

Diversity and seasonality of *Pseudo-nitzschia* (Peragallo) at two North Sea time-series monitoring sites

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Abstract Species within the diatom genus *Pseudo-nitzschia* (Peragallo) have been identified as producers of domoic acid, the toxin responsible for amnesic shellfish poisoning. Toxin- and non-toxin-producing species of *Pseudo-nitzschia* have been reported globally; however, as *Pseudo-nitzschia* species cannot be routinely identified to species level using light microscopy, cells are rarely recorded to species level during long-term monitoring studies. Here, we report the results of a comparative survey of *Pseudo-nitzschia* species at two monitoring sites in the North Sea: Stonehaven on the east coast of Scotland and Helgoland Roads in the German Bight. A difference in the seasonality of this genus was observed between the sites with *Pseudo-nitzschia* cells playing a major role in the spring bloom as well at the summer and autumn diatom community at Stonehaven. In contrast, *Pseudo-nitzschia* was abundant only during the summer months at Helgoland. *Pseudo-nitzschia* cells constitute a higher proportion of the diatom community at Stonehaven than at Helgoland, particularly during the late summer, autumn and winter and thus may be considered more ‘ecologically important’ at this site. A total of eight different species were recorded during this survey with five *Pseudo-nitzschia* species observed at the Helgoland site: *P. pungens*, *P.*

fraudulenta, *P. americana*, *P. cf. delicatissima* and the potentially toxic species *P. multiseriis*. Six species were also recorded at Stonehaven; *P. australis*, *P. cf. delicatissima*, *P. pungens*, *P. cf. pseudodelicatissima*, *P. subpaci-fica* and *P. seriata*. This study represents the first examination of the seasonality of *Pseudo-nitzschia* species around Helgoland and the first comparison between two long-term monitoring sites in the North Sea. *P. americana* and *P. multiseriis* are also recorded at the Helgoland Roads time-series site for the first time.

Keywords *Pseudo-nitzschia* · Diversity · North Sea · Time series · Helgoland · Stonehaven

Introduction

The diatom genus *Pseudo-nitzschia* (Peragallo) is the focus of considerable international scientific attention due to the production by some species of the amnesic shellfish toxin domoic acid (DA) (Hasle 2002; Trainer et al., 2012). Since the discovery of DA associated with human mortalities in the late 1980s, the presence of toxin-producing *Pseudo-nitzschia* species has been confirmed worldwide (Villac et al. 1993; Hasle 2002; Lelong et al. 2012; Trainer et al. 2012). DA contamination in shellfish has been reported from many European states particularly along the Atlantic (Hasle 2002) and Mediterranean coasts (Costa and Garrido 2004). In the North Sea, the occurrence of ASP has been reported from northern regions; Scotland, Norway, Denmark and Sweden (Gallacher et al. 2001; Hasle 2002; Fehling et al. 2006; Trainer et al. 2012). Reports of DA and shellfish closures from the southern North Sea are less frequently recorded although *Pseudo-nitzschia* cells are observed in the water column with reports from the

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Netherlands, English Channel and the north coast of France (Vrieling et al. 1996; Percy et al. 2006; Downes-Tettmar et al. 2013).

Long-term phytoplankton time series are considered essential for the examination of changes in the marine ecosystem with many of the current European environmental assessments being made on an ocean-scale system (McQuatters-Gollop 2012). Within the North Sea region, data from two long-term phytoplankton time series, Helgoland Roads, Germany (south east North Sea), and Stonehaven, Scotland (north-west North Sea), are currently being used to identify variations and changes to the phytoplankton community over time (Wiltshire et al. 2008; Bresnan et al. 2009; Löder et al. 2012)).

In Scotland, *Pseudo-nitzschia* is observed in phytoplankton samples throughout the year (Fehling et al. 2006; Turrell et al. 2008). This genus is considered a key member of the phytoplankton community in this region, and *P. australis* and *P. seriata* are confirmed as DA producers (Fehling et al. 2004). Although it is not possible to routinely identify *Pseudo-nitzschia* cells using light microscopy, they can be separated into different size categories. An examination of *Pseudo-nitzschia* in Scotland has identified a distinct seasonality within this genus. *Pseudo-nitzschia* ‘delicatissima-type’ cells (diameter < 3 µm) dominate in the spring time of the year, while *Pseudo-nitzschia* ‘seriata-type’ cells (diameter > 3 µm) dominate in the late summer/early autumn (Fehling et al. 2004; Brown and Bresnan 2008). The diversity of *Pseudo-nitzschia* is considered high in Scottish waters with transmission electron microscopy (TEM) used for identifying 13 species to date (Fehling et al. 2004; Bresnan et al. 2008; Brown and Bresnan 2008; Turrell et al. 2008). For comparison, nine species have been identified in sediments of the Mariager Fjord in Denmark (Lundholm et al. 2010). As well as being the identified cause of extensive closures of the offshore scallop fishing grounds at the end of the 1990s (Gallacher et al., 2001), DA has also been reported in the faeces, urine and amniotic fluid in seals (*Phoca vitulina*) from Scottish waters (Hall and Frame 2010). *Pseudo-nitzschia* has been less problematic in the southern North Sea; however, *P. multiseriata* was identified as a DA producer in the Wadden Sea and English Channel (Vrieling et al. 1996; Percy et al. 2006). Investigations into the dynamics of *Pseudo-nitzschia* in the English Channel has shown that DA detected in the water column was associated with the ‘*P. seriata*’ group (>3 µm diameter) as identified by light microscopy (Downes-Tettmar et al. 2013). TEM of *Pseudo-nitzschia* in the eastern English Channel associated DA in the water column with the presence of *P. australis* (Klein et al. 2010).

At the Helgoland long-term monitoring site, *Pseudo-nitzschia* is not routinely identified to species level.

