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Process length variation in cysts of the dinoflagellate *Protoceratium reticulatum*, from surface sediments of the Baltic–Kattegat–Skagerrak estuarine system: a regional salinity proxy

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Results are presented from a regional comparison of average process length variation in cysts of *Protoceratium reticulatum* and *Lingulodinium polyedrum*, extracted from surface sediments in the Skagerrak–Kattegat–Baltic estuarine system, with the environmental variables of seawater temperature and salinity. Although too few cysts of *Lingulodinium polyedrum* were recovered from the sediments to make reliable correlations, cysts of *Protoceratium reticulatum* were well represented, and average process length was correlated significantly with both salinity and temperature. Owing to dominant summer surface production, and regional covariation between salinity and density, we propose the use of the significant correlation with summer sea surface salinity (SSS_{summer}) by the equation $SSS_{summer} = 3.16\times$ average process length -0.84 ($R^2 = 0.8$). Application of this equation down-core in Limfjord (northern Denmark) shows its usefulness as a regional palaeosalinity proxy.

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Salinity contributes significantly to the density of seawater and is an important parameter for tracking changes in ocean circulation and climate variation. The salinity of the oceans has been affected by present-day global climate change (see Reid *et al*. 2009 for a review), and thus palaeosalinity reconstructions are important for understanding this phenomenon, as they enable us to trace past changes of the thermohaline circulation (Schmidt *et al*. 2004). Quantitative salinity reconstructions have been proposed on the basis of several approaches, using, for example, foraminiferal oxygen isotopes (e.g. Wang *et al*. 1995), $\delta^{18}O_{seawater}$ based on foraminiferal Mg/Ca ratios and $\delta^{18}O$ (e.g. Nürnberg & Groeneveld 2006; De Schepper *et al*. 2009), alkenones (e.g. Blanz *et al*. 2005) and δD in alkenones (e.g. van der Meer *et al*. 2008) and the modern analogue technique applied to dinoflagellate cyst assemblages (e.g. de Vernal & Hillaire-Marcel 2000). However, none of these approaches is unequivocal (e.g. alkenones; Bendle *et al*. 2005), and there is a need to improve our capability for utilizing palaeosalinity in climate reconstruction (Rohling 2007).

Some planktonic organisms show morphological changes reflecting salinity, for example variable noding in the ostracod *Cyprideis torosa* (van Harten 2000) and morphological variation in coccoliths of *Emiliania huxleyi* (Bollmann *et al*. 2009). A similar relationship was reported for the process length of *Lingulodinium machaeorophorum*, the cyst of the autotrophic dinoflagellate *Lingulodinium polyedrum*, in the Black Sea (Wall *et al*. 1973) and was subsequently used semi-quantitatively in other regions (Dale 1996; Matthiessen & Brenner 1996; Nehring 1997; Ellegaard 2000; Mudie *et al*. 2001; Brenner 2005; Sorrel *et al*. 2006; Head 2007; Marret *et al*. 2009). Kokinos & Anderson (1995) first demonstrated the occurrence of different biometrical groups of cysts of *Lingulodinium polyedrum* in culture experiments. Experiments by Hallett (1999) revealed a linear relationship between the average process length of this taxon and both salinity and temperature. This relationship was confirmed for cysts of *Lingulodinium polyedrum* from surface sediments by Mertens *et al*. (2009b). This proxy was applied to down-core salinity reconstructions in the Black Sea (Verleye *et al*. 2009) and the Cariaco Basin (Mertens *et al*. 2009a). However, the temperate to tropical coastal distribution of this taxon imposes biogeographic restrictions on the use of this cyst for salinity reconstructions. Two additional cyst types showing reduced process length in low-salinity waters were included as basic elements of the

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salinity signal proposed by Dale (1996), namely cysts of *Protoceratium reticulatum* and a small *Spiniferites* sp. The cyst of *Protoceratium reticulatum* (sometimes referred to as *Operculodinium centrocarpum* sensu Wall & Dale 1966) presents considerable potential for palaeosalinity reconstruction, as it has a long biostratigraphic range – similar morphotypes extend down to the Eocene (Head 1996) – and is the most cosmopolitan living cyst type so far documented (Wall *et al.* 1977; Dale 1996; Marret & Zonneveld 2003). Use of this taxon would allow palaeosalinity reconstructions in temperate to polar regions, where cysts of *Lingulodinium polyedrum* are absent and calcareous remains of coccoliths and foraminifera are often dissolved. Process length variation in cysts of *Protoceratium reticulatum* has previously been semi-quantitatively related to salinity variations by de Vernal *et al.* (1989), Dale (1996), Ellegaard (2000), Brenner (2005) and Head (2007).

This study aims to evaluate whether the average process length of cysts of *Protoceratium reticulatum* shows a linear relationship to salinity and/or temperature, and to assess its usability as a proxy for palaeosalinity reconstruction. To achieve this goal, cysts of both *Protoceratium reticulatum* and *Lingulodinium polyedrum* were studied from surface sediments representing the Baltic–Kattegat–Skagerrak estuarine system and from down-core in Limfjord, northern Denmark.

Environmental setting

The Baltic–Kattegat–Skagerrak has an estuarine circulation system with a pronounced two-layer stratification through outflowing low-salinity surface water of the Baltic Current (8–12 psu), which mixes with more saline waters from the North Sea and the Atlantic (30–35 psu). This 2000-km-long salinity gradient ranges from very low (0.5 psu) in the Bay of Bothnia to fully marine (35 psu) in the Skagerrak (Leppäranta & Myrberg 2009) (Fig. 1A). The relatively narrow channels between the Kattegat and the Baltic, with maximum sill depths of 18 m, reduce the inflow of fully saline marine water into the Baltic. The counterclockwise current pattern in the Skagerrak is determined by inflow from the Norwegian Sea at depths between 150 and 200 m and outflow of the Norwegian Coastal Current formed by surface water from the southern North Sea mixed with the Baltic Current (Svansson 1975, 1984; Larsson & Rodhe 1979; Rodhe 1987). These currents determine the extent of stratification of the water column, which is influenced to a lesser degree by wind (Svansson 1975). In the Kattegat, increasingly more bottom water is mixed upwards as vertical water exchange intensifies northwards (Svansson 1975; Stigebrandt 1983). A strong thermohaline stratification produces temperatures that are high (~18 °C) in summer and low (~0 °C) in winter (Svansson 1975). The tidal range of ~20 cm has little influence on the current system, facilitating a very stable salinity gradient.

Material and methods

Sample preparation and light microscopy

A total of 71 surface sediment samples were studied for biometric measurements of cysts of *Protoceratium reticulatum* and *Lingulodinium polyedrum*, using microscope slides from previous studies (Fig. 1B). For 58 of

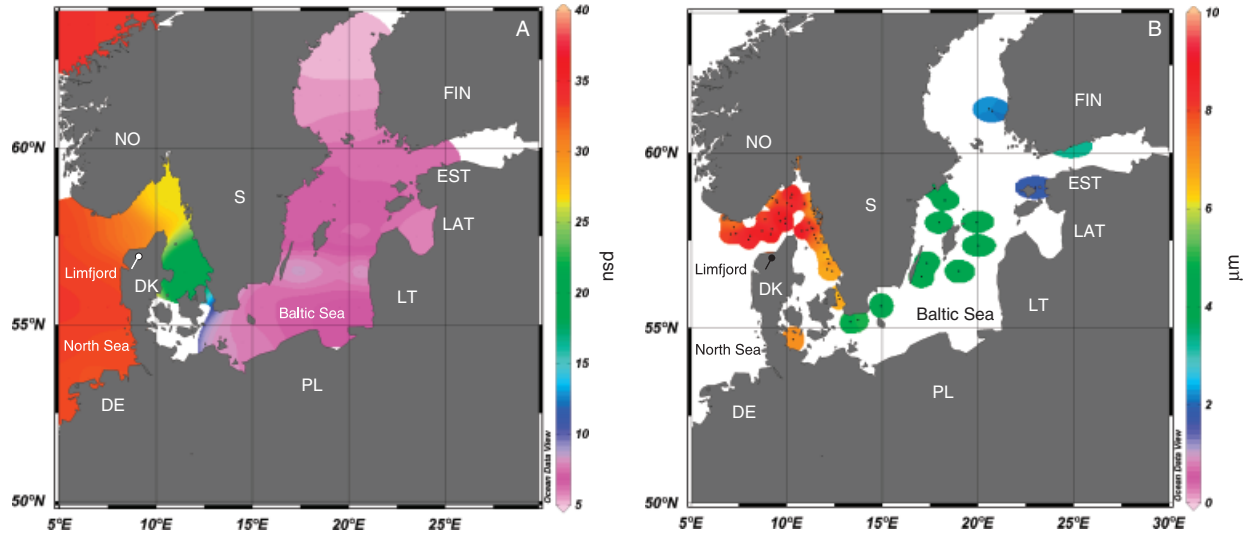

Fig. 1. A. Summer salinity in the Baltic–Kattegat–Skagerrak estuarine system. B. Distribution of studied surface sediment samples, indicating average process lengths (µm) of cysts of *Protoceratium reticulatum* (B) and major countries, the North Sea, Baltic Sea and the position of Limfjord. DE = Germany; DK = Denmark; EST = Estonia; FIN = Finland; LAT = Latvia; LT = Lithuania; NO = Norway; PL = Poland; S = Sweden.

the 71 samples, the dinoflagellate cyst assemblages have been studied previously (Konieczny 1983; Dale 1985; Gundersen 1988; Thorsen *et al.* 1995; Thorsen & Dale 1998; Dale *et al.* 1999; Ellegaard 2000; Persson *et al.* 2000; Grøsfjeld & Harland 2001; Clarke *et al.* 2006), and for 26 of the 71 samples, measurements of *Lingulodinium polyedrum* cysts have already been published (Mertens *et al.* 2009b). Most samples were core-top samples from areas with relatively high sedimentation rates and can be considered recent, that is, representing tens of years to a few centuries old (Table 1). It is assumed here that the environmental conditions affecting the morphological changes within the cysts are similar to recent environmental conditions. Identical morphological measurements were also applied down-core to dinoflagellate cysts extracted from 27 samples from a core from Limfjord, Denmark (Fig. 1A). Details for these samples are given in Ellegaard (2000). Dinoflagellate cysts were previously studied for these samples, and the core was later dated by Christensen *et al.* (2004).

