo-nitzschia* species. Toxigenicity requires both the presence of the requisite DA biosynthetic genes and the permissive conditions for their functional expression.

Based upon careful taxonomic and toxicity research studies it has been well established that *P. australis*, *P. multiseries* and *P. seriata* are the primary DA producing species in North European coastal regions and hence the main causes of DA accumulation in shellfish. These *Pseudo-nitzschia* species (or species complexes) can contain both non-toxicogenic and toxicogenic strains or populations, but which are virtually indistinguishable by taxonomic and morphological features by traditional LM most often employed in national HAB monitoring programs. From a commercial and seafood security perspective, it is difficult for shellfish aquaculture operations to make informed decisions on shellfish harvesting, particularly if the dominant *Pseudo-nitzschia* species and their respective toxigenicity cannot be identified with certainty. During March–May 2005, for example, an unprecedented AST event occurred in SW Ireland, which caused numerous closures of production of aquaculture mussels (*M. edulis*) and Pacific oysters (*Magallana gigas*) in several bays in the region. In this AST event, *Pseudo-nitzschia seriata* complex were counted at high cell densities. The causative DA-producing species, however, could not be identified or discriminated within field samples composed of mixed non-toxicogenic and toxicogenic *Pseudo-nitzschia* species. Since then, AST events have been near annual occurrence in the SW and also occasionally along the West coast of Ireland, resulting in short term mandatory closures for harvesting. Difficulties in attributing the causative agent of AST events to particular *Pseudo-nitzschia* species within toxicogenic blooms remains a chronic challenge for effective monitoring throughout the diverse national programs within northern Europe. The general strategy is to regulate closures based on DA levels in shellfish, but some North European countries employ a *Pseudo-nitzschia* cell density threshold as an early warning or precautionary closure alert for more intensive toxin monitoring.

The GlobalHAB program has advanced modelling and technology implementation in their science plan, and combining modelling into

EWS strategies is now a major element of the IOC-IPHAB task teams. In Europe, issues surrounding sustained funding for bloom monitoring continues to limit development and operational research in this area. Progress has been made by the ASIMUTH and PRIMROSE EU-funded projects for countries along the NE Atlantic margin. Empirical model validation and forecast skill are essential to build trust in the models.

A diverse array of alternative modelling strategies (described in Section 7) has already been applied to analyze *Pseudo-nitzschia* blooms on a local or regional scale, with models developed for various regions along the NE Atlantic margin (Cusack et al., 2016; Dabrowski et al., 2016; Silva et al., 2016). These models have yielded varying degrees of success with respect to predictive capacity. Regional models for *Pseudo-nitzschia* blooms remain in the development or prototype phase with little or no operational uptake. The variability and reliability of the historical dataset used to parameterize and validate the *Pseudo-nitzschia* bloom models limits their application. Climate-based modelling projections are typically based upon changes in physical parameters (temperature, salinity) and their predicted integrated effects on currents and stratification, and hence bloom dynamics and distribution. Process-based HAB models may be more suitable for extrapolation into future climate forcing conditions, as concluded by Ralston and Moore (2020). In the case of *Pseudo-nitzschia*, bloom prediction in the face of global climate change will require incorporation of biological properties (life history strategies, growth rate, grazing and parasitism, interspecific competition, buoyancy, gene regulation, etc.) at the infraspecific or even population level.

For management purposes, the goal is to incorporate output from multiple tests of predictive models with appropriate statistical power and spatio-temporal coverage. Short-term predictions for *Pseudo-nitzschia* blooms from regional and local models are frequently lacking applications to serve as EWS for discrete sites favored by the shellfish industry, and in other areas are still at the experimental stage of development. The applications must be validated in the real world to determine if the forecasts will be appropriate to the temporal and spatial scales relevant to the industry. Since stakeholders and policy makers are mostly interested in decadal predictions, the next step is to apply the GBM model approach to *Pseudo-nitzschia* blooms. The predictability of the climate service prototype approach as developed for SW Ireland is limited by a number of HAB sampling bias, such as the fact that all the plankton samples come only from coastal shellfish production area and not from regular offshore stations. This creates uncertainty and currently restricts the extent of climate models to the few variables included in the model. The CoCliME model, for example, includes only SST, salinity and a water column stratification index. It is important to establish regular offshore sampling of HABs to develop climate models with longer time scale and larger spatial area, e.g. to extend the GBM to the whole of Ireland, including the Celtic Sea, Irish Sea and NE Atlantic margin. The habitat suitability GBM model used for CoCliME could be improved by facilitative changes, as well as including other variables potentially important for predicting *Pseudo-nitzschia* blooms such as pH, pCO₂ and nutrients.