Although the most current phytoplankton checklists for Helgoland (Drebes and Elbrächter 1976; Hoppenrath 2004) list *Pseudo-nitzschia pungens*, *P. fraudulenta* and *P. delicatissima* as occurring around Helgoland, no data about the seasonality of these species are available. A recent cruise in the waters of the German Bight did not detect of DA in the area around Helgoland, and no information was reported about the *Pseudo-nitzschia* species present (Krock et al. 2013). There has yet to be a detailed study of the diversity of *Pseudo-nitzschia* species in this area.

Current European directives such as the Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD) require the marine waters of member states to achieve ‘Good Ecological Status’ and ‘Good Environmental Status’, respectively. Inherent in this is the requirement for an understanding of the dynamics of the phytoplankton community on a regional sea scale. This assessment is not straightforward owing to the paucity of baseline information available. Initiatives to address this are being led by the Oslo–Paris Commission (OSPAR) Intersessional Correspondence Group for Coordinated Biodiversity Assessment and Monitoring (ICG-COBAM). In this study, the diversity and seasonality of *Pseudo-nitzschia* is assessed at two long-term monitoring sites (Stonehaven and Helgoland) with a view to identifying regional differences in a key diatom genus.

Methods

Long-term monitoring sites

The location of the two monitoring stations can be seen in Fig. 1. The Helgoland Roads monitoring site (54°11.3′N, 7°54.0′E) is located approximately 70 km from the German mainland in a channel between the main island of Helgoland and a small sandy island (the dune). The water depth varies from 6–8 m in depth depending on the tide. Further information about this site can be found in Franke et al. (2004) and Wiltshire et al. (2010). Sampling for phytoplankton began at this site in 1962. The Stonehaven monitoring site (56°57.8′N, 02° 06.2′W) is located 5-km offshore and 50-m depth. Further details can be found in Bresnan et al. (2009). Sampling for phytoplankton began at this site in 1997.

Sampling and analysis methodology

Helgoland

Surface water samples were collected daily from the Helgoland Roads monitoring site using a bucket. The samples were mixed well, and 100 ml subsample was preserved in

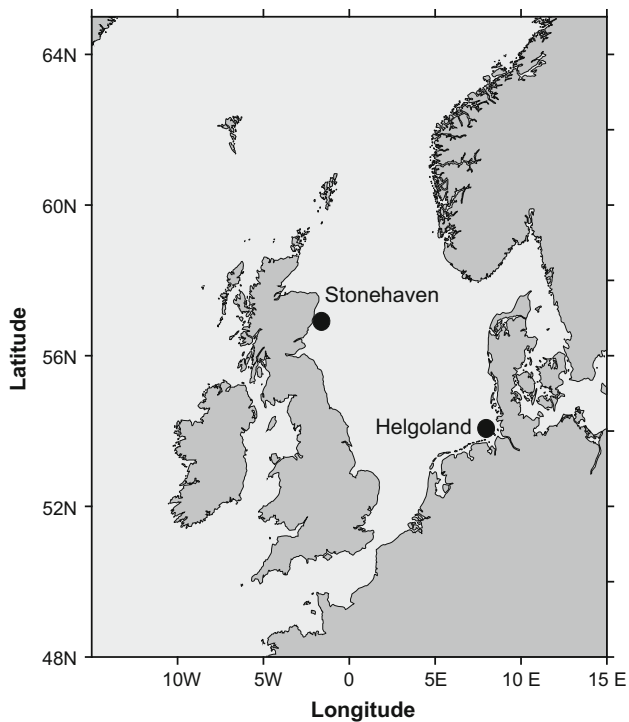


Fig. 1 Map showing location of the Stonehaven and Helgoland Roads time-series monitoring sites

0.1 % neutral Lugol's iodine and stored in an amber glass bottle before 25 ml is analysed using the Utermöhl method (Lund et al. 1958). These counts provided the baseline data used in this study (see Fig. 2b). In addition, to the bucket samples, net samples were taken twice weekly. Two horizontal hauls with a 20- and 80- μm net were carried out. Samples were fixed in hexamine-buffered formalin (4 %), (Thronsen 1978) and stored in 30-ml amber glass bottles for TEM analysis. Temperature and salinity at this site were measured in the bucket sample using a thermometer and a Guildline salinometer (Autosal, Gamma Analysen Technik GmbH), respectively. Temperature and salinity data from 2002 to 2011 are used to describe the seasonal cycle at this site.

Stonehaven

Samples from the Stonehaven site were collected weekly using a 10-m integrated tube sampler. The sample was well mixed, and 1 L subsample was preserved in 0.5 % Lugol's iodine (Thronsen 1978). Fifty millilitres of sample was analysed immediately for the presence of *Pseudo-nitzschia* cells using the Utermöhl method (Lund et al. 1958) and the remaining 950 ml stored in an amber glass jar, in the dark and at 5 °C before analysis by transmission electron

microscopy (TEM). Full community phytoplankton analysis was performed using the Utermöhl technique as described in Bresnan et al. (2009). Niskin bottles fitted with digital reversing thermometers were deployed to surface and 45-m depths to collect water for salinity and chemical nutrient analysis. In some instances, material from zooplankton samples collected by a vertical 40-cm-diameter bongo (68- μm mesh) net haul from 45 m to surface was used for TEM analysis. These samples were preserved in 4 % borax-buffered formaldehyde until analysis. Water samples for salinity analysis were stored in 200-ml salinity sample bottles in a temperature-controlled room (21 °C) for at least 24 h prior to analysis using a Guildline 8410A Portasal salinometer. Temperature and salinity surface water data from 2002 to 2011 are used to describe the seasonal cycle at this site.

