Chapter 12
Dinoflagellate Cysts as Proxies for Holocene Environmental Change in Estuaries: Diversity, Abundance and Morphology

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Abstract Dinoflagellates are important components of marine plankton as both primary producers and predators on bacteria and microeukaryotes. About 200 species, most of these estuarine, form a resting stage, called a cyst, as part of their life-cycle. Dinoflagellate cysts are mostly organic, composed of a very resistant material called dinosporin, and are often very well preserved in sediments. Dinoflagellate cysts have in many cases provided excellent records of changes through time in salinity, temperature and nutrients, and have also been used to reconstruct changes in sea ice cover, industrial pollution and coastal proximity through time. These environmental changes can be reconstructed from: (1) changes in dinoflagellate cyst communities; (2) changes in abundance; and (3) morphological variations in individual species. The two main methods for concentrating dinoflagellate cysts from sediment samples are palynological processing with HCl and

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HF, and density separation. As dinoflagellate cysts are mostly organic, they may be preserved where microfossils composed of silica or calcium carbonate are missing because of dissolution.

1   Introduction

Dinoflagellates are a diverse and intriguing group of organisms that are important components of marine plankton, both as primary producers and as heterotrophic or mixotrophic grazers on bacteria, phytoplankton and other protists (e.g. Schnepf and Elbrächter 1992). Dinoflagellates are found in the plankton as characteristically bi-flagellated motile cells, but many, particularly those inhabiting coastal and estuarine environments produce a resting stage (cyst) as part of their life cycle (Dale 1983). Cysts are often morphologically quite different from the vegetative stages (e.g. Ellegaard et al. 2002), are non-motile and enter the sedimentary cycle as silt parti-cles which accumulate together with fine-grained material in bottom sediments in areas with low physical forcing (e.g. Lewis 1988). Cysts can remain viable up to at least a century (Lundholm et al. 2011) and the resistant cyst walls are commonly found as microfossils in sediments dating from at least the Triassic (250 to 200 Ma; MacRae et al. 1996) to the present day. The existence of two very distinct life cycle stages has resulted in two taxonomic systems: one based on the morphology of living motile cells (used by biologists); and the other based on resting cyst morphology (used by paleontologists). Here we use the motile stage-based name when possible and, in addition, give the equivalent cyst-based name where relevant. Many dinofla-gellate cysts are organic-walled, composed of so-called dinosporin, which is possibly a carbohydrate (Versteegh et al. 2012), although at least 30 species have calcareous cyst walls (Zonneveld et al. 2005). Organic-walled cysts can be preserved in environ-ments where mineralised microfossils are absent due to dissolution and are found in aquatic sediments in all areas of the globe (e.g. Zonneveld et al. 2013).

Dinoflagellate cysts are excellent paleoecological indicators: cyst assemblages and their absolute abundances have been shown to reflect changes in, for example, temperature, salinity and primary productivity (N, P, chl *a*) (e.g. Dale 1996; Zonneveld et al. 2013), as well as the effects of industrial pollution (e.g. Sætre et al. 1997; Pospelova et al. 2002, 2005; Liu et al. 2012) and coastal proximity (Dale 1996). Furthermore, some of these environmental factors can also affect the morphology of individual species. This has been shown for salinity, both in labora-tory studies (Ellegaard et al. 2002) and in natural assemblages (Ellegaard 2000; Mertens et al. 2009, 2010, 2012a, b). A few studies also indicate that temperature affects cyst size (Ellegaard et al. 2002). Several factors may, however, distort the paleoecological signals. Some dinoflagellate cysts are sensitive to oxidation

(e.g. Zonneveld et al. 2008), which may complicate the interpretation of the environmental signal if cysts have been exposed to excessive oxidation in the water column or bottom sediments. Another potential complicating factor is cyst transport via dynamic processes in the sedimentary regime, such as ocean currents (Wall et al. 1977). In estuarine environments, however, these factors will rarely present problems as these systems are not usually influenced by long-distance transport, and estuaries are often very productive, with accompanying high sedimentation rates and low oxygen levels in bottom waters where the cysts are deposited (Howe et al. 2010a). The coastal proximity of estuaries means that cyst-producing dinoflagellates in these environments are often subjected to large salinity variations, as well as variable (and often high) levels of nutrients. The relatively shallow water in most of these areas also allows for the tight coupling between the benthic and planktic environments necessary for meroplanktic organisms.