All the cysts were extracted from the sediments using methods described in the respective literature (Table 1). Most were standard palynological methods involving hydrochloric acid and hydrofluoric acid, sieving and/or sonication. For a few samples (Koljo Fjord, Oslo Fjord, Anholt, Hakefjorden and Vinga), heavy-liquid separation using sodium polytungstate with a density of 2.1 g ml^{-1} was used (Bolch 1997). Regardless of the extraction method, comparison of cysts between samples showed no differences in terms of preservation (Figs 2, 3). This suggests that the difference in processing methods had no effect on measurements, as noted previously by Mertens *et al.* (2009b).

All measurements and light micrographs were made using a Zeiss Axioskop 2 and an Olympus BH-2 light microscope, equipped with an AxioCam MRc5 digital camera (AxioVision v. 4.6 software) and Color View II (Cell F Software Imaging System) respectively, and 100× oil immersion objectives. All measurements were made by K. N. Mertens. For each sample, the average of the length of the three longest visible processes and the largest body diameter of 50 cysts per sample were measured, when possible. Measuring 50 cysts yields reproducible results (Mertens *et al.* 2009b); like Head (2007), we found that average process length per sample for cysts of *Protoceratium reticulatum* is generally reproducible within about 0.5 µm, and for cysts of *Lingulodinium polyedrum*, within about 1 µm. The length of each process was measured from the middle of the process base to the process tip (Fig. 4). For cysts of *Protoceratium reticulatum*, only specimens carrying processes with the distinct platforms and hooklets were measured for morphological analysis. It is important to note that no cysts without processes (i.e. 'zero' process length) were included in the analysis, because of the difficulty of species identification associated with these forms, which would introduce observer bias into the measurements. For each cyst, three processes could always be found within the focal plane of the light microscope, so this number seemed a reasonable choice. Three reasons can be advanced for choosing the longest processes. First, the longest processes reflect unobstructed growth of the cyst. Second, measuring the longest processes increases the accuracy of the proxy as it documents the largest variation. Third, because only a few processes were parallel to the focal plane of the microscope, it was imperative to make a consistent choice. Sometimes fewer than 50 cysts were measured, if more were not available. Fragments representing less than half of a cyst were not measured, nor were cysts with mostly broken processes. All measurements are available as supplementary data.

For scanning electron microscope observation, residue from the sample GEOMAR Poseidon PO 65 POS 14747-1 (North Baltic) was washed with distilled water and dehydrated in a graded ethanol series (30 to 100% in six steps), critical-point-dried with CO_2 (CPD Bal-Tec 030), glued onto a stub, sputter-coated with platinum/palladium for 90 s (Jeol JFC-2300 HR) and examined using a JEOL 6330F scanning electron microscope.

Salinity and temperature data

Biometric measurements on cysts from all study areas were compared with both seasonal and annual temperature (T), salinity (S), the ratio between salinity and temperature (S/T) and seawater density (D) at various depths using the gridded 1-degree World Ocean Atlas 2005 (Antonov *et al.* 2006; Locarnini *et al.* 2006) and the Ocean Data View software (Schlitzer 2010) by calculating Pearson's correlation coefficient. The significance of Pearson's *r* was calculated using a *t*-test.

Results

Process length variation in surface sediments

Overall cyst biometrics. – For cysts of *Lingulodinium polyedrum*, the 3855 process length measurements gave an average of 13.5 µm with a standard deviation of 4.5 µm, and a range from 2 to 32 µm (Fig. 5A). Most cysts encountered were comparable to the forms described by Kokinos & Anderson (1995) and Ellegaard (2000), and cysts without processes were not observed. The range found is within the global range of 0–41 µm detailed by Mertens *et al.* (2009b). The skewness of the distribution was 0.39, as there was some tailing on the right side of the size–frequency curve. This is attributable to an overrepresentation of individuals with long processes. The 1285 body-diameter measurements gave an average body diameter of 47.7 µm with a standard

deviation of 5.9 μm, over a range from 31 to 73 μm (Fig. 5B). This relatively large range can be explained partly by cysts sometimes being compressed or torn, resulting in an anomalously large body diameter (see also Mertens *et al*. 2009b). This mechanical deformation of the cyst also explains the positive skewness of the size–frequency spectrum.

For cysts of *Protoceratium reticulatum*, the 9252 process length measurements gave an average of 7.0 μm with a standard deviation of 2.3 μm, and a range from 0.5 to 13.5 μm (Fig. 5C). The morphotypes encountered were comparable to morphotypes described by Nehring (1997), Matsuoka *et al*. (1997), Ellegaard (2000) and Head (2007). The range found is comparable to the ranges of 2.5–13 μm for the German coast reported by Nehring (1997), of 4–8 μm for Pacific specimens (Matsuoka 1990), of 7–14 μm for the British Isles (Reid 1974), of 6–16 μm for Atlantic specimens (Wall & Dale 1968) and of 8–16 μm for Caribbean specimens (Wall 1967). The skewness of the distribution was −0.46, as there was some tailing on the left side of the size–frequency curve. This was the result of a higher representation of individuals with short processes. The 3084 body-diameter measurements gave an average body diameter of 38.3 μm with a standard deviation of 4.6 μm, over a range from 23 to 62 μm (Fig. 5D). This range is comparable to the ranges of 31–44 μm for the German coast reported by Nehring (1997), of 33–46 μm for the British Isles (Reid 1974), of 28–50 μm for Pacific specimens (Matsuoka 1990) and of 40–56 μm for Caribbean specimens (Wall 1967). This relatively large range can again be explained partly by cysts sometimes being compressed or torn, resulting in an anomalously large body diameter (see also Mertens *et al*. 2009b). This mechanical deformation of the cyst also explains the positive skewness of the size–frequency spectrum.

Correlations between the parameters using a reduced data set. – All samples that contained fewer than 20 measured cysts were excluded from the analysis. Forty of the 63 samples that contained cysts of *Lingulodinium polyedrum* were excluded, while only 3 of the 71 samples containing cysts of *Protoceratium reticulatum* were excluded (see supplementary data). For cysts of *Lingulodinium polyedrum*, this meant that only measurements from coastal sites and fjords around the Kattegat and Skagerrak were available.

Pearson's correlation coefficient was calculated between the average process length of each species and S, T, S/T and D at various water depths, both annually and seasonally (Table 2). There was no significant correlation between average process length and any of the environmental parameters for cysts of *Lingulodinium polyedrum* ($p < 1 \times 10^{-12}$, max $R^2 = 0.36$). However, there was a significant correlation with nearly all parameters for cysts of *Protoceratium reticulatum* ($p < 1 \times 10^{-12}$; Table 2). This is related to the covariation of salinity with temperature in the Baltic–Kattegat–Skagerrak estuarine system. The correlation of average process length with S and D is larger than that with T and S/T, on both an annual and a seasonal scale.

The average process length of cysts of *Protoceratium reticulatum* was not significantly correlated with the average process length of cysts of *Lingulodinium polyedrum* ($R^2 = 0.02$). This was probably as a result of the underrepresentation of cysts of *Lingulodinium polyedrum* cysts in the measured samples.

No correlation was found between the process length and cyst body diameter for cysts of *Lingulodinium polyedrum* ($R^2 = 0.35$). This confirms previous observations by Mertens *et al*. (2009b). This was expected, as culture experiments have also revealed no correlation between body diameter and salinity (Hallett 1999). In addition, no significant relation between body diameter and average process length was found ($R^2 = 0.12$) for cysts of *Protoceratium reticulatum*. Variations in cyst body diameter are probably caused by compression or are formed during cyst germination.

No significant correlation was found between relative abundances of cysts of *Lingulodinium polyedrum* and average process length ($R^2 = 0.13$) nor body diameter ($R^2 = 0.04$), and between relative abundances of cysts of *Protoceratium reticulatum* and average process length ($R^2 = 0.45$) nor body diameter ($R^2 = 0.17$). The relationship between relative abundance and environmental parameters will be the subject of another publication. All the reduced-process forms of cysts of *Protoceratium reticulatum* were included in the use of these relative abundances (see below).

Process length variation down-core in Limfjord

Overall cyst biometrics. – For cysts of *Lingulodinium polyedrum*, the 2952 process length measurements gave a mean of 13.1 μm with a standard deviation of 4.6 μm, and a range from 1 to 30 μm (Fig. 5E). Most cysts encountered were comparable to the forms described by Kokinos & Anderson (1995), and cysts without processes occurred (as previously noted in Ellegaard 2000) but were not measured. The observed range is within the global range of 2–32 μm measured in surface sediments. The skewness of the distribution is −0.07, as there is some tailing on the left side of the size–frequency curve. This is caused by an overrepresentation of individuals with short processes. The 984 maximum body-diameter measurements gave an average maximum body diameter of 47.9 μm with a standard deviation of 5.6 μm, over a range from 32 to 72 μm (Fig. 5F). This range is again comparable to the range of 31–73 μm measured in the surface sediments.