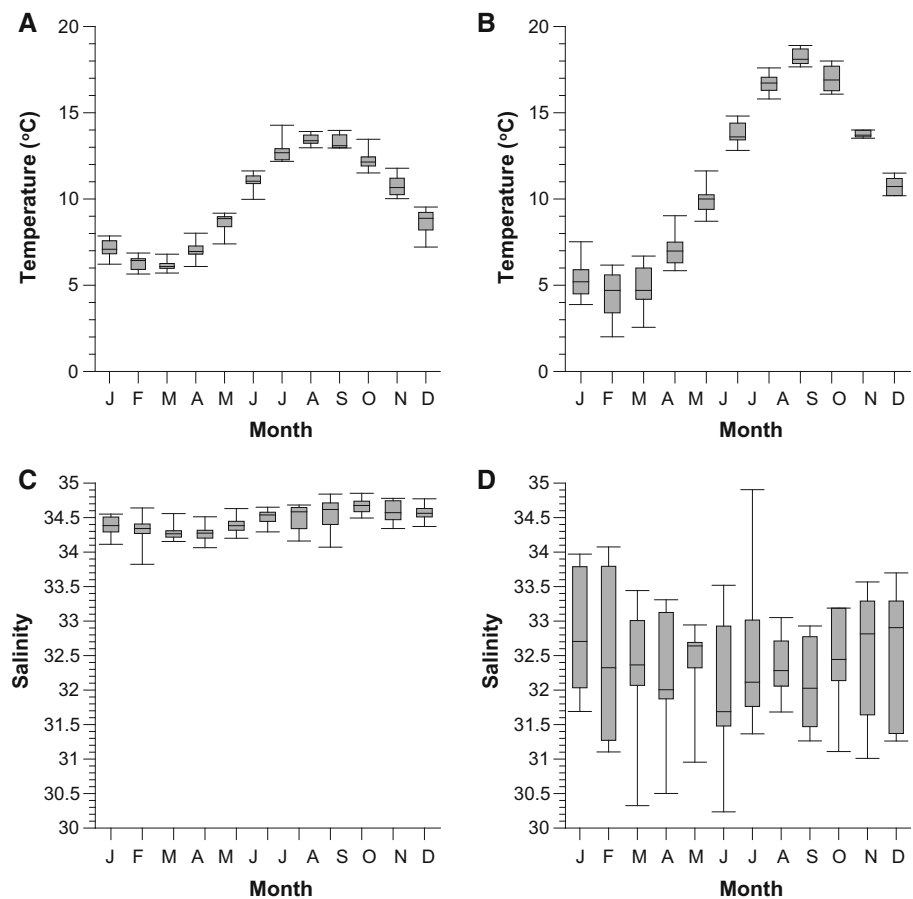
Pseudo-nitzschia cell counts from 2002 to 2011 were used to examine the temporal abundance of *Pseudo-nitzschia* at the two monitoring sites. All cell count data are subject to quality control examination. Identification and enumeration of *Pseudo-nitzschia* cells using light microscopy received ISO 17025 accreditation at Marine Scotland Marine Laboratory in 2005. Identification of *Pseudo-nitzschia* cells using TEM was accredited in 2008.

Transmission electron microscopy

Integrated tube samples from the Stonehaven site were left to stand for 1 week to allow the phytoplankton cells to sink to the bottom of the amber glass jars. The top 900 ml was gently siphoned off and the remaining 50 ml centrifuged at 3000 rpm for 15 min. The supernatant was gently removed leaving a final volume of 5 ml. Thirty millilitres of net sample from the Helgoland Roads site was concentrated by centrifuging at 3000 rpm for 15 min. The supernatant was removed leaving a final sample volume of 5 ml. Samples were cleaned using the method described by Lundholm et al. 2002, mounted onto 200-mesh Formvar-covered grids and left to air dry before being analysed using a Philips CM10 TEM.

Zooplankton net samples collected during August 2002, July 2003, August 2004 and September 2004 were used to examine material from the Stonehaven monitoring site. In this instance, 5 ml aliquot of material was suspended in 30 ml of distilled water in a 50-ml graduated cylinder. The mixture was inverted up to 20 times to ensure homogenisation. The samples were let settle for 10 min to let the heavy material (e.g. zooplankton) sink to the bottom. The top 25 ml was siphoned off and concentrated to a volume of 5 ml by centrifuging at 3000 rpm for 15 min. Samples

Fig. 2 Box plots of monthly temperature and salinity averages from 2002 to 2011 at both monitoring sites: **a** temperature—Stonehaven, **b** temperature—Helgoland, **c** salinity—Stonehaven and **d** salinity—Helgoland



were cleaned using the method described by Lundholm et al. 2002, mounted onto 200-mesh Formvar-covered grids and left to air dry before being analysed using a Philips CM10 TEM.

Samples from each monitoring site containing high numbers of *Pseudo-nitzschia* as determined by light microscopy were analysed using TEM. Samples were spread over the growing period from April to September. Helgoland samples from August/September 2005, April 2006, June 2006, July 2006, April 2008, August 2008 and June 2009 and Stonehaven samples from July 1998, July 2002, September 2002, August 2002, July 2003, August 2004, September 2004, April 2007 and June 2007 were analysed. *Pseudo-nitzschia* cells were identified to species level using criteria described in (Skov et al. 1999; Lundholm et al. 2002, 2003, 2006). As no molecular analysis was performed, *P. delicatissima* is identified as *P. cf. delicatissima* as it is not possible to separate this species from *P. arenysensis* using morphological criteria alone (Quijano-Scheggia et al. 2009). Owing to the difficulty in getting sufficient resolution of the poroids, *P. pseudodelicatissima*-like cells are referred to as *P. cf. pseudodelicatissima* in this study.

Results

Temperature and salinity

Figures 2a–d show the seasonal patterns of temperature and salinity at both sites. The seasonal cycle of temperature is typical of that in the temperate northern hemisphere with the warmest temperatures measured in August/September and the coldest temperatures in February/March. At Stonehaven, in the northern North Sea, the seasonal cycle is less extreme and the lowest temperature observed in the measurement period (2002–2011) was 5.1 °C, while at Helgoland, the lowest temperature measured (2002–2011) was 1.1 °C. The maximum temperatures were considerably higher at Helgoland (varying between 17.0 and 18.7 °C) than at Stonehaven (between 12.2 and 16.2 °C).