To date, a total of 2294 species of dinoflagellates have been described (Gómez 2012), and in a comprehensive review of living dinoflagellate cysts, Head (1996) listed some 200 known marine cyst-forming species (ca. 25 of these partly calcified). Since then, at least 20 marine cyst-forming species have been described (e.g. Reñé et al. 2011). Recent studies furthermore indicate that some species descriptions may be masking further cryptic diversity (e.g. Soehner et al. 2012, who uncovered 21 ITS ribotypes within the species *Scrippsiella trochoidea* (Stein) Balech ex Loeblich III 1965). In a recent comprehensive overview of global modern distributions of marine to brackish dinoflagellate cysts in sediments, 71 organic-walled species were included (Zonneveld et al. 2013). Individual paleoecological studies of dinoflagellate cyst assemblages in recent estuarine sediments typically encompass 15–35 species (Dale 1976), however, in some nutrient rich estuarine systems this number can be higher (Price and Pospelova 2011). Many studies report cysts of unknown affinity or new cyst types (e.g. Head 2007; Price and Pospelova 2011), indicating that the diversity of cysts is yet far from fully documented.

This chapter focuses on reports of changes in organic-walled dinoflagellate cyst assemblages and morphology, as well as their implications for paleoenvironmental interpretations in Holocene estuarine sediments. The main cyst signals that have been detected in estuarine environments are changes in temperature, salinity, nutrient levels and industrial pollutants, and each of these is addressed separately.

2 The Link Between the Metabolically Active, Flagellated, Vegetative Stage and the Resting Cyst

In dinoflagellates, the stage that is preserved in the sediment record is the resting cyst. This stage was in earlier studies (summarised by Dale 1983) shown to be the result of sexual reproduction, with the cyst representing the diploid, hypnozygote stage. In recent studies this general picture has been nuanced as some studies have shown sexual reproduction without cyst formation (e.g. Figueroa and Bravo 2005)

and others cyst formation without sexual reproduction (Kremp and Parrow 2006). Numerous laboratory studies, as well as field investigations, have shown effects of temperature, nutrients and salinity on dinoflagellate vegetative growth, planozygote and cyst production, cyst dormancy and longevity, and germination success (e.g. Lundholm et al. 2011; Kremp et al. 2009). Environmental conditions at different stages of the life cycle thus influence the final production of cysts, with the net result being the deposition of cysts in sediments under, or close to, where the vegetative stage thrives. Using temperature as an example, some species will then have a more restricted window for encystment than for vegetative growth (Anderson et al. 1985; Sgrosso et al. 2001), which can either be at the low or high end of the growth range. For example, for *Alexandrium minutum* Halim 1960, higher temperatures have a positive effect on both planozygote and cyst production (Figueroa et al. 2011). A threshold cell concentration is required for a minimum encounter rate between gametes to produce the zygote, which then forms the cyst (Wyatt and Jenkinson 1997). In other words, cyst production is dependent on the abundance of vegetative cells. Although dinoflagellate cyst production may be induced by stressful environmental conditions (e.g. nutrient limitation, darkness), there is solid evidence that encystment can also occur under favorable growth conditions, and may be triggered during the active growth phase (Rengefors 1998; Olli and Anderson 2002; Probert 2002). Kremp et al. (2009) suggested that multiple cues may trigger cyst formation—stressors (or ultimate causes) and signals (or token cues).

3   Methods for Preparing and Studying Dinoflagellate Cysts

The two main methods for concentrating dinoflagellate cysts from sediment samples are palynological processing (e.g. de Vernal et al. 1996) and density separation (Bolch 1997). Briefly, the palynological method involves removing mineral remains with HCl and HF and subsequently sieving for size fractionation. Typically the fractions between 10, 20 or 25 and 100 μm are kept. The density separation method is often used to concentrate living cysts, but may also be used for empty cysts. This method involves layering a size fractionated, waterlogged sediment sample onto a sodium polytungstate solution, centrifuging and isolating the fraction concentrated at the density interface. After rinsing, samples from both methods may be permanently mounted in glycerol gelatin and sealed with wax. Absolute concentrations may be calculated, either by counting an entire sample of a known weight of dry sediment, an aliquot of a sample, or by adding *Lycopodium clavatum* spore tablets to each sample (the marker-grain method; Stockmarr 1971). Typically, at least 300 dinoflagellate cysts are counted per sample (e.g. Mertens et al. 2009). Standard literature for nomenclature and identification include Rochon et al. (1999) and Head et al. (2001).

