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Recent dinoflagellate cysts from the Chesapeake estuary (Maryland and Virginia, U.S.A.): taxonomy and ecological preferences.

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Picture on the cover

An exceptionally dense bloom of *Alexandrium monilatum* was observed in lower Chesapeake Bay along the north shore of the York River between Sarah's Creek and the Perrin River on 17 August 2015.

Credit: W. Vogelbein/VIMS

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1. INTRODUCTION

Estuaries provide a home to many of the worlds' major harbours, contributing to their high economical value. Since a couple of centuries they receive treated sewage, wastes, pollution and contamination. Neilson and Cronin stated already in 1981 that excessive enrichment could be highly detrimental to estuaries and their uses. Expanding population and industrial activity have accelerated this nutrient over-enrichment. Examples of human activity, such as conversion of woodlands to agricultural use and the extensive application of fertilizers, have resulted in the input of large quantities of nutrients into estuaries. Eutrophication, the result from nutrient over-enrichment, is the most acute problem in estuarine systems (Pospelova, 2002). This leads to phytoplankton population increase and, eventually, blooms.

The timing and significance of the ecological change in estuaries caused by anthropogenic activity are revealed through paleoecological studies. Dinoflagellate cysts (from here on termed as dinocysts) assemblages reflect the ecology of planktonic dinoflagellates, which are influenced by environmental factors such as temperature, salinity and nutrients (de Vernal and Marret, 2007). In general, the ecology of phytoplankton communities in estuaries is less well resolved compared to open marine settings. In recent decades, estuaries of different types and locations have been studied for their ecological history (de Vernal and Marret, 2007). Of these studies, e.g. Thorsen and Dale (1997); Dale et al. (1999); Matsuoka (1999); Dale (2001); Dale and Dale (2002); Pospelova et al. (2002); Pospelova and Kim (2010) have examined dinocysts in their relationship with cultural eutrophication and industrial pollution.

Our research examines the organic remains in 21 surface sediment samples from Chesapeake Bay (Fig. 4.1), Maryland and Virginia (U.S.A.). Chesapeake Bay is America's largest and most productive estuary and is nowadays threatened by complex environmental issues, which are related to eutrophication, anoxia and toxic phytoplankton (Cronin et al., 2001). Many authors (e.g. Taft et al, 1980; Officer et al. 1984; Malone, 1987) described eutrophication since the 20th century in the Chesapeake Bay and found that anthropogenic factors are the culprits for eutrophication. This study aims to investigate the dinocysts in the Chesapeake Bay estuary and the relation of their assemblages to environmental factors, eutrophication and toxic pollution. This is the first analysis of this kind for Chesapeake Bay.

1.1 Research questions and objectives

1.1.1 Dinocyst ecology

Studies of dinocysts in surface sediments have a dual objective. First, they provide essential information concerning the marine biogeography and ecology of living dinoflagellates (Taylor, 1987). Dinocysts from coastal environments provide meaningful information on biological processes and interactions within the aquatic system (Pospelova et al., 2005). Furthermore, dinocysts, because of their highly resistant wall, have the potential to record environmental changes, like eutrophication, which will be further discussed in section 1.3.2.

Second, surface sediments offer a basis for interpreting fossil assemblages and paleoenvironments of the Quaternary (Wall et al., 1977). In extension of this, transfer functions can be applied to compare downcore dinocyst assemblages with a modern dataset in order to reconstruct the paleoenvironment (Guiot and de Vernal, 2007). Transfer functions have been used to reconstruct sea-surface parameters such as annual and seasonal temperature, salinity and duration of sea-ice cover (Rochon et al., 1999, de Vernal et al., 2001, de Vernal and Marret, 2007). The available dataset on modern assemblages is growing (Zonneveld et al., 2013), but more information on recent dinocyst assemblages is essential in order to improve the applicability of transfer functions.

The purpose of this study is to determine the relationship between dinocysts and their environmental context and to compare the results with other dinocysts studies in surface sediments of embayments and estuaries. This will be done qualitatively on the one hand, using the dinocyst counts and interpretation based on dispersed data from literature. On the other hand, the assemblages will also be analysed quantitatively using multivariate analysis in order to determine the main environmental factors that are influencing the distribution of cysts in sediments from Chesapeake Bay.