Light microscopy cell counts

A different seasonality in the occurrence of *Pseudo-nitzschia* spp. is evident at both sites (Fig. 3a, b). Two different periods of elevated *Pseudo-nitzschia* cell densities can be observed at the Stonehaven site with an initial increase in

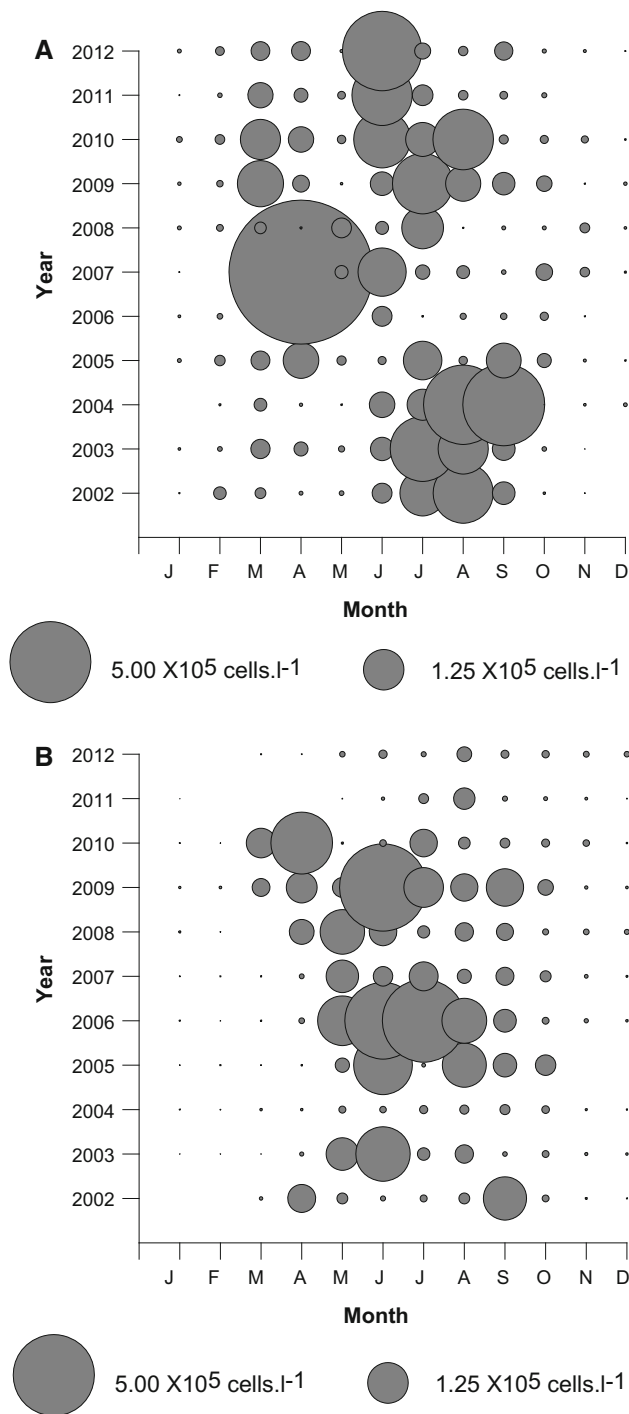


Fig. 3 Graphs showing monthly averages of *Pseudo-nitzschia* cell counts from **a** Stonehaven and **b** Helgoland Roads sites

numbers in spring during March and April and then a larger bloom in summer or early autumn (Fig. 3a). The intensity of these blooms varied from year to year over the time series with the timing of the highest abundance varying between spring, summer and autumn throughout the time series. The highest cell count of *Pseudo-nitzschia* species at Stonehaven (1.75×10^6 cells L^{-1}) was recorded during the

intense bloom observed during the spring of 2007. *Pseudo-nitzschia* cell densities at Helgoland Roads did not observe the same seasonality, and higher cell densities were observed during late spring and early summer (May–July). The highest cell densities were observed during June 2009 when cell densities reached a maximum of 9.25×10^5 cells L^{-1} (Fig. 3b). In common with the Stonehaven site, the cell densities at Helgoland Roads showed considerable internal variation from year to year with particularly low cell densities observed since 2010. The growing season for *Pseudo-nitzschia* species in general was shorter at Helgoland with *Pseudo-nitzschia* rarely appearing before April and virtually absent also after November in contrast to the Stonehaven site where *Pseudo-nitzschia* begin to increase from February onwards. Even during the winter months it is usual to record low numbers (100 cells L^{-1}) in the water column at the Stonehaven monitoring site.

Pseudo-nitzschia as a proportion of the total diatom community

Figure 4a, b show the % *Pseudo-nitzschia* cells in the total diatom community at both sites. At Stonehaven, although *Pseudo-nitzschia* is relatively abundant between February and May, it represents a relatively small % of the total diatom community (<10 %). This is due to the very high abundance of other diatom genera such as *Skeletonema*, *Thalassiosira* and *Chaetoceros*, which are dominant components of the spring bloom. During the late summer/early autumn months, the percentage of *Pseudo-nitzschia* cells in the diatom population increases, comprising between 20–25 % from July to September. In winter months, diatom cell density at Stonehaven is very low (<5,000 cells L^{-1}) and *Pseudo-nitzschia* cells are often the dominant part of this community (albeit at very low cell densities).

In contrast, *Pseudo-nitzschia* comprises a much smaller proportion of the diatom population at Helgoland (mostly <5 %), with the exception of June when *Pseudo-nitzschia* cell densities represent 15 % of the diatom population. The diatom community on Helgoland is very variable on annual scale with *Thalassionema nitzschioides*, *Asterionellopsis* and *Thalassiosira* species as well as *Chaetoceros debilis* being dominant bloom forming species. Higher diatom cell densities are recorded at Helgoland than at Stonehaven during the autumn and winter, and *Pseudo-nitzschia* cells comprise a much smaller part of the diatom population during this period.