4   Dinoflagellate Cysts as Proxies of Nutrient Levels and Pollution

Nutrients are one of the primary factors regulating dinoflagellate cyst distributions in marine and brackish water environments. Nutrients have the following effects on dinoflagellate cysts: (1) changes in dinoflagellate cyst assemblages; (2) changes in dinoflagellate cyst concentrations; and (3) changes in dinoflagellate cyst diversity. Two main sources of evidence are: cyst distributions in recent sediments from high nutrient waters today, and documented changes in cyst assemblages coincident with increased nutrients over time, as revealed from cored bottom sediments. Comparing both these lines of evidence has identified strong signals that are particularly useful for interpreting changes in the nutrient status of coastal environments due to eutrophication.

The link between cysts and nutrients was first identified from sites of coastal upwelling where cyst assemblages were heavily dominated by heterotrophic species (Wall et al. 1977). Over the last two decades extensive research has demonstrated that dinoflagellate cysts also reflect nutrient enrichment or eutrophication in coastal systems, by changing composition of cyst assemblages, their sedimentary concentrations or fluxes, and diversity. Pioneering studies of dinoflagellate cysts in Norwegian fjords (Dale and Fjellså 1994; Sætre et al. 1997; Thorsen and Dale 1997; Dale et al. 1999) proposed the utility of dinoflagellate cysts as indicators of eutrophication in the recent past. By correlating changes in dinoflagellate cyst assemblages with data on the anthropogenic nutrient enrichment in the inner part of the Oslofjord, Dale and Fjellså (1994) provided the first evidence that dinoflagellate cysts respond to cultural eutrophication, confirmed by subsequent studies (see Dale et al. 1999). The eutrophication signal in the Oslofjord comprised an increase in total dinoflagellate cyst abundances, for example, from 7000 to 156,000 cysts g^{-1}, interpreted as a reflection of increased primary production, with a marked increase in *Lingulodinium polyedrum* (Stein) Dodge 1989 (cyst name *Lingulodinium machaerophorum* (Deflandre & Cookson) Wall 1967) accounting for much of the total cyst abundances. *Lingulodinium polyedrum* is a warmer-water species that blooms in late summer in the Oslofjord. Its increase in response to eutrophication is interpreted to reflect the fact that the Oslofjord is a naturally nutrient limited system. Normally, blooms of this species would be limited by the amounts of nutrients remaining after the spring and early summer plankton production, but the year-round injection of sewage effluent extended the available nutrient supply that *L. polyedrum* could utilize (Dale 2001, 2009). Added support for the eutrophication interpretation was provided by the record of the main spring-blooming species, *Pentapharsodinium dalei* Indelicato & Loeblich III 1986, whose cysts showed no apparent response to eutrophication, presumably since it normally experienced an excess of nutrients prior to eutrophication. The follow-up studies of dinoflagellate cysts in the north-western Adriatic Sea, within proximity of the Po-river delta (Sangiorgi and Donders 2004; Zonneveld et al. 2009), confirmed that the increased

abundance of *L. polyedrum* can be used as a eutrophication signal in warm, deeper estuaries with stratified waters or with a large river input (see discussions in Dale 2009; Pospelova et al. 2002; Zonneveld et al. 2009). The Po-river study also illustrated the usefulness of cysts in cores where diatoms are absent due to dissolution (Sangiorgi and Donders 2004). In coastal areas, *L. polyedrum* has been identified as an indicator of warmer sea surface waters and stratified conditions such as those resulting from upwelling relaxation (Bringué et al. 2013; see also Sect. 6).

An alternative cyst signal from another Norwegian fjord subjected to higher levels of nutrient loading by sewage has been described by Thorsen and Dale (1997): basically a proportional increase in cysts of heterotrophic species very similar to the upwelling signals (see above). Similar signals have also been observed in many other, primarily cooler and/or well-mixed, heavily eutrophied estuaries of Japan (Matsuoka 1999, 2001; Matsuoka et al. 2003), eastern United States (Pospelova et al. 2002, 2005), Canada (de Vernal and Giroux 1991; Thibodeau et al. 2006; Radi et al. 2007; Krepakevich and Pospelova 2010), Denmark (Ellegaard et al. 2006), Korea (Pospelova and Kim 2010; Shin et al. 2010b), and China (Liu et al. 2012). These studies have recorded the proportional increase of additional cysts produced by heterotrophic dinoflagellates, in particular of mixed-dieted Polykrikaceae and Diplopsalidaceae (Matsuoka 1999, 2001; Pospelova et al. 2002, 2005; Matsuoka et al. 2003; Ellegaard et al. 2006; Dale 2009).