1.1.2 Taxonomy

We present the first extensive study of modern dinocyst assemblages in surface sediments from Chesapeake Bay. No overview of appearances of dinocysts has yet been published. According to Cronin et al. (2001) literature on the major microfossil groups in Chesapeake Bay is sparse. However, there is a large amount of information on the living phytoplankton. In this thesis a taxonomical overview will be provided with all species encountered in the sampled sites. Dinocysts will be identified, described and measured. Determination of the cysts is just a beginning in order to understand the relation between cyst-based nomenclature and motile-stage nomenclature. For harmful dinoflagellate species the challenge is even harder, sometimes due to encystment complications. A possible follow-up study would be to develop incubation experiments to conform cyst-theca relationships (e.g. Wall and Dale, 1968).

1.1.3 Cyst beds

An additional research goal for this thesis, outlined by the VDH (Virginia Health Department), is to localise seedbeds of HABs (Harmful Algal Blooms). Cysts of *Alexandrium monilatum* are of greatest interest (T. Egerton, pers. comm.), but other harmful algal, which have a

seasonal succession were also of concern. These seedbeds, also termed as bloom initiation areas, act as a source for the next bloom. It is of high importance to find these in order to mitigate the effects of harmful algae on the ecosystem. This is possible by remediation and monitoring. The cysts of *Alexandrium* spp. were attentively observed with the purpose to demonstrate their distribution in Chesapeake Bay.

1.2 State-of-the-art

1.2.1 Previous eutrophication studies in Chesapeake Bay

Studies based on diatom population and pollen were already carried out in the past years, e.g. Brush and Davis (1984); Cooper and Brush (1993); Willard et al. (2003). Cooper and Brush (1993) their purpose was to recognize the anthropogenic disturbances (i.e. sediment loadings from cleared and cultivated land, additional nutrient loading from fertilizers and toxic loadings from herbicides used in agricultural activities) on diatom populations. Ecological data from recent sediments indicate relationship between water quality and distribution of populations. Nutrient enrichment and land clearance effects coincide with decreases in species diversity and population size. Cooper and Brush (1993) found that ongoing monitoring programs for eutrophication are not sufficient to establish anthropogenic influences on the ecology of Chesapeake Bay. Diatoms are a very useful indicator for eutrophication and pollution (for example, Patrick, 1977; Köster et al., 2007) Instead of monitoring physical, chemical and biological characteristics in the water column over time, they used stratigraphic records from sediment cores to reconstruct historic sedimentation rates and water quality in order to understand the evolution of anoxia and eutrophication in the bay. The pollution and eutrophication are not yet studied in Chesapeake Bay using dinocysts.

1.2.2 Background on eutrophication studies in estuaries

Pospelova et al. (2002) investigated dinocyst records in two estuaries along the Northwestern Atlantic Ocean. The application of dinocyst studies is geographically limited and the utility of dinocysts as bio-indicators of anthropogenic changes in North American estuaries was till then unknown. They presented the first study of this kind for North American estuaries. In the New Bedford Harbor and Apponagansett Bay, Massachussetts (U.S.A), they demonstrated sensitivity dinoflagellates to environmental change caused by human activity. They proposed that the decline of species richness and fluctuations in cyst abundances express intensified anthropogenic disturbance in these watersheds, due to increased eutrophication and toxic pollution.

The greatest change in the dinocyst records coincides with the most intense human activity of the 20th century, which in this case are the periods of textile and post-textile industry. There was no observable increase in total dinocyst concentrations, opposing the findings of Saetre et al. (1997) that dinocyst abundances may decline with the development of pollution. This implies that industrial pollution can counteract the stimulating effects from nutrient enrichment. Cultural eutrophication (sewage effluent) and industrial pollution (e.g. chemical waste) give opposite signals in dinocyst assemblages: Increase in cyst concentrations and increase in autotrophs were related to cultural eutrophication while overall decreasing in cyst concentration and increased amounts of heterotrophs were seen on industrially polluted areas (Dale, 2001).

Species richness declined in the New Bedford Harbor during the 20th century due to overpopulation in addition to point source discharge of sewage and industrial pollutants. The population size in the 1920 was already 120,000, which is an increase of a factor of 4.5 compared to the population in 1880 (Latimer et al., 2003). Furthermore, there is an increase in heterotrophic taxa coinciding with eutrophication and industrial pollution. This could be due to an increased diatom production (Matsuoka, 1999) and/or a reduced production of autotrophic dinoflagellates caused by reduced light penetration (Dale, 2001).