Pseudo-nitzschia diversity

Six species of *Pseudo-nitzschia* were found in samples from Stonehaven (Fig. 5: *P. cf. delicatissima*, *P. pseudo-delicatissima*, *P. pungens*, *P. seriata*, *P. australis* and

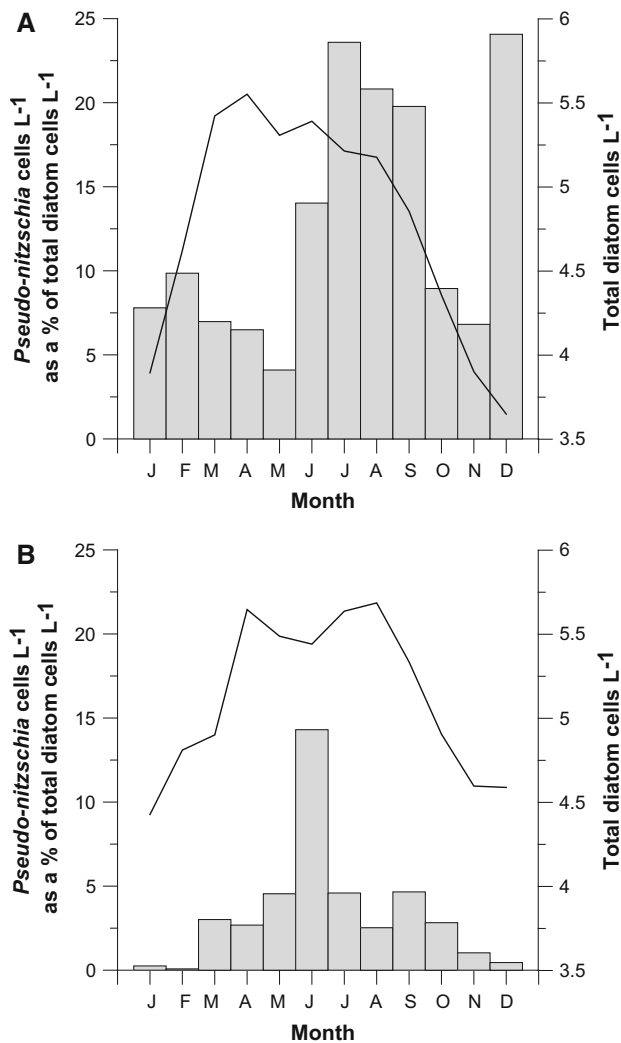


Fig. 4 Plots showing *Pseudo-nitzschia* cells L⁻¹ as a % of the monthly averaged total diatom cell count at **a** Stonehaven and **b** Helgoland over the duration of the study. *Pseudo-nitzschia* cells are represented by a bar and the total diatom cell count by the solid line

P. subpacificus) and five species were found from Helgoland (Figs. 6, 7: *P. americana*, *P. cf. delicatissima*, *P. fraudulentus*, *P. pungens* and *P. multiseriatus*). The morphological dimensions recorded in the identification of these cells are presented in Table 1. The diversity of the *Pseudo-nitzschia* populations at the two sites during different times of year is summarised in Table 2. *P. delicatissima* and *P. pseudo-delicatissima* were more abundant at the Stonehaven site during the spring time, while *P. australis*, *P. seriata* and *P. subpacificus* were observed only at the Stonehaven site during late summer/early autumn. Only one cell of *P. subpacificus* was observed, collected during August 2004. In contrast to the Stonehaven site, *P. multiseriatus* was only observed at the Helgoland site and was detected during both spring and late summer/early autumn.

Although not observed in samples analysed from the Stonehaven site during the course of this study, *P. multiseriatus* and *P. americana* have previously been recorded at Scottish sites from the west coast (Fehling et al. 2006; Brown and Bresnan 2008). This study is the first record of these species at Helgoland Roads.

Discussion

Analysis of time-series data is becoming increasingly important to understand long-term changes in marine ecosystems (Wiltshire et al. 2010). A thorough knowledge of regional biodiversity is particularly important with respect to identifying the impacts from environmental and anthropogenic impacts as detailed biodiversity inventories form the baseline for judging future changes (Edwards et al. 2010). Such knowledge is particularly important as recent scenarios of climate change (IPCC 2007) predict considerable temperature increases in marine waters, which are likely to be associated with changes in marine biodiversity including that of harmful species (Peperzak 2003, Moore et al. 2008).

Several *Pseudo-nitzschia* species are now considered to be cosmopolitan or at least to have a very wide distribution (Hasle 2002; Casteleyn et al. 2008, 2009). Nevertheless, at a regional-scale analysis of time-series data from both the Helgoland and Stonehaven, time series showed considerable differences in the species composition in the examined seasonal samples, with only two species, *P. delicatissima* and *P. pungens* occurring at both sites. New records of two *Pseudo-nitzschia* species, *P. multiseriatus* and *P. americana*, were established at Helgoland Roads.

While *Pseudo-nitzschia multiseriatus* might be considered a cosmopolitan species (Evans 2004, Doucette et al. 2008) and has also been observed in the southern North Sea, i.e. the Dutch Wadden Sea (Vrieling et al. 1996), the distribution of *P. americana* is much less known, possibly because it is very inconspicuous and under the light microscope does not show the typical *Pseudo-nitzschia* morphology. It has only been reported infrequently from different locations globally, e.g. the English Channel (Klein et al. 2010), Scotland (Bresnan et al. 2002; Fehling et al. 2006), Bay of Fundy (Kaczmarek et al. 2005), from the Japan and Okhotsk Seas (Orlova and Shevchenko 2002) and also from Australian waters (Hallegraeff 1994) and Denmark (Lundholm et al. 2010). It is sometimes observed on the setae of *Chaetoceros* cells (Horner 2002).