One of the major point sources of nutrient enrichment in estuarine waters in densely urbanised areas is sewage and storm water effluent. Direct impact of increased nutrient enrichment from sewage outfalls was observed in dinoflagellate cysts from surface sediment samples (Pospelova et al. 2005; Krepakevich and Pospelova 2010). It has been shown that even at small spatial scales, where changes in salinity and temperature were negligible, cyst assemblages vary along gradients of nutrient enrichment, in direct correlation to distance from the sewage outfalls (Pospelova et al. 2005). Gradual change in composition of the cyst assemblages with increased proportions of cysts of heterotrophic dinoflagellates, particularly Polykrikaceae and Diplopsalidaceae, towards the sewage outfalls was accompanied by large fluctuations in the total cyst concentration. Temporal (sediment core) records have also shown that large fluctuations in the total cyst abundances (Pospelova et al. 2002; Shin et al. 2010b), or individual taxa (e.g. cysts of *L. polyedrum* in the Oslofjord; Thorsen and Dale 1997; Dale et al. 1999; and Po River deltaic system (Zonneveld et al. 2012), *Spiniferites* spp. in Buzzards Bay embayments (Pospelova et al. 2002), can be considered as eutrophication signals and be indicative of an increased number or duration of dinoflagellate blooms. However, in this context it should be noted that short-term climatic oscillations (e.g. the North Atlantic Oscillation, NAO; El Niño/La Niña events) may sometimes enhance these fluctuations (Dale 2009; Pospelova et al. 2010). For example, the NAO may induce periods of increased availability of dissolved nutrients and stratification by increasing the amount of relatively nutrient-rich water flowing from the Kattegat and North Sea-Skagerrak areas towards the Swedish west coast (Lindahl et al. 1998; Harland et al. 2006; see discussion in Dale 2009). The El Niño-Southern Oscillation (ENSO) climatic events also affect nutrient availability, sea-surface temperature (SST), and

freshwater discharge along the Pacific coast of North America, including the Strait of Georgia (British Columbia, Canada), where large variations in dinoflagellate cyst fluxes and assemblage composition were recorded during the 1997–1998 El Niño event (Pospelova et al. 2010).

An increase of algal blooms, including those caused by harmful species due to instability of an ecosystem, is one of the effects of cultural eutrophication on phytoplankton dynamics (Smayda 1990; Dale 2001). Harmful blooms of *Alexandrium* are common in many nutrient rich estuarine systems and have been studied worldwide in order to understand mechanisms of costly paralytic shellfish poisoning associated with this genus (e.g. Anderson 1994, 1997; Glibert et al. 2005). Recent work by Hattenrath et al. (2010) has shown that sewage sources of nitrogen enrichment, especially in the form of ammonium (McGillicuddy et al. 2014), have played an important role in increasing *A. fundyense* Balech in D.M. Anderson, A.W. White, & D.G. Baden 1985 blooms and cell toxicity in Northport Bay (New York, USA). This study supports observations of increased sedimentary abundances of cysts produced by *Alexandrium* within proximity of sewage treatment plant outfalls in Buzzards Bay embayments (Massachusetts, USA) (Pospelova et al. 2005), Southern Vancouver Island bays (Krepakevich and Pospelova 2010), and Bedford Basin embayment (our interpretation of Lacasse et al. 2013 data).

Only a few published studies have addressed the effect of industrial pollution on dinoflagellate cysts (e.g. Sætre et al. 1997; Pospelova et al. 2002, 2005; Liu et al. 2012). Quite commonly, sites with the highest levels of industrial pollution are also highly eutrophic (or hypertrophic). Presence of pollutants, particularly high concentrations of copper, which is toxic to dinoflagellates, leads to decreased cyst concentrations in sediments, as well as to a reduction of diversity or species richness. Similar trends have been shown in spatial distributions of dinoflagellate cysts from Buzzards Bay embayments (Pospelova et al. 2005), and in temporal cyst records in the industrially polluted Frierfjord (Sætre et al. 1997) and New Bedford Harbor (Pospelova et al. 2002). Low dinoflagellate cyst concentrations associated with industrial pollution are likely to reflect suppressed dinoflagellate production due to high levels of metals and other toxic pollutants in the water column and sediments at the study sites.