Pospelova et al. (2002) concluded that the variability in total dinocyst concentrations and fluxes indicate human disturbance in an estuary. Effects of pollution are clearest visible when species richness, abundances and composition of the dinocyst assemblages are taken into account together. At the very end they acknowledge that eutrophication signals in dinocyst assemblages can be different in fjords and estuaries. More research must be carried out to better understand dinocysts as a record of paleoenvironmental changes in estuaries.

Another study from Pospelova et al. (2005) analysed the spatial distribution of the dinocysts assemblages in Buzzards Bay, in the region of Massachusetts. It was the first study that investigated the spatial distribution of dinocysts in relation to eutrophication and toxic pollution in estuarine systems. Pospelova et al. (2005) found that nutrient and toxic pollution were the major controls that influenced the distribution of dinocysts, as temperature and salinity variations were small. Dinocyst assemblages reflect gradients of nutrients and toxic pollution. They concluded by highlighting the complexity of the interaction and reaction between dinocyst records in sediments and environmental parameters.

A more recent paper (Pospelova and Kim, 2010) aimed at locating the most eutrophic sites in estuaries in southern South Korea. This study area is comparable with Chesapeake Bay: a rich ecosystem, surrounded by large cities and affected by human activity (industrial, agriculture and wastewater discharges). The dinocyst diversity is high, which broadly agrees with observations made in other shallow estuarine environments. It was already noticed that the proportion of cysts, produced by heterotrophic dinoflagellates, increases with increasing nutrient enrichment (Matsuoka, 1999; Pospelova et al., 2002; Ellegaard et al., 2006). Pospelova and Kim (2010) concluded that the amount of heterotrophic dinoflagellates is a sign of eutrophication.

It is important to mention that the character and extent of eutrophication varies from one estuary to another depending on the intensity of the anthropogenic activity within the watershed, the basic nutrient level of the system and the characteristics of the system itself (Pospelova et al., 2002 and Pospelova and Kim, 2010). Dinocysts could give different signals for different estuaries.

From these studies, it is clear that there is not yet a universally accepted cyst assemblage parameter that indicates nutrient over-enrichment or industrial pollution in estuaries (Pospelova et al., 2002). From more recent publications, however, it seems that the proportion of cysts of heterotrophic

dinoflagellates proves to be the most commonly used parameter for eutrophication (e.g. Dale, 2009; Pospelova and Kim, 2010). In addition, proportions and the increase or decrease of individual species could be evaluated as second parameter.

1.3 Thesis outline

This dissertation tackles as well dinocysts as the ecology of Chesapeake Bay in order to understand the relationship between both subjects. A concise overview of the basic morphology of dinoflagellates and the ecological applications of the dinocysts is given in Chapter 2. Chapter 3 provides a background on Chesapeake Bay, its geological history and the basic aspects of its ecosystem in order to establish the link between the problems in estuaries and their consequences on the environment. Chapter 4 details the materials and applied methodology. Results and discussion are given respectively in chapters 5 and 6. The descriptions of the species (i.e. the chapter of taxonomy) are listed in a separate chapter. In chapter 7 and 8, the conclusions of this study are given and some further research possibilities are formulated.

2. DINOFLAGELLATES

This chapter gives a concise overview of the general and morphological characteristics of dinoflagellates and their cysts. The information provided in this section is mainly obtained from the following sources: Spector (1984), Evitt (1985), Taylor (1987), Fensome et al., (1993) and Bravo & Figueroa (2014). Additional consulted sources are referenced in the text.

2.1 General characteristics

Dinoflagellates are eukaryotic, single-celled organisms (i.e. protists) between 8 (Nezan et al., 2014) and 100 μm in diameter. They are considered important primary producers. Their primitivity is mainly reflected by their 'dinokaryon', a special nucleus containing fibrillary chromosomes that remain condensed during mitosis. Dinoflagellates include a range of trophic styles from autotrophic (photosynthetic) to heterotropic with intermediate mixotrophic species. There are even some parasitic and symbiotic species (Schnepf and Elbrachter, 1992). A detailed introduction to the biology is given by Fensome et al. (1993).

According to Spector (1984), dinoflagellates are classified as the Division Pyrrophyta (Greek *pyrrhos*, flame-colored) and are a large and diverse group of biflagellate, coccoid, filamentous and amoeboid organisms. Recently the phylogeny of dinoflagellates changed. Adl et al (2012) proposed a new higher rank classification of the protists and multicellular groups. The phylum Dinoflagellata (defined by Bütschli in 1885) is placed within the Kingdom Alveolata. The classification by Fensome et al. (1993) is generally accepted.