Both sites differ in their general hydrography with the Helgoland site being very shallow (maximum 8 m depending on the tide) and exposed to strong currents. It is therefore almost always well mixed. The Stonehaven site is

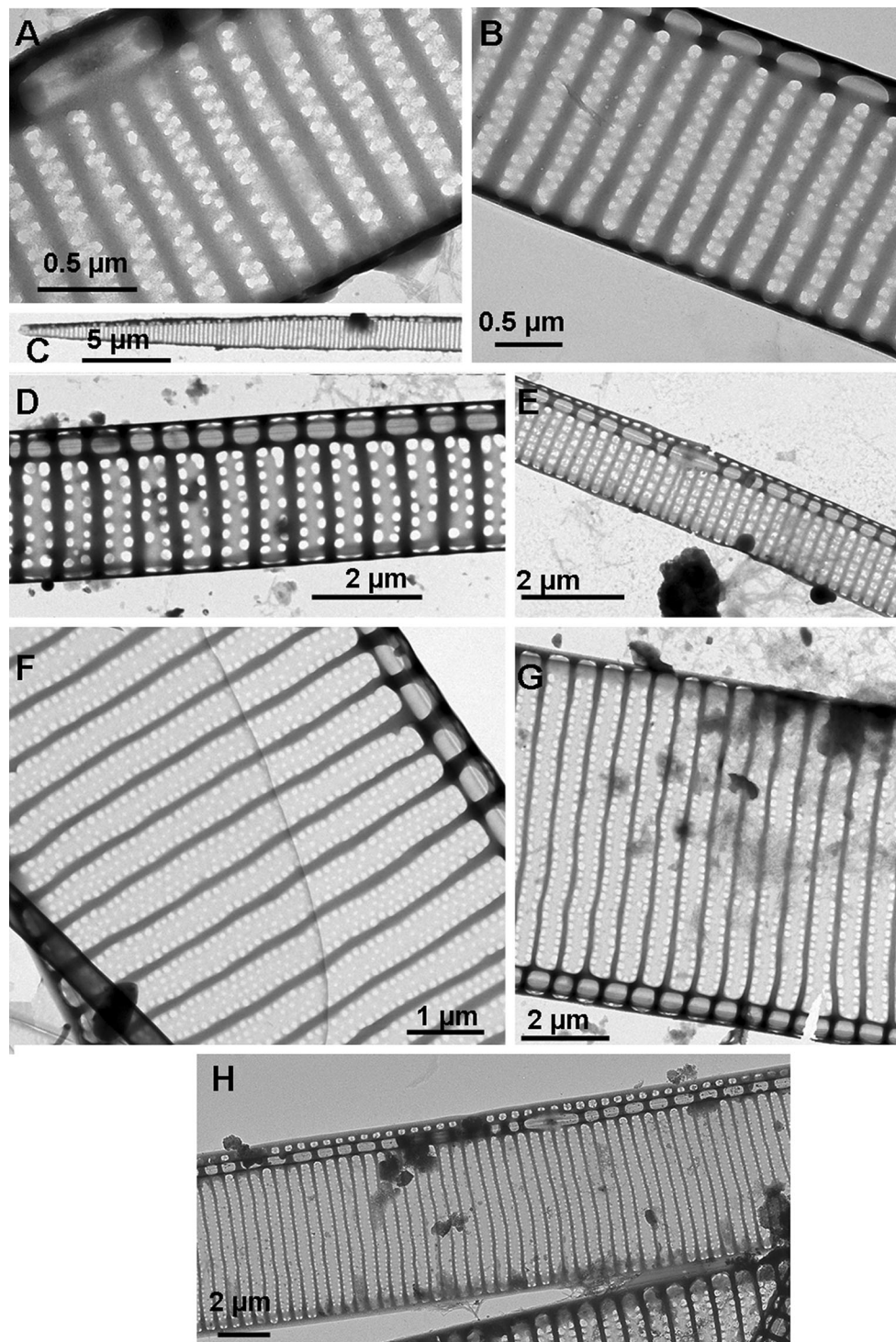


Fig. 5 TEM micrographs of *Pseudo-nitzschia* species from Stonehaven: **a–c** *P. cf. delicatissima*, **d** *P. pungens*, **e** *P. pseudodelicatissima*, **f** *P. seriata*, **g** *P. australis* and **h** *P. subpacific*a

also well mixed but is 50 m deep with a strong southerly current. At both sites, following the general pattern observed in the North Sea, there is also a long-term trend of increasing temperatures. At the Helgoland Roads site, sea

surface temperatures have increased by 1.67° in the past 45 years (Wiltshire et al. 2010; Holt et al. 2012), a warming rate of 0.37 °C per decade (Hughes et al. 2009; Wiltshire et al. 2010). Stonehaven follows the warming