5   Dinoflagellate Cysts as Proxies of Salinity

Estuarine systems are characteristically prone to pronounced salinity fluctuations and gradients caused by, for example, the precipitation-evaporation balance, river discharge and influence of ocean currents. Reconstructions of salinity levels are, therefore, important for determining the historical development of an estuarine system, and dinoflagellate cysts have been shown to be useful indicators of changes in salinity through time. Many dinoflagellates show a restricted tolerance to salinity, such that the motile stages and their cysts are absent when salinity exceeds their limits (e.g. Dale and Dale 2002; de Vernal and Marret 2007). Some species are truly

marine (occurring in salinities higher than ca. 30 ppt; e.g. *Impagidinium* species), others can only withstand freshwater conditions (less than 0.5 ppt; Mertens et al. 2012c), while most range between marine and freshwater conditions (Dale 1996). As seen in many other organism groups, the diversity of cyst-forming dinoflagellates often decreases over a decreasing salinity gradient in estuaries and semi-enclosed seas (Dale 1996). Such spatial changes in dinoflagellate cyst assemblages have been documented along the salinity gradient in the Baltic Sea (Dale 1996) and the Gulf of St. Lawrence (de Vernal and Giroux 1991). Also, Pospelova et al. (2004) found that lagoons in New England (USA) with low salinities (<10 ppt) are characterized by low species diversities and cyst concentrations, with cysts of freshwater dinoflagellates found alongside species of broad salinity tolerance ranges. Quantitative reconstructions of salinity have been achieved by using transfer functions, which use modern dinoflagellate cyst assemblages as modern analogues, more particularly from the North Atlantic (e.g. de Vernal et al. 2001). There has been recent debate about this methodology centering on issues of taxonomic ambiguities, autocorrelation and issues arising from large-scale transport of cysts found in oceanic sediments (Telford 2006; Telford and Birks 2005, 2009, 2011; Guiot and de Vernal 2011). This should be taken into account when assessing reconstructions using transfer functions and likewise, caution should be used when salinity data is extracted from gridded data to delimit ecological ranges (Zonneveld et al. 2013).

Observations from surface sediment assemblages and from culture studies have been used to infer (approximate) salinity levels in temporal records of dinoflagellate cyst assemblages, e.g. in Yeoya Bay, Korea (Shin et al. 2010a), Bjørnsholm Bay, the Limfjord, Denmark (Ellegaard 2000), the Black Sea (Mudie et al. 2001, 2002; Marret et al. 2009; Bradley et al. 2012) and the Baltic Sea (Brenner 2005).