The majority of the free-living dinoflagellates are planktonic or benthic. They have a greatest diversity in tropical waters, but occur also in freshwater environments. Some marine species produce toxins and can form blooms (commonly referred as red tides) in circumstances with abundant light and nutrients. These blooms can lead to shellfish poisoning. Not all dinoflagellates produce toxins, but also other dinoflagellates can form 'red tides'.

Dinoflagellates produce different types of cysts during their life. According to Dale (1983), three types of cysts are prominent, based upon their function. Resting cysts are the most common and represent a dormant stage. These cysts are the result of sexual fusion and are termed hypnozygotes (see discussion in section 2.3). Temporary cysts are formed when a motile dinoflagellate sheds its flagella and outer wall. Vegetative cysts are non-motile cells that remain metabolically and/or reproductively active (Fensome et al., 1996).

The morphology of the dinocysts knows a rapid evolution and left a rich fossil record in the Mesozoic and Cenozoic sediments. The dinocysts are an excellent biostratigraphical tool because of their distinctive morphology, rapid morphological evolution and their abundance in sediments.

2.2 Motile cell

Dinoflagellates typically occur as motile, biflagellate cells. The motile flagellate cells constitute the haploid part of their life cycle. They possess a longitudinally and transversally oriented flagella used for displacement in spirals. The latter flagellum is ribbon-like and located in the cingulum (or girdle), which is an equatorial groove in the external surface. The cingulum divides the dinoflagellate into an epitheca (anterior) and hypotheca (posterior). The longitudinal flagellum is located in an elongate ventral depression on the hypotheca called the sulcus. The detailed morphology is showed in figure 2.1.