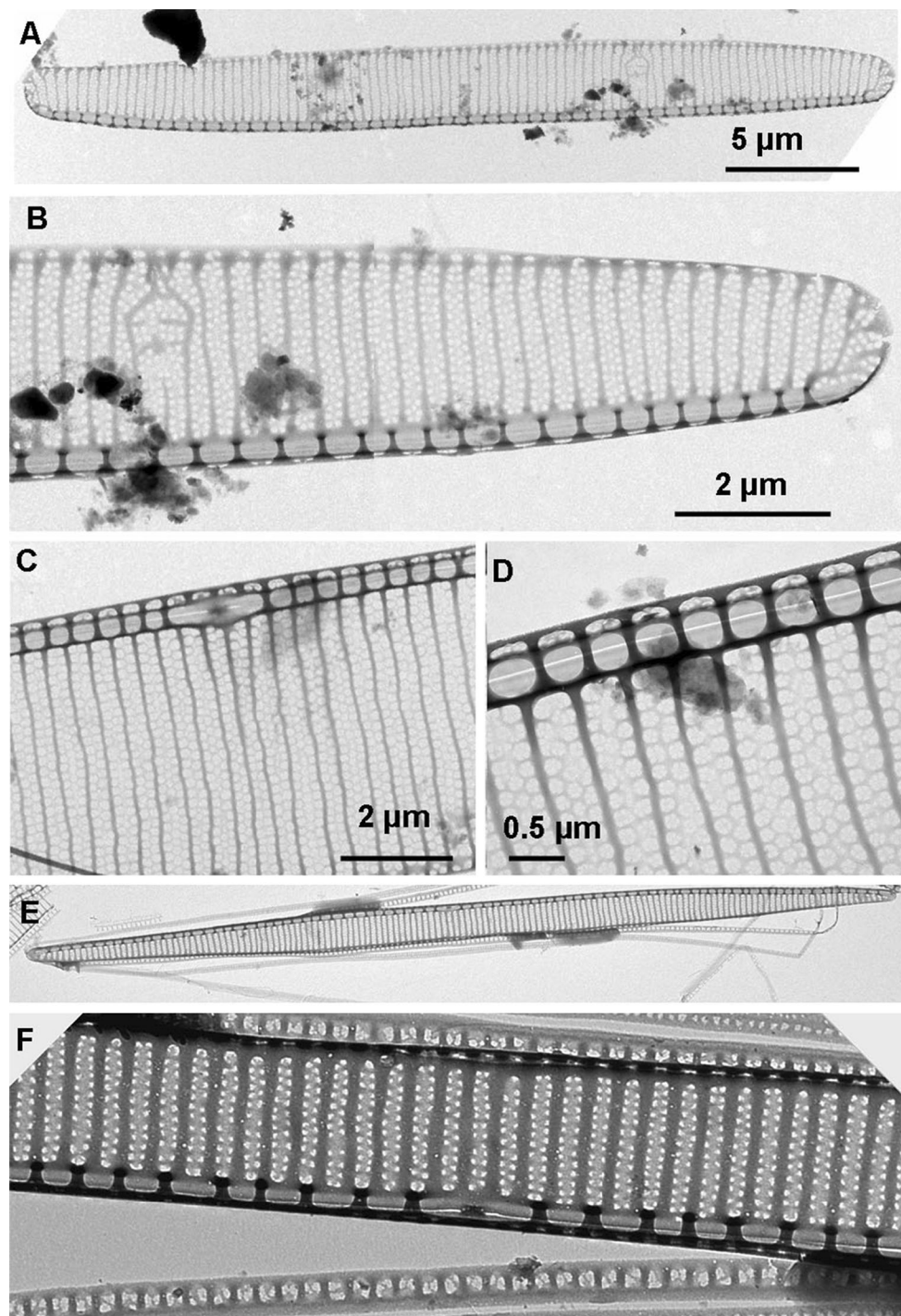


Fig. 6 TEM micrographs of *Pseudo-nitzschia* species from Helgoland Roads: **a–b** *P. americana*, **c–d** *P. fraudulententa* and **e–f** *P. cf. delicatissima*

trend observed in the northern North Sea (Hughes et al. 2009), which is slightly lower than that observed further south. In the northern North Sea, warming has occurred at a rate of 0.23° per decade over the period 1970–2010 (Hughes et al. 2010).

Both sites experience different salinity regimes. At Stonehaven, surface salinity is influenced by a small out-flow of freshwater from a nearby river and varies between 32.91 and 34.95. These fresher water episodes only happen once or twice per year, are very short in duration lasting

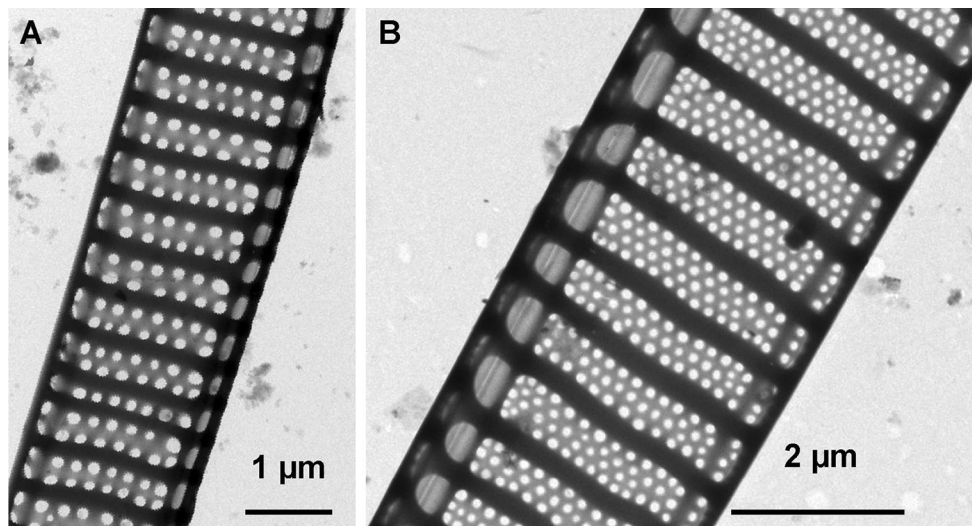


Fig. 7 TEM micrographs of **a** *P. pungens* and **b** *P. multiseriis* from Helgoland Roads time series