In addition to the changes in cyst-assemblages over salinity gradients, some species also show changes in morphology in response to lowered salinity (examples shown in Fig. 12.1). This has been shown, both in analyses of surface sediment assemblages over known salinity gradients and in controlled laboratory studies. Salinity variations affect the morphology of gonyaulacoid dinoflagellate cysts, more particularly the length of processes (e.g. solid or hollow outgrowths from the cyst wall), which broadly speaking is reduced in synchronicity with salinity (e.g. Dale and Dale 2002). A relation between process length and salinity has been shown for cysts of *Gonyaulax baltica* Ellegaard et al. 2002; = *Spiniferites bulloideus* sensu Wall and Dale 1968 and other *Spiniferites* species formed in culture (Ellegaard et al. 2002; Rochon et al. 2009), for cysts of *Lingulodinium polyedrum* formed in culture (Hallett 1999) and extracted from surface sediments (Mertens et al. 2009) and for cysts of *Protoceratium reticulatum* (Claparède & Lachman) Bütschli 1885 (often reported by the cyst name *Operculodinium centrocarpum* (Deflandre & Cookson) Wall 1967; however see Paez-Reyes and Head 2013) extracted from surface sediments (Mertens et al. 2010, 2012a; Verleye et al. 2012). Other species with processes do not show an effect of salinity on the process length, as shown for cysts of *Pyrophacus steinii* (Schiller) Wall & Dale 1971 through culture experiments (Zonneveld and Susek 2007). Yet some species still need investigation (e.g. *Operculodinium israelianum* (Rossignol) Wall 1967, *Polysphaeridium zoharyi*
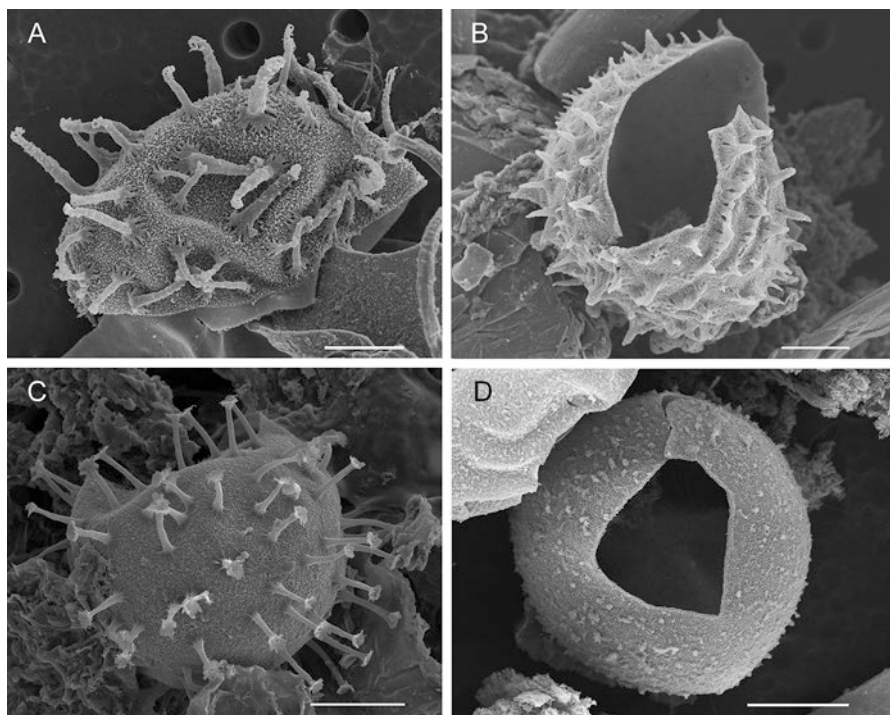
Fig. 12.1 Morphological variation of dinoflagellate cysts related to salinity variations as illustrated by Scanning electron microscopy (SEM). (**a**) Cyst of *Lingulodinium polyedrum* with long processes from the marine Vilaine Bay (South Brittany, France). (**b**) Cyst of *Lingulodinium polyedrum* with processes reduced in length from the brackish southern Caspian Sea, showing archeopyle. (**c**) Cyst of *Protoceratium reticulatum* with long processes from the marine southwest African coast. **d**) Cyst of *Protoceratium reticulatum* with extremely reduced processes from the brackish Northern Baltic, showing archeopyle. All scale bars = 10 μm

(Rossignol) Davey & Williams 1966 or further study (cysts of *Pentapharsodinium dalei* have been suggested to reduce in process length by Nehring (1994, 1997), but this still needs further confirmation).

For *G. baltica*, a linear relationship between process length and salinity was found within a salinity range of 10–35 ppt. However, at higher salinity (45 ppt) the process length decreased again (Ellegaard et al. 2002). Furthermore, when the length/salinity relationship was applied to natural assemblages deposited at known salinity ranges, the culture-derived model tended to overestimate the salinity (relative to historical data and macrofaunal based reconstructions), with the average process length minus one standard deviation achieving the most accurate salinity reconstruction (unpublished data). This indicates that although culture experiments may be very valuable for developing quantitative reconstructions of salinity levels (and probably other proxies), caution should be used in applying results from culture experiments directly to natural assemblages.

Relationships between the process length of cysts in sediment samples and the salinity of the overlying water mass have been used to reconstruct quantitative salinity changes using measurements of process lengths of cysts in sediment cores. This technique has been applied with success to reconstruct Holocene salinity variations in two regions: the Black Sea—Caspian Sea and the Baltic Sea. Using well-dated sediment cores at four core sites from the Black Sea, Mertens et al. (2012b) showed a gradual change of salinity from ~14 ± 0.91 ppt around 9900 cal years BP to a minimum ~12.3 ± 0.91 ppt around 8500 cal years BP, reaching current salinities of ~17.1 ± 0.91 ppt around 4100 cal years BP based on measurements of process lengths of *L. polyedrum* cysts. These variations are much smaller than was thought before, and this record failed to reveal a catastrophic salinization event at ~9140 cal years BP, called Noah's flood by other researchers (e.g. Ryan et al. 1997). Using cysts of *P. reticulatum,* Willumsen et al. (2013) showed that in Fårö Deep (Baltic Sea), average salinities were probably highest during 7200 to 5200 cal years BP, being ~15–17 ppt versus 7.5 ppt of the present day. It should be stressed that the relationship between process length and salinity may have a regional imprint (Mertens et al. 2012a, b). It may, therefore, be problematic to try to reconstruct salinity over time in a sediment core using process length/salinity relationships derived from other regions.