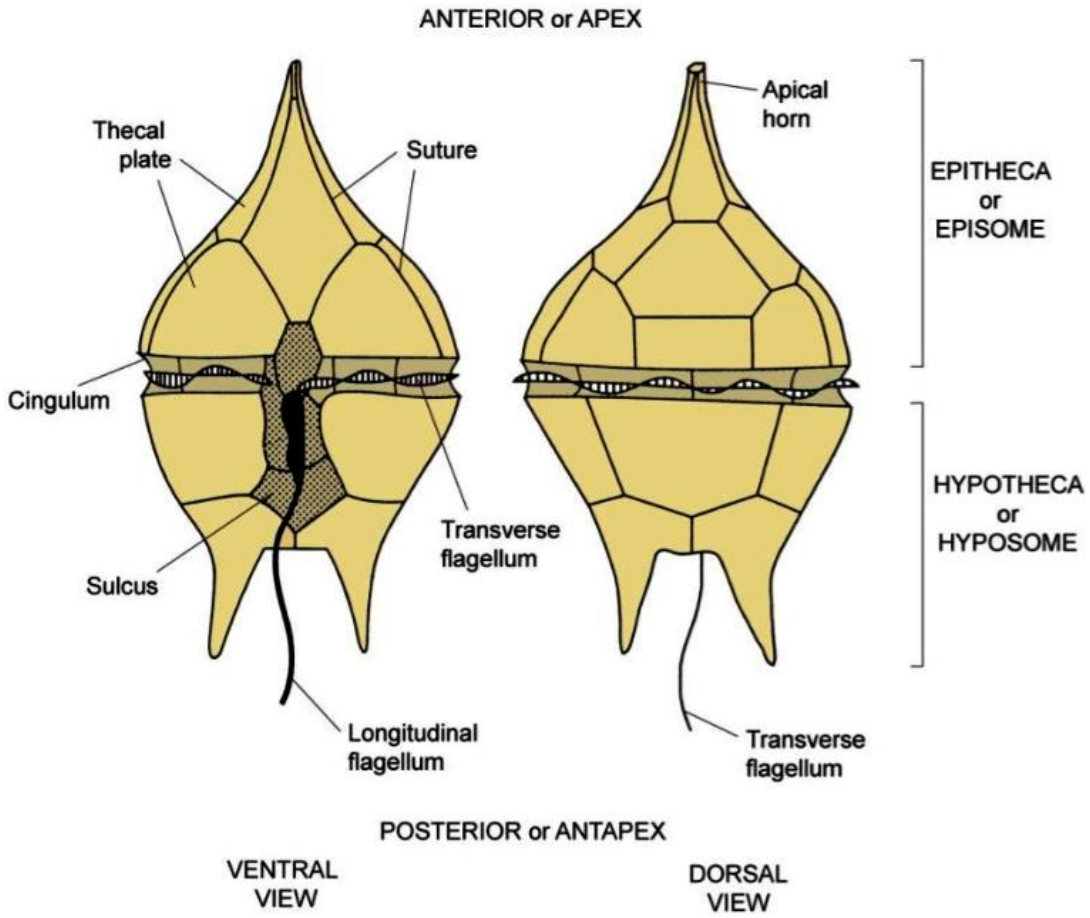


Fig. 2.1. General morphology of a thecate dinoflagellate cell. The cingulum divides the dinoflagellate into an epitheca and hypotheca. Modified from Fensome et al. (1996), originally from Evitt (1985).

Dinoflagellate cells are usually surrounded by the cell membrane called plasmalemma. The amphiesma (or cortex) is the complex outer region, which includes the wall of the cell (Loeblich III, 1970). A single layer of vesicles, the amphiesmal vesicles, is typically present beneath the cell membrane. These vesicles can contain cellulose plates (the thecal plates). Such taxa are termed thecate or armoured, the taxa without thecal plates are referred to as athecate or naked.

The cellulosic material does not preserve well in the sediments since it is often consumed by bacteria. Some species have below the theca a pellicle. This is a thin, fibre-like layer, which is

sometimes cellulosic, but possibly also contains of dinosporin. The pellicle could be the potential ancestor of the resistant cell wall.

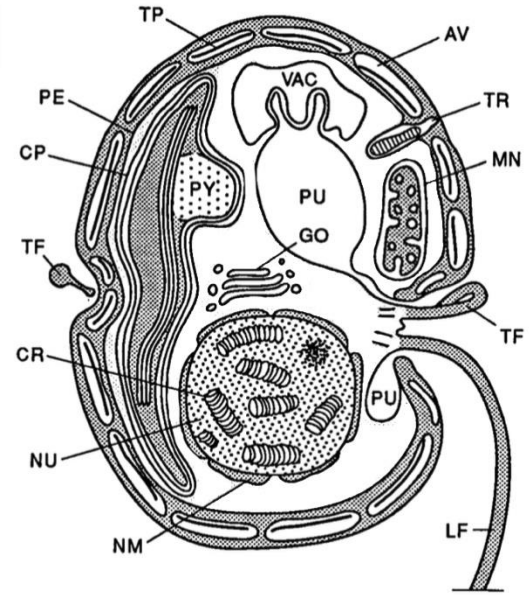


Fig. 2.2. Generalized section through a typical thecate motile dinoflagellate. AV = amphiesmal vesicle, CP = chloroplast, CR = chromosome, GO = golgi body, LF = longitudinal flagellum, MN = mitochrondrion, NM = nuclear membrane, NU = nucleus, PE = pellicle, PU = pusule, PY = pyrenoid, TF = transverse flagellum, TP = thecal plate, TR = trichocyst, VAC = part of cell vacuome. (Fensome et al, 1993, adapted from Taylor (1987)).

The arrangement of thecal plates displays a pattern and is termed the tabulation. Since thecal plates do not occur within the amphiesmal vesicles, tabulation can also be regarded as the arrangement of amphiesmal vesicles, with or without thecal plates. These thecal plates do not touch each other due to the fact that they are contained within these vesicles, which are separated by membranes. This results in junctions between adjacent thecal plates. In the sulcal and cingular regions tongue-and-groove contacts and butt joints are observed, while in other regions an overlapping flange is present in neighbouring plates.

Dinoflagellate tabulations can be subdivided into six standard tabulation types (Fig. 2.3). According to Netzel and Dürr (1984), gonyaulacoid and peridinoid types could be grouped together as one tabulation type besides the other five, as they show both a similar arrangement (based on the number of plate series).