Table 1 Morphometric dimensions of *Pseudo-nitzschia* cells identified using TEM

Species\Metric	Shape	Length	Width	Central Interspace (Y/N)	No. of fibulae (10 µm)	No. of striae (10 µm)	No. of rows of poroids	No. of poroids (1 µm)
<i>P. americana</i>	rect	31.6–32.6	2.8–3.2	N	19–21	28	2	9
<i>P. australis</i>	lanc	80.2–121	7.2–8.2	N	14–17	15–18	2	5
<i>P. cf. delicatissima</i>	lanc	45.4–57.3	2.0	Y	22–24	36	2	8–9
<i>P. fraudulenta</i>	lanc	73.3–81.8	5.4–7.0	Y	21–24	20–24	2–3	6
<i>P. multiseriis</i>	lanc	99.2–190.4	4.5–4.7	N	11–14	10–12	3–4	6
<i>P. cf. pseudodelicatissima</i>	lin	63.34	1.21	Y	23	39	1	5–6
<i>P. pungens</i>	lin	106–148	3.4–4.6	N	10–12	10–12	2	3
<i>P. seriata</i>	lanc	80.6–120	7.5–8	N	14–15	16–17	2 (+1/2)	8
<i>P. subpacificica</i>	Lanc	79	5.3	Y	16	27	2	9

Linear, lanceolate and rectangular shape of valves is notated as lin, lanc and rect, respectively

Table 2 Seasonal diversity of *Pseudo-nitzschia* species from TEM results

Species	Spring (March/April)	Summer (May/June/July)	Autumn (August/September)
<i>P. americana</i>	H,-	H,-	-, -
<i>P. australis</i>	-, -	-, S	-, S
<i>P. cf. delicatissima</i>	H, S	-, -	-, -
<i>P. fraudulenta</i>	H, S	H,-	-, -
<i>P. multiseriis</i>	H,-	H,-	-, -
<i>P. pungens</i>	H,-	H, S	H, S
<i>P. pseudodelicatissima</i>	-, S	-, -	-, -
<i>P. seriata</i>	-, -	-, S	-, S
<i>P. subpacificica</i>	-, -	-, -	-, S

The observation of the species from in samples from a season is represented by H (samples from Helgoland), S (samples from Stonehaven) or - (not observed in samples)

less than a week and are confined to the surface layers. Nearbed salinity is generally 34.1, with a median value of 34.53. The coastal waters surrounding Helgoland are much

fresher as a result of the large quantities of freshwater entering the coastal regions in the German Bight (Holliday et al. 2010). Salinity at Helgoland ranged from 23.71 to

36.11 (although such extreme values are recorded very infrequently), and interannual variability was high.

It is possible that Atlantic inflow may influence the species composition within the *Pseudo-nitzschia* genus in the North Sea and may contribute to the higher diversity in areas exposed to Atlantic waters. For instance, *Pseudo-nitzschia australis* and *seriata* as well as closures of shellfish harvesting areas as a result of high concentrations of ASP in shellfish flesh have been observed along the Atlantic coasts of Spain, Portugal, France, Ireland and Scotland (Lelong et al. 2012; Trainer et al. 2012) while the occurrence of confirmed DA-producing species and ASP closures in the southern North Sea has been much less frequently observed. Examination of the ecological importance of *Pseudo-nitzschia* genus at both sites shows that *Pseudo-nitzschia* comprises a larger portion of the diatom population at the Stonehaven monitoring site than at the Helgoland. This is particularly apparent from July to December when diatom cell abundance can be dominated by *Pseudo-nitzschia*, and this may have implications for the transfer of DA through the marine food web. In contrast, at Helgoland, blooms of other genera such as *Chaetoceros*, *Guinardia*, *Leptocylindrus* and since 2010, *Mediopyxis helysia* can dominate the community.

Historic investigations into the diversity of *Pseudo-nitzschia* species in the North Sea are scarce. Hasle et al. (1996) observed a shift from *P. multiseriata* to *P. pungens* in the Skaggeerak during the 1970s and 1980s and attributed this to increasing water temperatures. *P. multiseriata* has a greater tolerance for low water temperatures, and this may be reflected in the distribution observed in this study (Hasle 1995). Averaged winter temperatures in Scottish coastal waters rarely dip below 5 °C (Holt et al. 2012), while in Helgoland these temperatures are frequently observed. Despite the observed increase in annually averaged temperatures since 1962 and the disappearance of incidences of sea ice in winter, winter temperatures frequently drop as low as 2–3 °C.

The diversity of *Pseudo-nitzschia* species in a sediment core from a Danish fjord also revealed a number of diversity changes in European waters over several decades (Lundholm et al. 2010). A shift towards a *P. pungens* dominated community was correlated with increasing nitrate input and increasing temperatures, while an isolated bloom of *P. americana* was correlated with ammonia input. Both of these studies highlight the transient nature of *Pseudo-nitzschia* community diversity and how species dominance can be influenced by environmental variables. A recent investigation by Hinder et al. (2012) has related the increase in *Pseudo-nitzschia* abundance observed in the Continuous Plankton Recorder data since the mid-1990 s with an increase in wind speed and intensity. No investigations have been carried out yet to investigate whether

this change in abundance is associated with a change in diversity; however, Hasle et al. (1996) and Lundholm et al. (2010) both observe a decrease in the DA-producing *P. multiseriata* in the Northern North Sea over the last number of decades.

This study provides the first genus-level comparison study of the diversity of *Pseudo-nitzschia* between two long-term time series within the North Sea. *Pseudo-nitzschia* is a key member of the phytoplankton community at both of these sites, and differences observed in seasonal abundances and community composition within a single diatom genus highlight the difficulties in making ecological status based assessments on a regional sea scale. For the genus *Pseudo-nitzschia*, this is particularly pertinent, as this genus contains a large number of species that are difficult to identify with the easy-to-use methodologies available to those carrying out routine monitoring. In most areas, including those in this study where detailed assessments were carried out, species diversity within this genus was much higher than previously known. This shows the importance of having baseline diversity data which can facilitate the identification of community changes which may occur over a number of decades. For toxin-producing genera such as *Pseudo-nitzschia*, this is particularly important as the recent observation of DA in seals in the North Sea (Hall and Frame 2010) highlights the requirement to understand the dynamics of this toxin-producing genus from an ecological view as well as to advise the aquaculture industry.

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