6 Dinoflagellate Cysts as Proxies of Temperature

Temperature is perhaps the main factor affecting cyst-forming dinoflagellates in estuarine environments. Temperature is an important factor controlling species distributions and abundance, reflected in cyst assemblage composition and it may have an influence on cyst morphology. The role of cysts as a resting stage in the life cycle of dinoflagellates greatly influences their potential use as temperature proxies in the sedimentary record. In effect, the cyst allows a species to extend its biogeographic range beyond the environmental tolerance-levels (e.g. to sea surface temperatures, SSTs) of the motile stages to the full extremes of annual variation. Thus, a warmer-water species such as *Lingulodinium polyedrum* would be limited to waters with lowest annual temperatures no less than about 10 °C (the tolerance of motile stages), whereas the resting cyst allows "over-wintering", followed by planktonic activity of excysted motile cells in even a brief summer period of >10 °C at higher latitudes (Dale 1983; Lewis and Hallett 1997). Sediment trap studies give very useful information on the timing of *in situ* cyst production and cyst fluxes at ecologically relevant time-scales (e.g. Montresor et al. 1998; Morquecho and Lechuga-Devéze 2004; Fujii and Matsuoka 2006; Pospelova et al. 2010; Price and Pospelova 2011; Bringué et al. 2013). In cases where sediment transport is minimal and good age control on sediment samples is available, the cyst assemblage from nearby surface sediments has been shown to be very similar to an annual average sediment trap assemblage (Pospelova et al. 2010; Price and Pospelova 2011; Bringué et al. 2013).

Sediment trap studies have shown SST to be related positively with the production of both autotrophic and heterotrophic dinoflagellates (Pospelova et al. 2010) and to be the main environmental driver of changes in species occurrence (Morquecho and Lechuga-Devéze 2004). In two Arctic fjords of the Svalbard archipelago, cyst production was related to the influence of distinct water masses, with *Islandinium minutum* (Harland & Reid in Harland et al. 1980) Head et al. 2001 reflecting Arctic water influence (Howe et al. 2010b).

6.1   *Effect of Temperature on Cyst Assemblage Composition*

Several authors have studied the distribution of dinoflagellate cysts in relation to SST (e.g. Wall et al. 1977; Dale 1983, 1996; Harland 1983; Rochon et al. 1999; Pospelova et al. 2004, 2008; Marret and Zonneveld 2003). Some studies have also used dinoflagellate cyst assemblages to reconstruct sea ice cover in the Arctic (e.g. de Vernal et al. 2013). However, dinoflagellate cysts have mainly been used to reconstruct temporal temperature changes (or sea ice) in oceanic and coastal settings, and only a limited number of studies have focused specifically on estuarine environments. Dale (1983, 1996) showed selected cyst distribution data from more than 100 surface sediments from mainly estuarine/coastal sites in the North and South Atlantic in relation to latitude and SST. This showed relatively sharp biogeographic boundaries for some individual species (e.g. *Lingulodinium polyedrum* on the Norwegian coast), and far more comprehensive biogeographic boundaries where some warmer species meet colder species from higher latitudes (e.g. the subpolar/temperate cyst boundary of the North Atlantic; Dale 1996). Shifts in these biogeographic boundaries have proved to be sensitive indicators of climatic change within estuarine environments of the Holocene, for example, allowing recognition of the "Little Ice Age" and the "Medieval Warm Period" (Thorsen and Dale 1997; Harland et al. 2013). Interestingly, some of these main biogeographic boundaries for cysts appear to coincide with the main established, marine biogeographic boundaries based on the distribution of other organisms such as mollusks and benthic algae, and some of the defining cyst species are bi-polar, allowing recognition of coincident boundaries in both hemispheres (Dale 1996). These boundaries, established from coastal/estuarine surface sediments, seem robust, but are not recognized in offshore cyst distributions (e.g. Harland 1983), more prone to long distance transport of coastal cysts by ocean currents (Dale 1996).