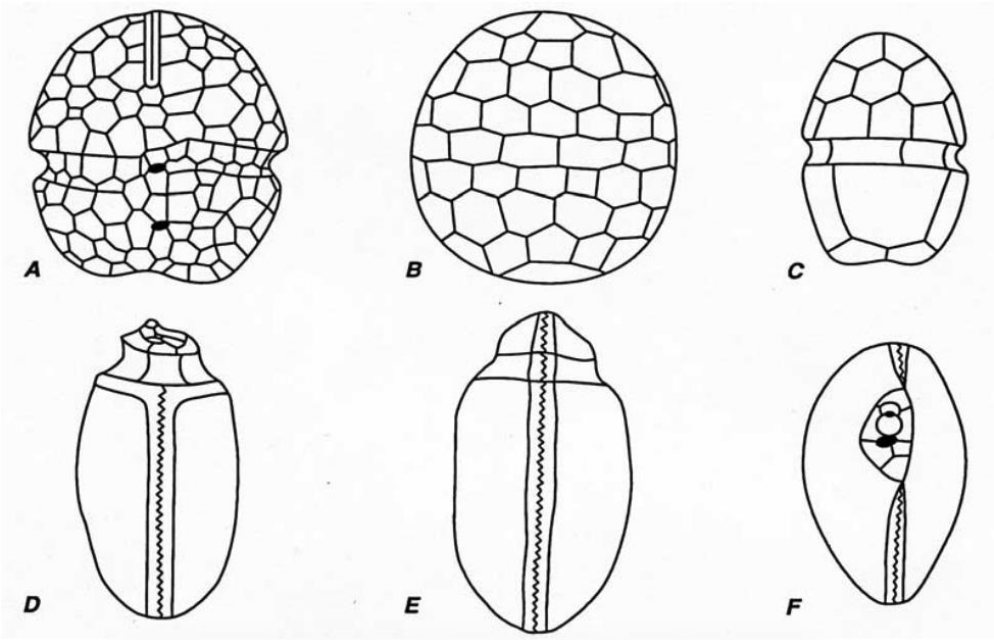


Fig. 2.3. Tabulation types; **A.** Gymnodinioid tabulation type in ventral view. **B.** Suessioid tabulation type in dorsal view. **C.** Gonyaulacoid-peridinioid tabulation type in dorsal view. **D.** Nannoceratopsioid tabulation type in dorsal view. **E.** Dinophysioid tabulation type in dorsal view. **F.** Prorocentroid tabulation type in oblique view (Fensome et al., 1993; A and F by Taylor, 1990; B by Evitt, 1985; C by Loeblich III, 1968; D and E by Piel and Evitt, 1980).

2.3 Life cycle

The life cycle (Fig. 2.4) of dinoflagellates may involve both phases of vegetative and sexual reproduction (Evitt, 1985). In most cases, the dinoflagellate species are known to have a haplontic life cycle. This means that the vegetative cells are haploid and the zygote is the only diploid cell in the cycle (Fensome et al., 1993). Exceptions to this life cycle are *Polykrikos*, *Noctiluca* (Fukuda and Endoh, 2006) and *Thoracosphaera helmii* (Inouye and Pienaar, 1983).

2.3.1 Asexual reproduction

Evitt (1985) stated that vegetative fission (asexual reproduction) dominates during the period favourable for rapid growth and expansion. Three different types of cell divisions can take place. The first and most simple one involves simple division of the cell into two halves by binary fission, resulting in two new haploid schizonts. The second type only takes place in atheca taxa and is known as *desmoschisis*, where the parent theca separates along the sutures as the protoplast divided. The result is that each daughter cell retains one half of the old theca. The other type of vegetative cell division is referred to *eleutheroschisis*. Here the parent theca is completely shed. Naked schizonts develop from the beginning a new theca. The protoplast may divide just before or just after the ecdysis, which is the stage of losing the theca (Evitt, 1985). Fensome et al. (1993) even mentioned a fourth form of cell division called *sporogenisis*. Multiple spores are formed (planospores, dinospores, gymnospores, etc.) and this usually occurs within the complex life cycle of parasitic taxa.

2.3.2 Sexual reproduction of thecate dinoflagellates

Some of the schizonts will function as gametes and fuse to form zygotes. The gametes can appear similar, but sexual dimorphism can also occur (Beam and Himes, 1984). The resulting diploid zygote constructs a new theca that is larger and thicker than the original vegetative theca. This so-called planozygote now has paired flagella, one from each gamete. The cell size now enlarges and the thecal plates are now visible. After some time (the activity level diminishes after 15 days) the cell becomes non-motile and reaches the hypnozygote stage. The protoplasm contracts and the flagella are lost. The inner membrane, which will be the future cyst wall, is then already visible. Shortly thereafter, the thecal plates completely break apart or are destroyed. The hypnozygote (now a resting cyst) is exposed and behaves as a sediment particle. The protoplast excysts through the archeopyle and the cycle closes with a meiotic division to produce new haploid cells, each with two flagella and a theca (Evitt, 1985).