For cysts of *Protoceratium reticulatum*, the 3105 process length measurements gave an average of

Table 1. Station name, longitude, latitude, water depth, core type, sampling date, estimated age, sedimentation rate and reference to the literature.

Station	Longitude (°E)	Latitude (°N)	Water depth (m)	Core type	Sampling date	Estimated age (cal. a)	Sedimentation rate (cm ka^{-1})	Reference
Finske Bukta (Station 20)	23.00	59.0000	50	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 1 (Station 19)	11.16	58.00	110	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 2 (Station 17)	11.43	57.66	80	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 3 (Station 18)	11.30	57.86	109	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 4 (Station 16)	11.64	57.44	50	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 5 (Station 15)	11.97	57.16	51	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 6 (Station 14)	12.14	56.97	56	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 7 (Station 13)	12.37	56.62	32	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 8 (Station 12)	12.71	55.92	40	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 9 (Station 11)	12.76	55.82	21	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 10 (Station 10)	13.33	55.17	44	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 11 (Station 9)	17.05	56.47	78	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 12 (Station 8)	17.33	56.85	100	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 13 (Station 7)	17.97	58.01	216	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 14 (Station 6b)	18.27	58.64	440	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 15 (Station 5)	19.93	58.01	188	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 16 (Station 4)	20.01	57.35	245	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 17 (Station 3)	19.00	56.62	140	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 18 (Station 2)	14.93	55.62	80	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
NG 19 (Station 1)	13.73	55.22	42	Gravity core	10–18 September 1984	200	14	Gundersen (1988)
Skagerrak15330-4 0–5 cm	7.08	57.67	325	Piston core	8 November 1980	25	100	Dale (1985)
8903 0–2 cm	12.21	56.84	44	Boxcore	October 1989	Recent	670	Thorsen *et al.* (1995)
9307 0–1 cm	7.11	58.07	75	Gravity core	August 1993	Recent	670	Thorsen & Dale (1998)
9308 0–2 cm	7.11	58.07	75	Gravity core	August 1993	Recent	670	Thorsen & Dale (1998)
B88 1–3	10.72	59.78	150	Gravity core	April 1988	30	100	Dale *et al.* (1999)
B95-BC 0–1 cm	10.70	59.84	100	Gravity core	September 1995	10	100	Dale *et al* (1999)
V95-BC 0–1 cm	10.54	59.81	100	Gravity core	September 1995	10	100	Dale *et al.* (1999)
GEOMAR Meteor M 26/2 M 436 - 3	9.66	58.05	332	Gravity core	1993	Recent	?	Unpublished
GEOMAR Littorina LIT 12880-1	10.33	54.67	23	Piston core	1975	Recent	?	Unpublished
GEOMAR Poseidon PO 65 POS 14747-1	20.58	61.25	102	Gravity core	1980	Recent	?	Unpublished
GEOMAR Poseidon PO 15525-4	8.09	57.62	247	Piston core	1980	Recent	?	Unpublished
GEOMAR Poseidon PO 65 POS 14753-1	20.75	61.19	75	Gravity core	1980	Recent	?	Unpublished
GEOMAR Planet PL12/84 GIK-PLA II core	8.71	57.77	245	Gravity core	1991	Recent	?	Unpublished
Himmerfjorden	17.73	58.98	32	Gravity core	March 23 2009	Recent	?	Unpublished
Koljo Fjord K4	11.59	58.28	45	Piston core	6 April 2006	Recent	?	Unpublished
Oslo Fjord	10.70	59.91	3	Sediment sucker	November 2009	Recent	100	Unpublished
Laajalahti Bay	24.87	60.18	4	Mackereth core	1998	Up to 100 years	?	Clarke *et al.* (2006)
V7715 (Station 1)	7.24	57.87	423	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
V7716 (Station 2)	7.35	57.69	333	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
S37 (Station 4)	8.01	57.52	125	Petersen Grab	1978	Up to 100 years	?	Konieczny (1983)
V7548 (Station 5)	8.87	58.10	515	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
S9 (Station 7)	9.22	57.82	127	Petersen Grab	1978	Up to 100 years	?	Konieczny (1983)
S8 (Station 8)	9.17	57.67	46	Petersen Grab	1978	Up to 100 years	?	Konieczny (1983)
A0821 (Station 9)	9.27	58.70	71	Gravity core	1982	Up to 100 years	?	Konieczny (1983)
S45 (Station 10)	9.45	58.64	322	Petersen Grab	1978	Up to 100 years	?	Konieczny (1983)
S46 (Station 11)	9.49	58.58	480	Petersen Grab	1978	Up to 100 years	?	Konieczny (1983)
S41 (Station 13)	9.59	58.27	700	Petersen Grab	1978	Up to 100 years	?	Konieczny (1983)
V7527 (Station 14)	9.91	58.12	285	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)

V7525 (Station 15)	9.93	57.98	88	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
G23 (Station 16)	10.28	58.81	165	Petersen Grab	1981	Up to 100 years	?	Konieczny (1983)
G24 (Station 17)	10.21	58.72	250	Petersen Grab	1981	Up to 100 years	?	Konieczny (1983)
G26 (Station 18)	10.02	58.56	534	Petersen Grab	1981	Up to 100 years	?	Konieczny (1983)
V7538 (Station 19)	10.92	58.54	70	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
V7536 (Station 21)	10.25	58.48	420	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
V7517 (Station 22)	11.37	57.83	61	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
V7515 (Station 24)	11.07	57.80	43	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
V7514 (Station 25)	10.80	57.77	35	Van Veen Grab	1973–1978	Up to 100 years	?	Konieczny (1983)
Anholt	12.12	56.67	53	Boxcore	1994	Recent	?	Unpublished
Hakefjorden	11.71	57.96	42	Boxcore	2002	Recent	?	Unpublished
Vinga	11.52	57.55	78	Boxcore	1994	Recent	?	Unpublished
Fjallbacka	11.26	58.62	18	Boxcore	September 1995	Recent	?	Persson *et al.* (2000)
S141	11.44	58.35	21	Boxcore	September 1995	Recent	?	Persson *et al.* (2000)
Valö	11.81	57.54	22	Boxcore	September 1995	Recent	?	Persson *et al.* (2000)
Byfjorden	11.89	58.34	42	Boxcore	September 1995	Recent	?	Persson *et al.* (2000)
K2	11.85	58.16	30	Boxcore	September 1995	Recent	?	Persson *et al.* (2000)
Havstenfjorden	11.86	58.21	20	Boxcore	September 1995	Recent	?	Persson *et al.* (2000)
G2	11.80	58.10	25	Boxcore	September 1995	Recent	?	Persson *et al.* (2000)
S112	11.40	58.34	46	Boxcore	September 1995	Recent	?	Persson *et al.* (2000)
Gullmar fjord	11.42	58.26	63	Boxcore	September 1995	Recent	?	Mertens *et al.* (2009b)
Risor Site	9.09	58.73	70	Boxcore	13–22 June 1991	Recent	?	Grøsfjeld & Harland (2001)
Limfjord 9–12 cm	9.13	56.88	10	Piston core	1993	183	140	Ellegaard (2000)

6.51 μm with a standard deviation of 2.5 μm, and a range from 1.0 to 15.0 μm (Fig. 5G). The morphotypes encountered were comparable to morphotypes described by Nehring (1997), Matsuoka *et al.* (1997) and Head (2007). The range found is comparable to results obtained from surface sediments. The skewness of the distribution is 0.34, as there is some tailing on the right side of the size–frequency curve. This is caused by a higher representation of individuals with large processes. The size–frequency curve is slightly bimodal. The 1035 body diameter measurements resulted in an average body diameter of 37.9 μm with a standard deviation of 4.4 μm, over a range from 25 to 55 μm (Fig. 5H). This range is comparable to the range of 23–62 μm measured in surface sediments.

Process length variation down-core. – Significant changes in process lengths of cysts of both *Lingulodinium polyedrum* and *Protoceratium reticulatum* can be observed down-core in Limfjord (Fig. 6). The average process length of cysts of *Lingulodinium polyedrum* varied between 16.3 and 8.0 μm, and that of cysts of *Protoceratium reticulatum* between 9.0 and 4.4 μm. Although there are similar trends in the two records, there is no significant correlation between process lengths of cysts of *Lingulodinium polyedrum* and *Protoceratium reticulatum* ($R^2 = 0.01$).

Discussion

The average process length in cysts of *Lingulodinium polyedrum* did not have any significant correlations with environmental variables. This is probably a result of the restriction of short-lived blooms of *Lingulodinium polyedrum* to fjords around the Skagerrak–Kattegat area, where the environmental conditions are very variable, complicating the use of the average process length of cysts of *Lingulodinium polyedrum* as a salinity proxy in this very particular environment (see also Mertens *et al.* 2009b). This discussion will therefore focus on the use of the average process length of cysts of *Protoceratium reticulatum* as a proxy. Before assessing the value of average process length as a salinity proxy and testing the proxy down-core, it is worth considering factors that may affect its reliability. These include extreme reduction of processes, timing of cyst formation and cyst transport.