10. Technological challenges and solutions for species detection and identification

The phenomena of *Pseudo-nitzschia* blooms and DA origin and biosynthesis have been investigated globally for more than three decades. Yet knowledge gaps remain in taxonomy, phylogeny, species biogeographical distribution, competition and selection within the plankton, and bloom dynamics on the mesoscale. At the cellular level, lack of knowledge on regulation of life history transitions and, although parts of the biosynthetic pathways was recently discovered (Brunson et al., 2018; Harðardóttir et al., 2019b), details and regulation of DA biosynthesis have continued to hamper efforts to define the eco-evolutionary significance of production of DA and its isomers. The fact that a few toxigenic strains of *Nitzschia* species have also been

discovered (Smida et al., 2014) poses unanswered questions regarding the chemodiversity and phylogenetic relationships with genus *Pseudo-nitzschia*. Addressing these gaps in basic research will assist in the development of coherent monitoring and early bloom warning systems based on quantitative detection of species and their respective toxigenicity.

The frequent taxonomic revisions and nomenclatural changes for *Pseudo-nitzschia*, and the proliferation of new “species” defined by critical taxonomy, highlight the difficulties in maintaining reporting coherence for national HAB monitoring programs and data entry, e.g., into HAEDAT. Discrimination of *Pseudo-nitzschia* species by optical microscopy is a time-consuming and tedious task requiring a high level of training in phytoplankton identification and taxonomy because most of the frustule morphometrics needed for species determination are close to the limit of resolution of the light microscope and often critical features are visible only by scanning or transmission electron microscopy (Lelong et al., 2012). Accordingly, for routine plankton monitoring by light microscopy, the genus is often operationally defined into two species complexes: *P. delicatissima* and *P. seriata sensu lato*, defined loosely and grouped by cell size <3 µm and >3 µm, respectively. Unfortunately, such simplifying grouping merges taxa with vastly different eco-physiological behavior and characteristic traits and provides little specificity of potential DA toxigenicity.

In these cases, confirmatory application of molecular techniques, such as DNA barcoding (Sildever et al., 2019; Dermastia et al., 2020; Guilietti et al., 2021b) and qPCR (Fitzpatrick et al., 2010; Kim et al., 2017; Wohlrab et al., 2019), are required for species identification. Particularly in an automated high-throughput format, molecular approaches are warranted for species diagnostics and perhaps also cell quantitation from field samples. Some promising probes have been developed for *Pseudo-nitzschia*, and in current formats they appear to capture also geographically distant populations of the same species (Bowers et al., 2017; Bates et al., 2018). Nevertheless, there remain gaps in the design and availability of appropriately selective probes for the different local- and regional-scale populations for *Pseudo-nitzschia* due to the limits of phylogenetic resolution of commonly applied phylogenetic markers such as 18S rRNA gene and D1/D2 region of the 28S rRNA gene. Increased efforts to develop markers for more extensive sequencing of the 5.8S/28S operon and in particular the ITS regions would facilitate discrimination of taxa at the infra-specific level.

A number of molecular assays based on the variability of ribosomal RNA (rRNA) genes between taxa have been applied to discriminate *Pseudo-nitzschia* at the species level. Assays involving, for example, whole-cell fluorescence *in situ* hybridization (FISH, Scholin et al., 1996), sandwich hybridization (SH, Scholin et al., 1997; Miller and Scholin, 1998), or qPCR (Fitzpatrick et al., 2010) are now in functional research mode, if not commonly deployed in species monitoring programs. Fluorescence *in situ* hybridization allows the specific detection of target taxa through the binding of oligonucleotide probes and enables the visualization of the fluorescent whole target cell (Groben et al., 2004). In qPCR assays a specific primer set in combination with a fluorescent intercalating nucleic acid stain (e.g. SYBR Green), or a combination of primers and a fluorescent probe (e.g., TaqMan approach), targeting the specific DNA region of the respective *Pseudo-nitzschia* taxa are applied to identify the target organisms. In general, qPCR is more sensitive for detection of target species at low cell abundance than FISH and provides a higher sample throughput and a potential for automation. Phylogenetic analysis of the genus *Pseudo-nitzschia* is performed by analyzing the sequence data of various phylogenetic markers (Dermastia et al., 2020). Most commonly used markers for detecting *Pseudo-nitzschia* are the large ribosomal subunit gene (28S), internal transcribed spacer regions (ITS), plastid encoded genes (e.g., RuBisCo large subunit, rbcL) and the mitochondrially encoded *cox1* gene (Armbricht et al., 2021; Guilietti et al., 2021a, and references therein).