Estuaries are typically subjected to large temperature variations and may prove very useful in refining the use of dinoflagellate cysts as proxies of temperature. In fact, Pospelova et al. (2004) identified variations in temperature as the main factor driving changes in dinoflagellate cyst composition in shallow neighboring lagoons of southern New England (USA). Cyst assemblages had a marked seasonal variation, with species of Arctic affinity present in the winter, and replaced by subtropical taxa in the summer (Pospelova et al. 2004). In Arctic fjords from the Labrador coast, cyst

assemblages vary greatly from north to south, and appear linked to changes in SST as well as with sea ice conditions, salinity and productivity (Richerol et al. 2012). In the Gulf of Mexico, annual temperature was found to be the second most important factor driving cyst distributions, after water depth/proximity to the coast (Limoges et al. 2013).

Radi et al. (2007) conducted a large-scale study of dinoflagellate cyst assemblages from 60 sites from the Georgia Strait, the Effingham and the Seymour–Belize Inlets (southern British Columbia, Canada). Using the multiple regression technique and the modern analogue technique, the authors concluded that SST (winter and summer), primary productivity and salinity could be reconstructed using this dataset with a reasonable degree of accuracy. Patterson et al. (2011) applied this dataset for paleoenvironmental reconstructions of mid- to late-Holocene temperature changes in the Effingham Inlet. Dinoflagellate cyst assemblages were also used qualitatively as a part of a multi-proxy climate reconstruction from the mid-Holocene to present in the Ría de Vigo, northwest Iberia, Spain (Sobrino et al. 2012). Interestingly, there was a correlation between SST warm anomalies, and an increase in the relative abundance of *Lingulodinium polyedrum*, possibly associated with periods of decreasing upwelling intensity and stronger stratification (Sobrino et al. 2012). Sharp increases in *L. polyedrum* in the Caspian Sea and in the Santa Barbara Basin have also been attributed to sea surface warming (Leroy et al. 2013; Bringué et al. 2014, respectively)

In a high-resolution study from the Gullmar Fjord covering the past 2500 years, dinoflagellate cyst species were grouped into thermophilic and cryophilic taxa, and their ratios were used in order to assess changes in temperature in the region (Harland et al. 2013). These studies illustrate that there are several approaches to the use of dinoflagellate cysts as proxies for temperature in estuarine environments. These include quantitative (e.g. modern analogue technique), semi-quantitative (relative abundances/ratios) and qualitative methods (e.g. indicator species).

6.2   Effect of Temperature on Cyst Morphology

It is a common trend in phytoplankton and other protists, that cell size is inversely related to temperature, and it has been estimated to correspond to ca. 2.5 % decrease in cell volume with each 1 °C increase in temperature (Atkinson et al. 2003). In dinoflagellates, the inverse relationship of size and temperature applies to both vegetative cells and cysts, as vegetative cell size is strongly linearly related to cyst size (Finkel et al. 2007). This relationship appears to hold at the species level (Ellegaard et al. 2002), as well as at the total assemblage level (Mousing et al. 2016), and over geological times (Finkel et al. 2007), indicating that dinoflagellate cyst size might be a promising proxy for paleotemperature. The process length of cysts of two dinoflagellate species, *Lingulodinium polyedrum* and *Protoceratium reticulatum*, appear to vary inversely with SSTs (Mertens et al. 2009, 2010; Verleye and Louwye 2010), but further studies are required to explore this possibility.

7 Summary

Dinoflagellate cysts are useful and robust indicators of environmental change in estuarine systems. Environmental shifts can be seen in and inferred from changes in species composition, total abundance, diversity and morphology of dinoflagellate cysts. The evidence for correlations between these micropaleontological changes and specific environmental factors has been inferred from three study methods of modern cysts: (1) cyst distributions in recent sediments deposited under gradients of environmental factors; (2) temporal cyst fluctuations under known *in situ* conditions in sediment trap studies; and (3) laboratory studies that induce cyst formation under particular conditions of cultures established from a single strain of a particular species. Furthermore, dinoflagellate cysts are often preserved where microfossils composed of silica (e.g. diatoms) or calcium carbonate (e.g. coccolithophores and foraminifera) are dissolved.

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