Complete reduction of processes of cysts of Protoceratium reticulatum

One distinct problem encountered in this study was the occurrence of the species *Pyxidinopsis psilata* (ex. *Tectatodinium psilatum*, transferred by Head (1994)) in the Baltic, as noted by Gundersen (1988), Dale (1996) and

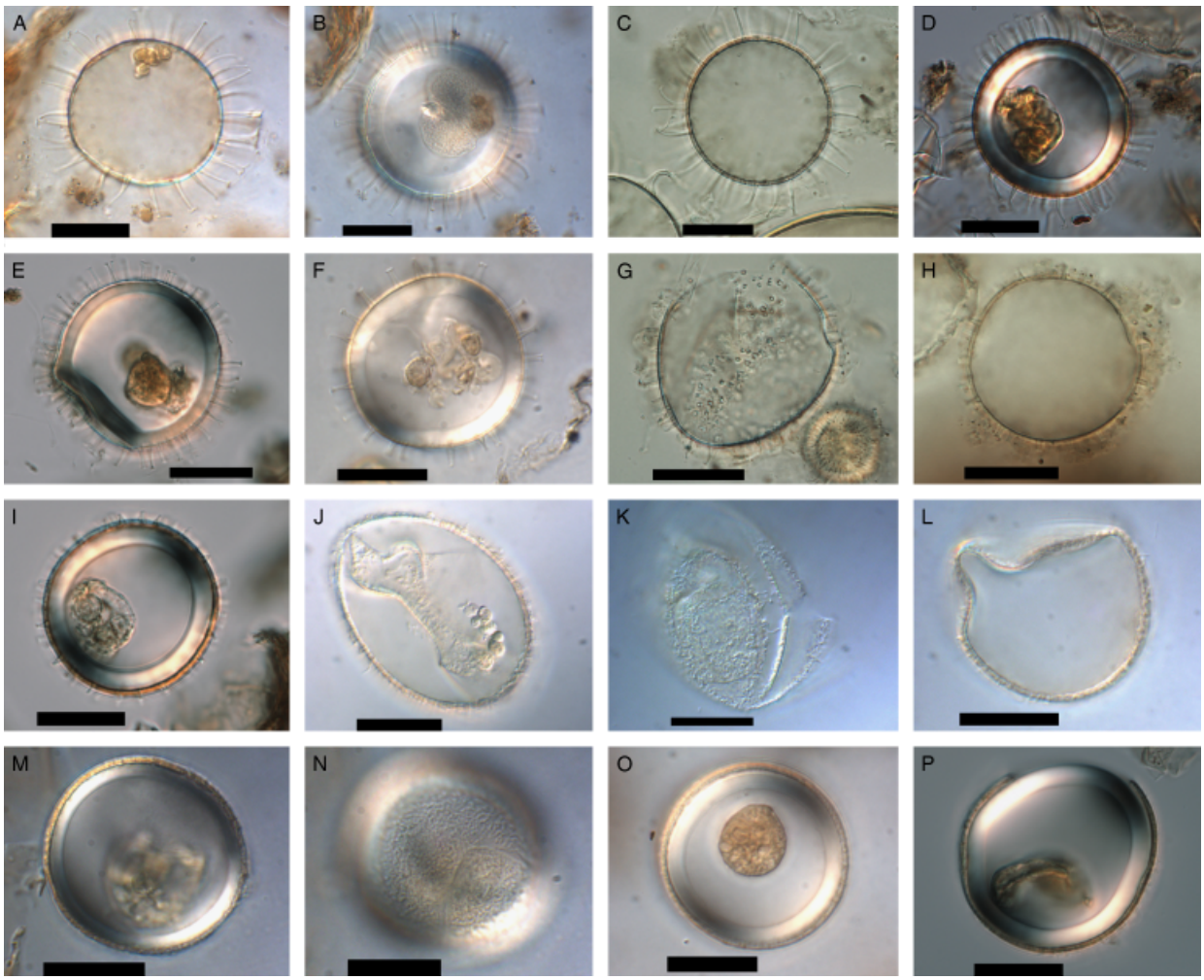

Fig. 2. Morphological variation of cysts of *Protoceratium reticulatum* in the Skagerrak–Kattegat–Baltic, sorted from long (A) to completely reduced (P) processes. A. Empty cyst with remains of red bodies from the Kattegat, core 8903 (Thorsen *et al.* 1995). B. Cyst with cell contents and birefringent endospore and remains of red bodies from the Norwegian coast of the Skagerrak, core 9307 (Thorsen & Dale 1998). C. Empty cyst from Gullmar Fjord, Swedish coast, adjacent to the Kattegat (Mertens *et al.* 2009b). D. Cyst with remains of red bodies from the Kattegat, station NG7 (Gundersen 1988). E. Cyst with cell contents, birefringent endospore and remains of red bodies from the Skagerrak, station NG1 (Gundersen 1988). F. Cyst with cell contents, birefringent endospore and remains of red bodies from the inner Oslo Fjord, station B95 (Dale *et al.* 1999). G. Empty cyst from Gullmar Fjord, Swedish coast, adjacent to the Kattegat (Mertens *et al.* 2009b). H. Empty cyst from Gullmar Fjord, Swedish coast, adjacent to the Kattegat (Mertens *et al.* 2009b). I. Cyst with cell contents, birefringent endospore and remains of red bodies from the Baltic (southern Sweden), station NG19 (Gundersen 1988). J. Empty cyst with dense, hair-like processes, from Laajalahti Bay, southern Finland (Clarke *et al.* 2006). K and L. Empty cysts with dense, hair-like processes, from the Estonian west coast (station 20) (Gundersen 1988). M and N. Cyst with cell contents and a few hair-like processes from the Estonian west coast (station 20) (Gundersen 1988), mid-focus view (M) and focus on wall texture and preformed operculum (N). O. Completely reduced cyst with cell contents from the Baltic (southern Sweden), station NG10 (Gundersen 1988). P. Cyst with cell contents and a few hair-like processes on the lower side from the Baltic (southern Sweden), station NG19 (Gundersen 1988). All scale bars represent 20 µm. This figure is available in colour at http://www.boreas.dk.

Brenner (2005). This species was originally described from the Black Sea as having a psilate, minutely punctate to finely scabrate wall surface with a spherical to cruciform shape and a complete lack of processes (Wall *et al.* 1973). However, Baltic cysts assigned to this species germinated in incubation experiments to give a swimming cell of *Protoceratium reticulatum*, indicating that these cysts possibly represent an ecophenotypic form of *Protoceratium reticulatum* in low-salinity waters (Dale 1996). In the studied samples, cysts of *Protoceratium reticulatum* intergrade morphologically into the cysts previously designated in the Baltic as *Pyxidinopsis psilata*, in terms of reduction of process length and slight changes in wall texture (Fig. 2). Cysts without processes very often had a few (very) short 'hairs' left. All wall surface textures we observed in the Baltic forms previously assigned to *Pyxidinopsis psilata* were microgranular, as in cysts of *Protoceratium reticulatum*, and built up of fibrils (Figs 2, 3). The observed texture cannot be termed psilate, punctate or scabrate as described for *Pyxidinopsis psilata* from the Black Sea. This suggests that *Pyxidinopsis psilata* observed by Wall *et al.* (1973) from the Black Sea is not

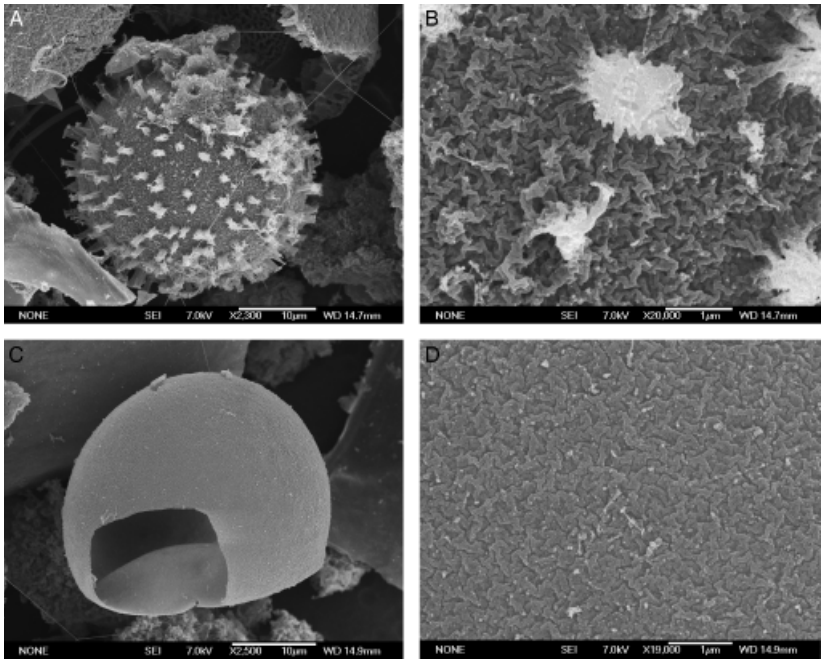

Fig. 3. Field emission SEM micrographs of cysts of *Protoceratium reticulatum* from sample GEOMAR Poseidon PO 65 POS 14747-1. A and B. Empty short-process-bearing cyst at two magnifications showing a loosely packed fibrillar texture. C and D. Empty reduced cyst lacking any processes at two magnifications showing a similar fibrillar but densely packed texture.

necessarily affiliated to the cysts we observed in the Baltic. New observations reported here showing comparable wall structure in *Protoceratium reticulatum* cysts and cysts previously assigned to *Pyxidinopsis psilata* strongly suggest that the Baltic form is a phenotypic form of *Protoceratium reticulatum* rather than *Pyxidinopsis psilata*, although it is interesting to note that both show a tendency towards a cruciform ambitus.