Molecular probes designed from sequences of *Pseudo-nitzschia* taxa from different geographical areas have to be tested for specificity and

cross-reactivity with non-target organisms before applying them for species detection, because of the high genetic variability within the genus (Parsons et al., 1999; Orsini et al., 2002). A study in Scottish coastal waters showed historically that not all FISH probes originally designed for *Pseudo-nitzschia* from the US west coast could be successfully applied due to a single-base pair change in some populations from Scotland (Turrell et al., 2008).

A series of Fluorescence Resonance Energy Transfer (FRET) hybridization probe assays targeting *P. australis*, *P. delicatissima*, *P. fraudulenta*, *P. pungens*, as well as *P. seriata* and *P. multiseriata* (Keady, 2010) have been developed and validated for distinguishing *Pseudo-nitzschia* cell abundance at the species level. The developed assays for all six *Pseudo-nitzschia* species target the ITS1 region of the rRNA gene. The sensitivity of the two assays were assessed through serial dilutions of known cell concentrations and verified in spiked field samples. The limit of detection (LOD) for the *P. multiseriata* and *P. seriata* assays was 400 cells L⁻¹, whereas for the other four species the LOD was consistently established at <10 copies for each target, which equates to approximately one cell. Performance evaluations and results of the molecular assays were compared to the microscopic method for determination of cells of *Pseudo-nitzschia* species in field samples from the Irish National Monitoring Program, with high correlation between the two methods, and with no false positives by the FRET hybridization technique.

Metabarcoding is a high throughput method (reviewed in Hoerstmann et al., 2022), in which PCR amplicons are generated using molecular marker targeting, e.g., parts of the 18S or 28S rRNA gene, to generate a wide variety of amplicons of diverse taxa for simultaneous detection and discrimination of individual taxa. The method has been demonstrated to be suitable for detection of HAB taxa even within complex environmental samples (e.g., Jacobs-Palmer et al., 2021). Metabarcoding can thus reveal the presence of *Pseudo-nitzschia* species at an early stage of a toxic bloom initiation and could be a useful tool in future routine monitoring. In a recent study of two years harmful microalgae monitoring in Tokyo Bay, Japan (including *Pseudo-nitzschia* species) using metabarcoding and applying universal primers targeting the 18S ribosomal RNA gene, has been shown to enable the detection of twice as many HAB-associated species than by LM. However, some species were detected only based on morphology using light microscopy. These findings show the strong need of using several markers in metabarcoding approaches (Sildever et al., 2022, 2023).

Metabarcoding has been deployed for detection of *Pseudo-nitzschia* along with other microeukaryotic taxa in natural phytoplankton assemblages, primarily on the Pacific coast of North America, including British Columbia coastal waters (Esenkulova et al., 2020) and the adjacent Salish Sea, USA (Gallego et al., 2020), and from the Barbara Basin, California, USA (Armbrecht et al., 2021). Compared to the eastern and western Pacific, there are fewer published research studies on barcoding approaches to diatom diversity that have also targeted *Pseudo-nitzschia* distribution from coastal European seas (Piredda et al., 2018), and none have been comprehensively integrated into monitoring strategies. Research metabarcoding surveys of *Pseudo-nitzschia* in northern European and Arctic gateway waters have included Oslofjord and the northern Skagerrak (Piredda et al., 2018; Gran-Stadniczenko et al., 2019), as well as the coast of Brittany and the Western English Channel (Piredda et al., 2018), the Arctic gateway to the Svalbard archipelago and adjacent northern Norwegian coast (e.g. Fig. 4 herein), and Iceland and west Greenland (Elferink et al., 2020) have been addressed only to a very limited extent.

Currently, the problem of species and toxin confirmation could be addressed by direct analysis of DA in plankton and species identification within particular blooms or even under pre-bloom conditions as an element of an early warning system (EWS) of an impending AST event. Semi-quantitative DA-antibody tests are already available, and the biosynthetic genes are known for *Pseudo-nitzschia*. Risk assessment of *Pseudo-nitzschia* blooms could be enhanced by incorporation of rapid screening for the toxin and biosynthetic genes rather than just the

species-type from molecular probes or microscopic identification. As an example of a national response strategy, in 2007, the Marine Institute, Ireland established a phytoplankton molecular unit focusing on the development of molecular assays targeting specific DNA gene regions to distinguish between toxigenic and non-toxigenic species and strains of the same species. These assays are highly sensitive and specific, with high sample throughputs resulting in rapid turnaround times. This technology has been established in routine monitoring for the detection of toxigenic species of the genera *Pseudo-nitzschia*, *Alexandrium* and *Azadinium*. This method supports and compliments the routine microscopy monitoring and has provided valuable early warning information to the aquaculture industry and regulatory authorities.