Further incubation experiments in combination with genetic analysis and detailed morphological comparison of *Pyxidinopsis psilata* from the Black Sea and the Baltic are required to resolve this problem. Pending such investigations, we will refer to these Baltic cysts as 'reduced cysts of *Protoceratium reticulatum*'. Because such cysts were excluded from our study, this does not affect the reliability of our proxy.

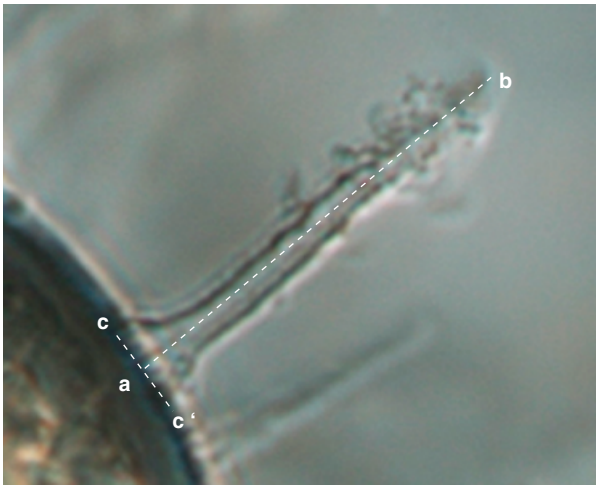

Fig. 4. Spine length in *Lingulodinium polyedrum* cysts is measured as the distance between the middle of the process base (a) and the process tip (b). The middle of the process base is defined as the middle between the process base margins (c and c′). This figure is available in colour at http://www.boreas.dk.

When and where are the cysts formed?

Assuming a relationship between cyst morphology and environment, it is not known when or how the environmental parameters that are reflected in the process length were recorded. Process length can be affected by the environment at the time of formation of the cyst and/or can be determined genetically. Evidence from culture work on the closely related species *Lingulodinium polyedrum* by Hallett (1999) supports both of these hypotheses. This author found high process length variability between strains grown in similar conditions of temperature and salinity. Furthermore, within a strain, changes in salinity and/or temperature cause changes in average process length, which suggests that the process length can be affected by the salinity and temperature of the surrounding medium. In any case, because cysts of *Protoceratium reticulatum* are formed after the bloom reaches its exponential phase (Wall & Dale 1968), the cysts probably reflect environmental conditions similar to those in which the motile cells thrive.

The abundance of *Protoceratium reticulatum* is known to peak during summer in Norwegian waters. Braarud & Bursa (1939) mention the occurrence of motile cells of *Protoceratium reticulatum* in the Oslofjord in June, July and October. Braarud *et al.*

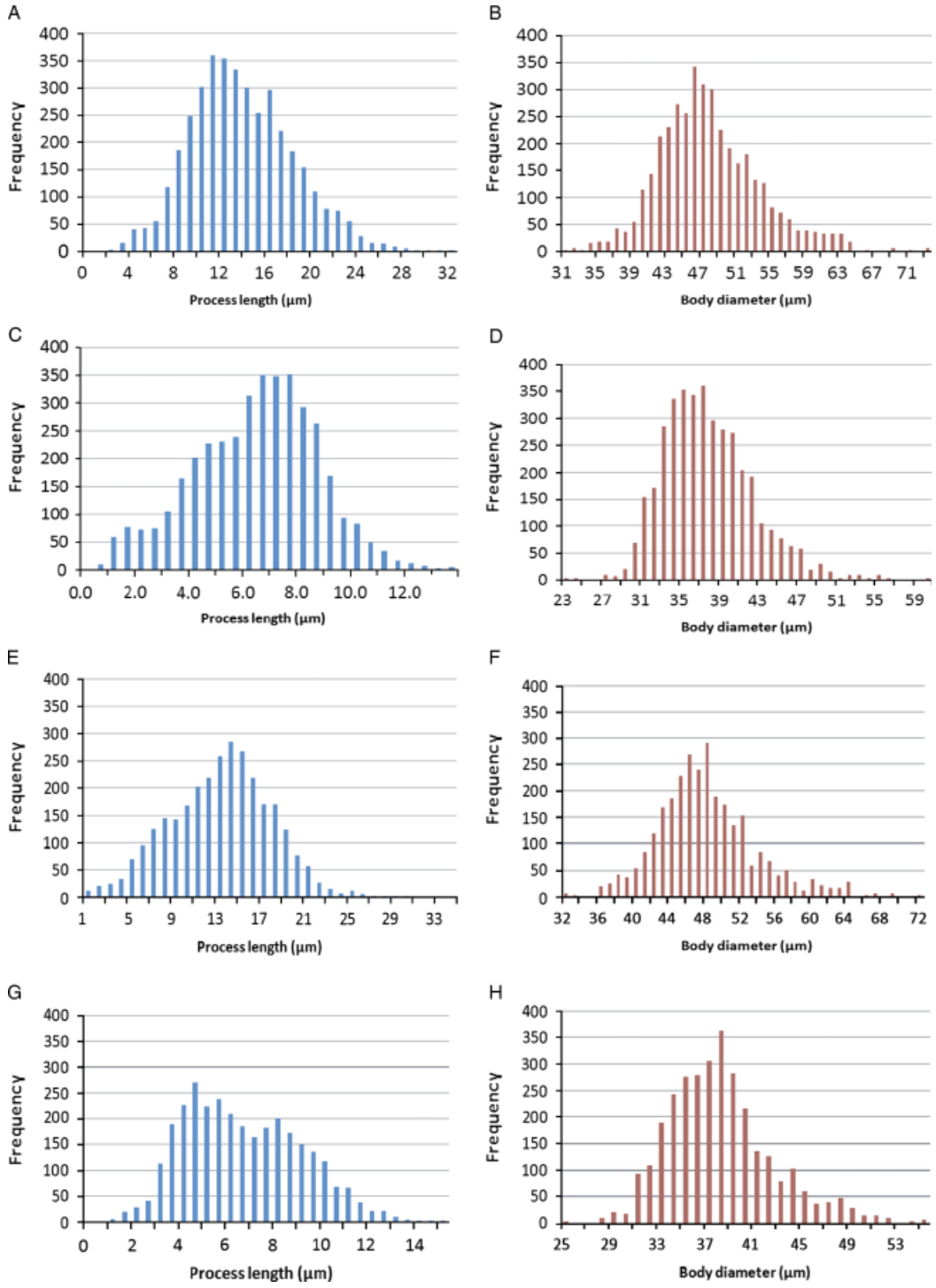

Fig. 5. Size–frequency spectra of process length and body diameter measurements of cysts of *Lingulodinium polyedrum* and *Protoceratium reticulatum* (A–H). A–D. Size–frequency spectra of 3855 process length (A) and 1285 body diameter (B) measurements of cysts of *Lingulodinium polyedrum* and 9252 process length (C) and 3084 body diameter (D) measurements of cysts of *Protoceratium reticulatum* in surface sediments of the Baltic–Kattegat–Skagerrak. E–H. Size–frequency spectrum of 2952 process length (E) and 984 body diameter (F) measurements of cysts of *Lingulodinium polyedrum* and 3105 process length (G) and 1035 body diameter (H) measurements of cysts of *Protoceratium reticulatum* from down-core Limfjord. This figure is available in colour at http://www.boreas.dk.

(1974) and Braarud (1976) note high abundances of *Protoceratium reticulatum* cells in Hardangerfjord from June to August. An early summer bloom in Flødevigen Bay, Norway, has also been noted by Aasen *et al.* (2005). There are reports from other regions of the world showing bloom formation during summer and subsequent cyst production. Wall & Dale (1968) report that motile cell and cyst production in

Table 2. Pearson's correlation coefficient calculated between environmental parameters and average process length of cysts of *Lingulodinium polyedrum* (left column) and cysts of *Protoceratium reticulatum* (right column) at water depths of 0 to 30 m. Significant correlations using the *t*-test ($p < 1 \times 10^{-12}$) are indicated in bold.

Parameter depth [m]	Cysts of *Lingulodinium polyedrum*				Cysts of *Protoceratium reticulatum*			
	0 m	10 m	20 m	30 m	0 m	10 m	20 m	30 m
Annual T	0.24	0.20	0.15	0.07	**0.64**	**0.63**	**0.69**	**0.74**
Annual S	0.08	0.02	0.02	0.02	**0.82**	**0.75**	**0.76**	**0.76**
Annual S/T	0.10	0.10	0.09	0.11	**0.70**	**0.71**	**0.71**	**0.68**
Annual D	0.03	0.03	0.02	0.02	**0.74**	**0.75**	**0.76**	**0.76**
Summer T	0.31	0.33	0.26	0.12	0.01	0.07	0.48	**0.72**
Summer S	0.01	0.01	0.01	0.01	**0.80**	**0.81**	**0.82**	**0.82**
Summer S/T	0.06	0.07	0.06	0.06	**0.79**	**0.80**	**0.81**	**0.81**
Summer D	0.02	0.02	0.01	0.01	**0.80**	**0.81**	**0.82**	**0.82**
Autumn T	0.01	0.02	0.13	0.03	**0.69**	**0.79**	**0.79**	**0.77**
Autumn S	0.28	0.30	0.23	0.19	**0.83**	**0.84**	**0.83**	**0.82**
Autumn S/T	0.35	0.34	0.27	0.20	**0.82**	**0.82**	**0.82**	**0.82**
Autumn D	0.28	0.30	0.23	0.19	**0.83**	**0.84**	**0.83**	**0.82**
Winter T	0.03	0.03	0.03	0.03	**0.79**	**0.81**	**0.83**	**0.85**
Winter S	0.02	0.01	0.00	0.00	**0.83**	**0.84**	**0.84**	**0.84**
Winter S/T	0.09	0.06	0.02	0.01	0.45	0.43	0.45	0.49
Winter D	0.01	0.01	0.00	0.00	**0.83**	**0.84**	**0.84**	**0.85**
Spring T	0.08	0.28	0.15	0.25	**0.75**	**0.76**	**0.84**	**0.85**
Spring S	0.19	0.26	0.09	0.09	**0.79**	**0.83**	**0.82**	**0.82**
Spring S/T	0.18	0.31	0.01	0.11	**0.67**	**0.77**	**0.73**	**0.69**
Spring D	0.19	0.26	0.09	0.09	**0.79**	**0.83**	**0.82**	**0.82**

T = temperature; S = salinity; D = density.

late-spring to summer of *Protoceratium reticulatum* occurred at Woods Hole, USA. Around the British Isles, the cyst is documented in the plankton from spring to late summer (April–September; Reid 1978). High fluxes of cysts of *Protoceratium reticulatum* were found in sediment trap material collected during summer (June and September) from the Gulf of Naples (Montresor *et al*. 1998). In the Strait of Georgia, British Columbia, a sediment trap study showed that the highest cyst fluxes are found in summer (Pospelova *et al*. 2010). Around South Africa, blooms of *Protoceratium reticulatum* with the formation of cysts are known to occur in December (Reinecke 1967; Grindley & Nel 1968, 1970), as well as in September (Horstman 1981). Recent sediment trap studies in South Africa show that the cyst occurs during spring and summer (Pitcher & Joyce 2009). In Okkirai Bay, Northern Japan, the species is recorded mainly through the summer (Koike *et al*. 2006). This last study also shows a vertical distribution of the motile cells in the water column, and suggests a dominant sea surface production of the motile cells. We therefore assume that cyst production occurs predominantly in surface waters.

The literature thus suggests that the process length will reflect environmental conditions at the sea surface during the summer.

Transport issues

Cyst transport away from the core area of cyst formation has the potential to affect the reliability of the environmental signal. In particular, the possibility for long-distance transport of coastal and neritic cysts into the deep sea (Dale 1996) may limit the application of this salinity proxy in the open ocean (but see Dodge and Harland, 1991,and below). However, long-distance transport is not likely to be an important factor in our study area. First and foremost, all sites with known ages have relatively high sedimentation rates (between 14 and 670 cm/ka; Table 1), which reduces the impacts of bioturbation and possible lateral transport. Furthermore, as motile cells of *Protoceratium reticulatum* are recorded all over the Baltic (Hällfors 2004), high occurrences of cysts are not surprising, because *Protoceratium reticulatum* is also suggested to have a high cyst production per motile cell (Dale 1976). More generally, comparison of plankton records of *Protoceratium reticulatum* with cyst records in the northeast Atlantic show a good correspondence (Dodge & Harland 1991), compared with other species such as *Lingulodinium polyedrum*, which reflect transport. It can also be noted that the preservation of the cysts is excellent, with the cysts analysed here often still containing the remains of cell contents (Fig. 2).

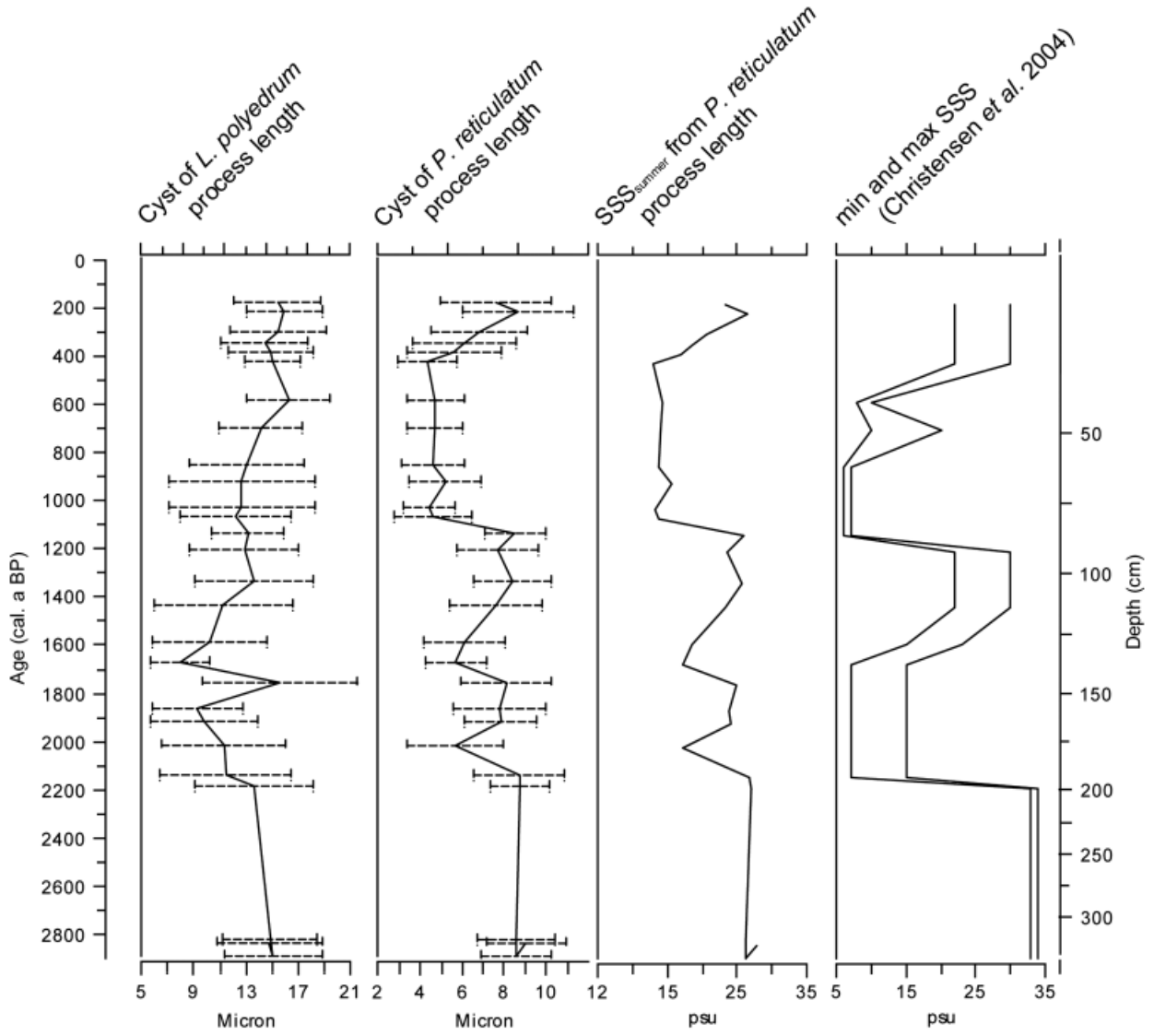

Fig. 6. Average process length variation of cysts of *Lingulodinium polyedrum* and cysts of *Protoceratium reticulatum*, next to summer sea surface salinity down-core in Limfjord reconstructed from average process length of *Protoceratium reticulatum* and from macrofaunal analysis (Christensen *et al.* 2004). All are plotted against both age and depth. The error bars on the process lengths represent ±1 SD.

Salinity and temperature?

For cysts of *Lingulodinium polyedrum*, both salinity and temperature cause morphological variations, both in culture (Hallett 1999) and in the field (Mertens *et al.* 2009b). Because of the close taxonomic affinity of *Protoceratium reticulatum* with *Lingulodinium polyedrum*, a similar relationship may be expected for the former. However, the covariation of salinity and temperature in the studied region makes it impossible to disentangle the influence of the two parameters. Pearson's correlation coefficients between sea surface salinity and sea surface temperature are relatively high, both annually ($R^2 = 0.71$) and seasonally, with a notable exception during summer (summer $R^2 = 0.06$, autumn $R^2 = 0.70$, spring $R^2 = 0.62$ and winter $R^2 = 0.96$). This correlation is similar for values deeper in the water column, namely at 10, 20 and 30 m. The covariation between salinity and temperature also results in a correlation between sea surface density and sea surface salinity, both annually ($R^2 = 0.95$) and seasonally (summer $R^2 = 0.99$, autumn $R^2 = 1.00$, spring $R^2 = 0.99$ and winter $R^2 = 1.00$). Thus, from our data alone we cannot determine whether salinity, density or any other combination of salinity and temperature is the most important factor in causing changes in process length. Extending this research to other regions could resolve this issue. Nevertheless, taking into account that the cysts probably reflect summer conditions of the surface waters (see above) we consider it safe to assume that our proxy can

be used for reconstruction of sea surface salinity (SSS) during summer in the Skagerrak–Kattegat–Baltic, as both density and salinity – which gave the more significant correlations – are strongly correlated in this area. Hence, for this region, we suggest the following equation (see Fig. 7): $SSS_{summer} = 3.16 \times$ average process length -0.84 ($R^2 = 0.8$). The standard error on the reconstructed SSS_{summer} is 0.81 psu.

Application of salinity reconstruction down-core in Limfjord

Using the above equation, SSS_{summer} was reconstructed from average process lengths of *Protoceratium reticulatum* (Fig. 6). Fluctuations are similar to the maximum and minimum salinity ranges proposed from a macrofaunal analysis on the same core (Christensen *et al*. 2004) (Fig. 6), showing more brackish (13–17 psu) conditions between 2000 and 1400 cal. a BP and 1100 and 400 cal. a BP, reverting back to normal salinity conditions in between (22–30 psu, the current salinity range in the Limfjord). A notable difference is the excursion to more marine conditions around 1800 cal. a BP shown by our salinity proxy. This and other discrepancies between the two proxies can be related to effects of seasonality, resulting in higher average salinities reconstructed from the process length record.