At this stage there are no definitive molecular markers to unequivocally discriminate between toxigenic and non-toxigenic *Pseudo-nitzschia* populations in accordance with molecular taxonomic characteristics of “species” nor to discover sexually reproducing populations in the field. Recent increased knowledge on *Pseudo-nitzschia* genomics and biosynthetic capacity and pathways of DA production in *Pseudo-nitzschia* (Brunson et al., 2018; Harðardóttir et al., 2019b; Dermastia et al., 2022) will allow in the near future the development of toxin biosynthesis gene-specific qPCR assays providing evidence of DA production within field populations, independent of the species detected. Such a technique would be analogous to the qPCR assays developed for detection of elements of the saxitoxin STX-gene cluster among various species and populations of the dinoflagellate *Alexandrium* Halim and other potentially PST-producing taxa (Murray et al., 2019).

Within the last two decades there have been significant advances in the simultaneous detection of *Pseudo-nitzschia* species in environmental samples based on automated systems. The Environmental Sample Processor (ESP, Scholin et al., 2009), for example, is an autonomous robotic device with deployment capabilities for *in situ* near real-time semi-quantitative detection of marine organisms in complex environmental samples via a Sandwich Hybridization Assay (SHA, Bowers et al., 2017). The ESP autonomously collects discrete water samples and automates application of molecular probes to identify specific plankton (Davidson et al., 2016). This method is based on cell homogenates and takes advantage of large pools of cellular rRNA transcripts (Cangelosi et al., 1997). The SHA can quantify molecular signatures from multiple species in crude lysates across a broad range of sample matrices (e.g., Harvey et al., 2013; Bowers et al., 2017) and has routinely proven comparable to traditional methodologies for species detection (e.g. Anderson et al., 2005; Goffredi et al., 2006; Doll et al., 2014). The ESP system has also been designed for on-line extraction of DA from *Pseudo-nitzschia* cells for near-simultaneous immunodiagnostic detection of toxin during ESP deployments for *Pseudo-nitzschia* blooms (Doucette et al., 2009; Bowers et al., 2016).

11. Concluding perspectives

The number of modelling investigations that combine physical and/or chemical variables with *Pseudo-nitzschia* presence and abundance data have increased dramatically in recent years. Yet there is still a major deficit in generating plausible long-term scenarios applicable at the species level. Current *Pseudo-nitzschia* models are not usually species specific, or have not defined the habitat type/ecological niche to provide reliable projections on favorable/optimal conditions for bloom populations with multiple ecotypes. The incorporation of biological factors, e.g. from autecological studies in culture or marine enclosures (mesocosms) into hydrodynamics model would help to define the ecological niche for *Pseudo-nitzschia* species and describe species interactions (e.g., with grazers or competitors) for growth dynamic equations.

Conceptual models coupling hydrodynamics with biological responses to environmental variables can be effective in designing monitoring programs and scenario building, but capacity building in statistical expertise and data base management remains a critical requirement for effective short-term forecasting and EWS development.

Short term HAB forecasts are already successful to some extent in providing information to shellfish farmers and monitoring agencies. Increasing the projection length for these forecasts to monthly, and then seasonal time-scale would fill a critical gap between urgent real-time responses and long-term regional climate scenarios.

Beyond conceptual models, non-linearity of processes and subtle changes that can lead to community assemblage disruption need to be elucidated. Unfortunately, blooms that are producing DA are not discriminated from non-toxicogenic ones in existing models. Ensemble numerical modelling approach is a future need in order to estimate uncertainties in global numerical ocean model, however, this requires a substantial increase in financial investment and expertise. Increased model resolution by downscaling (ensemble, spatial and temporal expansion) must incorporate observational data from off-shore HAB sampling stations. Parametrization of such models at the regional spatial scale will require finer resolution of data on species discrimination and cell abundance, as well as particulate DA toxin data to feed into models.

At this stage, *Pseudo-nitzschia* models do not fit the criteria of turn-key climate services for delivery to aquaculture planning and development and resource managers for long-term risk assessment. Models defined as climate services are designed by scientists and technical experts but are often not useful for the non-scientific or non-technical community. Effective application of models for *Pseudo-nitzschia* cell abundance, biogeographical distribution and bloom dynamics requires attention to the knowledge for interpretation, as well as criteria for spatial planning and multiple risks responses by the end-user communities, comprising the shellfish industry, regulatory and public health authorities and marine resource managers.

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.

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Supplementary materials

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