Conclusions

This study documents the morphological variation of dinoflagellate cysts extracted from sediments from the Skagerrak–Kattegat–Baltic estuarine system. The average process length of cysts of *Lingulodinium polyedrum* does not show any significant correlation with environmental parameters, probably as a result of its restriction to fjords around the Skagerrak–Kattegat. By contrast, the average process length of cysts of the cosmopolitan species *Protoceratium reticulatum* is correlated with summer salinity through the equation $SSS_{summer} = 3.16 \times$ average process length -0.84 ($R^2 = 0.8$). This equation can be used as a proxy to reconstruct salinity quantitatively in the Skagerrak–Kattegat–Baltic estuarine system, as shown by the salinity reconstruction from a late Quaternary core at Limfjord. Further research on process length variation of cysts of *Protoceratium reticulatum* in other regions should broaden the area of application and contribute to a better understanding of the possible temperature influence on the proxy.

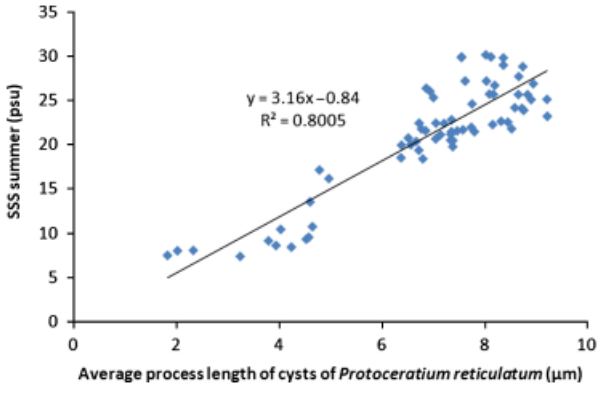

Fig. 7. Relationship between average process length of cysts of *Protoceratium reticulatum* and summer sea surface salinity (SSS), and linear trend-line. This figure is available in colour at http://www.boreas.dk.

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References

Aasen, J., Samdal, I. A., Miles, C. O., Dahl, E., Briggs, L. R. & Aune, T. 2005: Yessotoxins in Norwegian blue mussels (*Mytilus edulis*): uptake from *Protoceratium reticulatum*, metabolism and depuration. *Toxicon 45*, 265–272.

Antonov, J. I., Locarnini, R. A., Boyer, T. P., Mishonov, A. V. & Garcia, H. E. 2006: *World Ocean Atlas 2005, Volume 2: Salinity*. Levitus, S. (ed.): NOAA Altlas NESDIS 62. U.S. Government Printing Office, Washington, D.C, 182 pp.

Bendle, J., Rosell-Melé, A. & Ziveri, P. 2005: Variability of unusual distributions of alkenones in the surface waters of the Nordic seas. *Paleoceanography 20*, PA2001, doi:10.1029/2004PA001025.

Blanz, T., Emeis, K.-C. & Siegel, H. 2005: Controls on alkenone unsaturation ratios along the salinity gradient between the open ocean and the Baltic Sea. *Geochimica et Cosmochimica, Acta 69*, 3589–3600.

Bolch, C. J. S. 1997: The use of polytungstate for the separation and concentration of living dinoflagellate cysts from marine sediments. *Phycologia 37*, 472–478.

Bollmann, J., Herrle, J. O., Cortés, M. Y. & Fielding, S. R. 2009: The effect of sea water salinity on the morphology of *Emiliania huxleyi* in plankton and sediment samples. *Earth and Planetary Science Letters 284*, 320–328.

Braarud, T. 1976: The natural history of the Hardangerfjord. 12. The ecology of taxonomic groups and species of phytoplankton related to their distribution patterns in a Fjord area. *Sarsia 60*, 41–62.

Braarud, T. & Bursa, A. 1939: The phytoplankton of the Oslo Fjord 1933–1934. *Hvalrådets Skrifter, Scientific results of marine biological research 19*, 1–49.

Braarud, T., Föyn Hovsfang, B., Hjelmfoss, P. & Överland, A.-K. 1974: The natural history of the Hardangerfjord. 10. The phytoplankton in 1955–56. The quantitative phytoplankton cycle in the fjord waters and in the offshore coastal waters. *Sarsia 55*, 63–98.

Brenner, W. 2005: Holocene environmental history of the Gotland Basin (Baltic Sea) – a micropalaeontological model. *Palaeogeography, Palaeoclimatology, Palaeoecology 220*, 227–241.

Christensen, J. T., Cedhagen, T. & Hylleberg, J. 2004: Late-Holocene salinity changes in Limfjorden, Denmark. *Sarsia 89*, 379–387.

Clarke, A. L., Weckström, K., Conley, D. J., Anderson, N. J., Adser, F., Andrén, E., de Jonge, V. N., Ellegaard, M., Juggins, S.,

Kauppila, P., Korhola, A., Reuss, N., Telford, R. J. & Vaalgamaa, S. 2006: Long-term trends in eutrophication and nutrients in the coastal zone. *Limnology and Oceanography 51*, 385–397.

Dale, B. 1976: Cyst formation, sedimentation, and preservation: factors affecting dinoflagellate assemblages in recent sediments from Trondheimsfjord, Norway. *Review of Palaeobotany and Palynology 22*, 39–60.

Dale, B. 1985: Dinoflagellate cyst analysis of Upper Quaternary sediments in core GIK 15530-4 from the Skagerrak. *Norsk Geologisk Tidsskrift 65*, 29–34.

Dale, B. 1996: Dinoflagellate cyst ecology: modelling and geological applications. *In* Jansonius, J. & McGregor, D. C. (eds.): *Palynology: Principles and Applications 3*, 1249–1275. AASP Foundation, Dallas, TX.

Dale, B., Thorsen, T. A. & Fjellså, A. 1999: Dinoflagellate cysts as indicators of cultural eutrophication in the Oslofjord, Norway. *Estuarine, Coastal and Shelf Science 48*, 371–382.

De Schepper, S., Head, M. J. & Groeneveld, J. 2009: North Atlantic Current variability through marine isotope stage M2 (circa 3.3 Ma) during the mid-Pliocene. *Paleoceanography 24*, PA4206, doi: 10.1029/2008PA001725.

Dodge, J. D. & Harland, R. 1991: The distribution of planktonic dinoflagellates and their cysts in the eastern and northeastern Atlantic Ocean. *New Phytologist 118*, 593–603.

Ellegaard, M. 2000: Variations in dinoflagellate cyst morphology under conditions of changing salinity during the last 2000 years in the Limfjord, Denmark. *Review of Palaeobotany and Palynology 109*, 65–81.

Grindley, J. R. & Nel, E. 1968: Mussel poisoning and shellfish mortality on the west coast of Africa. *South African Journal of Sciences 64*, 420–422.

Grindley, J. R. & Nel, E. 1970: Red water and mussel poisoning at Elands Bay, December 1966. *Fisheries Bulletin South Africa 6*, 36–55.

Grøsfjeld, K. & Harland, R. 2001: Distribution of modern dinoflagellate cysts from inshore areas along the coast of southern Norway. *Journal of Quaternary Science 16*, 651–659.

Gundersen, N. 1988: *En palynologisk undersøkelse av dinoflagellatcyster langs en synkende salinitetsgradient I recente sedimenter fra Østersjø-området*. Cand. Scient. thesis, University of Oslo, 96 pp.

Hallett, R. I. 1999: *Consequences of environmental change on the growth and morphology of Lingulodinium polyedrum (Dinophyceae) in culture*. Ph.D. thesis, University of Westminster, 109 pp.

Hällfors, G. 2004: Checklist of Baltic Sea phytoplankton species (including some heterotrophic protistan groups). Helsinki Commission, Baltic Marine Environment Protection Commission. *Baltic Sea Environment Proceedings 95*, 208 pp.

van Harten, D. 2000: Variable noding in *Cyprideis torosa* (Ostracoda, Crustacea): an overview, experimental results and a model from Catastrophe Theory. *Hydrobiologia 419*, 131–139.

Head, M. J. 1994: Morphology and palaeoenvironmental significance of the Cenozoic dinoflagellate cyst genera *Tectatodinium* and *Habibacysta*. *Micropaleontology 40*, 289–321.

Head, M. J. 1996: Modern dinoflagellate cysts and their biological affinities. *In* Jansonius, J. & McGregor, D. C. (eds.): *Palynology: Principles and Applications 3*, 1197–1248. AASP Foundation, Dallas, TX.

Head, M. J. 2007: Last Interglacial (Eemian) hydrographic conditions in the southwestern Baltic Sea based on dinoflagellate cysts from Ristinge Klint, Denmark. *Geological Magazine 144*, 987–1013.

Horstman, D. A. 1981: Reported red-water outbreaks and their effects on fauna of the west and south coasts of South Africa, 1959–1980. *Fisheries Bulletin South Africa 15*, 71–88.

Koike, K., Horie, Y., Suzuki, T., Kobiyama, A., Kurihara, K., Takagi, K., Kaga, S. N. & Oshima, Y. 2006: *Protoceratium reticulatum* in northern Japan: environmental factors associated with seasonal occurrence and related contamination of yessotoxin in scallops. *Journal of Plankton Research 28*, 103–112.

Kokinos, J. P. & Anderson, D. M. 1995: Morphological development of resting cysts in cultures of the marine dinoflagellate *Lingulodinium polyedrum* (= *L. machaerophorum*). *Palynology 19*, 143–166.

Konieczny, R. 1983: *En miljørettet palynologisk analyse av dinoflagellat-cyster i resente marine sedimenter fra Skagerrak*. Cand. Scient. thesis, University of Oslo, 106 pp.

Larsson, A.-M. & Rodhe, J. 1979: Hydrographical and chemical observations in the Skagerrak. *Gøteborg University, Department of Oceanography, Report 29*, 1–39.

Leppäranta, M. & Myrberg, K. 2009: *Physical Oceanography of the Baltic Sea*. 378 pp. Springer, Berlin.

Locarnini, R. A., Mishonov, A. V., Antonov, J. I., Boyer, T. P. & Garcia, H. E. 2006: World Ocean Atlas 2005, Volume 1: Temperature. Levitus, S. (ed.): NOAA Atlas NESDIS 61, U.S. Government Printing Office, Washington, D.C., 182 pp.

Marret, F. & Zonneveld, K. 2003: Atlas of modern organic-walled dinoflagellate cyst distribution. *Review of Palaebotany and Palynology 125*, 1–200.

Marret, F., Mudie, P., Aksu, A. & Hiscott, R. N. 2009: A Holocene dinocyst record of a two-step transformation of the Neoeuxinian brackish water lake into the Black Sea. *Quaternary International 193*, 72–86.

Matsuoka, K. 1990: *Protoceratium reticulatum* (Claparède and Lachmann) Bütschli. *In* Fukuyo, Y., Takano, H., Chihara, M. & Matsuoka, K. (eds.): *Red Tide Organisms in Japan – An illustrated taxonomic guide*, 110–111. Uchida-Roukakuho, Tokyo.

Matsuoka, K., McMinn, A. & Wrenn, J. 1997: Restudy of the holotype of *Operculodinium centrocarpum* (Deflandre and Cookson) Wall (Dinophyceae) from the Miocene of Australia, and the taxonomy of related species. *Palynology 21*, 19–33.

Matthiessen, J. & Brenner, W. 1996: Chlorococcalalgen und Dinoflagellaten-Zysten in rezenten Sedimenten des Greifswalder Boddens (südliche Ostsee). *Senckenbergiana Maritima 27*, 33–48.

van der Meer, M. T. J., Sangiorgi, F., Baas, M., Brinkhuis, H., Sinninghe-Damsté, J. S. & Schouten, S. 2008: Molecular isotopic and dinoflagellate evidence for Late Holocene freshening of the Black Sea. *Earth and Planetary Science Letters 267*, 426–434.

Mertens, K. N., González, C., Delusina, I. & Louwye, S. 2009a: 30 000 years of productivity and salinity variations in the late Quaternary Cariaco Basin revealed by dinoflagellate cysts. *Boreas 38*, 647–662.

Mertens, K. N., Ribeiro, S. & Bouimetarhan, I. *et al.* 2009b: Process length variation in cysts of a dinoflagellate, *Lingulodinium machaerophorum*, in surface sediments: Investigating its potential as salinity proxy. *Marine Micropaleontology 70*, 54–69.

Montresor, M., Zingone, A. & Sarno, D. 1998: Dinoflagellate cyst production at a coastal Mediterranean site. *Journal of Plankton Research 20*, 2291–2312.

Mudie, P. J., Aksu, A. E. & Yasar, D. 2001: Late Quaternary dinoflagellate cysts from the Black, Marmara and Aegean seas: variations in assemblages, morphology and paleosalinity. *Marine Micropaleontology 43*, 155–178.

Nehring, S. 1997: Dinoflagellate resting cysts from recent German coastal sediments. *Botanica Marina 40*, 307–324.

Nürnberg, D. & Groeneveld, J. 2006: Pleistocene variability of the Subtropical Convergence at East Tasman Plateau: Evidence from planktonic foraminiferal Mg/Ca (ODP Site 1172A). *Geochemistry, Geophysics, Geosystems 7*, Q04P11, doi: 10.1029/2005GC000984.

Persson, A., Godhe, A. & Karlson, B. 2000: Dinoflagellate cysts in recent sediments from the West coast of Sweden. *Botanica Marina 43*, 66–79.

Pitcher, G. C. & Joyce, L. 2009: Dinoflagellate cyst production on the southern Namaqua shelf of the Benguela upwelling system. *Journal of Plankton Research 31*, 865–875.

Pospelova, V., Esenkulova, S., Johannessen, S. C., O'Brien, M. C. & Macdonald, R. W. 2010: Organic-walled dinoflagellate cyst production, composition and flux from 1996 to 1998 in the central Strait of Georgia (BC, Canada): A sediment trap study. *Marine Micropaleontology 75*, 17–37.

Reid, P. C. 1974: Gonyaulacacean dinoflagellate cysts from the British Isles. *Nova Hedwigia 25*, 579–637.

Reid, P. C. 1978: Dinoflagellate cysts in the plankton. *New Phytologist 80*, 219–229.

Reid, P. C., Fischer, A. C. & Lewis-Brown, E. *et al.* 2009: Impacts of the oceans on climate change. *Advances in Marine Biology 56*, 1–150.

Reinecke, P. 1967: A dinoflagellate causing a red tide at Elands Bay, Cape Province, in December 1966. *The Journal of South African Botany 33*, 157–160.
Rodhe, J. 1987: The large-scale circulation in the Skagerrak; interpretation of some observations. *Tellus 39A*, 245–253.
Rohling, E. J. 2007: Progress in palaeosalinity: overview and presentation of a new approach. *Paleoceanography 22*, PA3215, doi: 10.1029/2007PA001437.
Schlitzer, R. 2010: *Ocean Data View*. Available at: http://odv.awi.de (accessed 01.03.10).
Schmidt, M. W., Spero, H. J. & Lea, D. W. 2004: Links between salinity variation in the Caribbean and North Atlantic thermohaline circulation. *Nature 428*, 160–163.
Sorrel, P., Popescu, S. M., Head, M. J., Suc, J. P., Klotz, S. & Oberhänsli, H. 2006: Hydrographic development of the Aral Sea during the last 2000 years based on a quantitative analysis of dinoflagellate cysts. *Palaeography, Palaeoclimatology, Palaeoecology 234*, 304–327.
Stigebrandt, A. 1983: A model for the exchange of water and salt between the Baltic and Skagerrak. *Journal of Physical Oceanography 13*, 415–427.
Svansson, A. 1975: Physical and chemical oceanography of the Skagerrak and Kattegat. *Open Sea Conditions*, *Fishery Board of Sweden, Institute of Marine Research, Lysekil, Report 1*, 88 pp.
Svansson, A. 1984: Hydrographic features of the Kattegat. *Rapport et Proces Verbaux Reunion Conseil International Exploration de la Mer 185*, 78–90.
Thorsen, T. A. & Dale, B. 1998: Climatically influenced distribution of *Gymnodinium catenatum* during the past 2000 years in coastal sediments of southern Norway. *Palaeogeography, Palaeoclimatology, Palaeoecology 143*, 159–177.
Thorsen, T. A., Dale, B. & Nordberg, K. 1995: 'Blooms' of the toxic dinoflagellate *Gymnodinium catenatum* as evidence of climatic fluctuations in the late Holocene of southwestern Scandinavia. *The Holocene 5*, 435–446.
Verleye, T. J., Mertens, K. N., Louwye, S. & Arz, H. W. 2009: Holocene salinity changes in the southwestern black sea: a reconstruction based on dinoflagellate cysts. *Palynology 33*, 77–100.
de Vernal, A. & Hillaire-Marcel, C. 2000: Sea-ice cover, sea-surface salinity and halo/thermocline structure of the northwest North Atlantic: Modern versus full glacial conditions. *Quaternary Science Reviews 19*, 65–85.
de Vernal, A., Goyette, C. & Rodrigues, C. G. 1989: Contribution palynostratigraphique (dinokystes, pollen et spores) á la connaissance de la Mer Champlain: coupe de Saint-Césaire, Québec. *Canadian Journal for Earth Sciences 26*, 2450–2464.
Wall, D. 1967: Fossil microplankton in deep-sea cores from the Caribbean Sea. *Palaeontology 10*, 95–123.
Wall, D. & Dale, B. 1966: "Living fossils" in western Atlantic plankton. *Nature 211*, 1025–1026.
Wall, D. & Dale, B. 1968: Modern dinoflagellate cysts and evolution of the Peridiniales. *Micropaleontology 14*, 265–304.
Wall, D., Dale, B. & Harada, K. 1973: Description of new fossil dinoflagellates from the Late Quaternary of the Black Sea. *Micropaleontology 19*, 18–31.
Wall, D., Dale, B., Lohman, G. P. & Smith, W. K. 1977: The environmental and climatic distribution of dinoflagellate cysts in modern marine sediments from regions in the North and South Atlantic Ocean and adjacent seas. *Marine Micropaleontology 2*, 121–200.
Wang, L., Sarnthein, M., Duplessy, J. C., Erlenkeuser, H., Jung, S. & Pfaumann, U. 1995: Paleo sea surface salinities in the low-latitude Atlantic: the $\delta^{18}O$ record of *Globigerinoides ruber* (White). *Paleoceanography 10*, 749–761.

Supporting information

Additional supporting information may be found in the online version of this article:

Table S1. All morphological measurements in surface sediments.
Table S2. Summary of morphological data in surface sediments.
Table S3. All morphological measurements from Limfjord.
Table S4. Summary of morphological data from Limfjord.

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