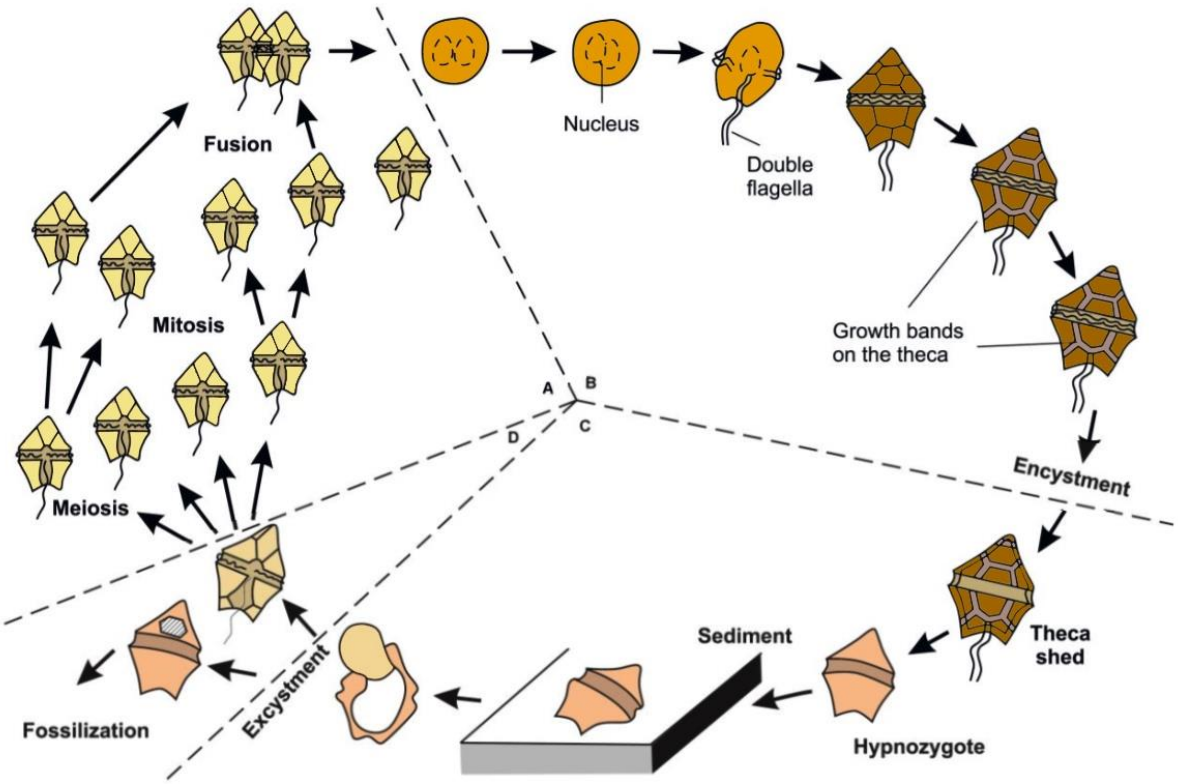


Fig. 2.4. Schematic overview of the life cycle of dinoflagellates. **A** = motile haploid stage, **B** = motile diploid stage, **C** = non-motile diploid stage, **D**: non-motile cyst fossilization and protoplast produces new haploid cells via meiotic divisions. Modified from Armstrong and Brasier (2005), originally from Stover et al. in Jansonius and McGregor (1996), vol.2, pp. 641-750.

2.4 Dinocysts

A major part of the fossil dinocysts is considered to represent non-motile cysts that are produced during the sexual phase of the dinoflagellate life cycle. Fossilized motile stages are extremely rare. The walls of the fossil cyst can consist of calcareous or siliceous material, but the majority of the cysts are composed of the organic material called dinosporin. Dinosporin is highly resistant to chemical and mechanical degradation. Even after two hundred million years, the thin, flexible walls of the fossil cysts retain the delicate structural relief and their original shape.

The description of the dinocyst is based on two main characteristics. The first one is the archeopyle, which corresponds with to one or more polygonal plates and is an unique feature of dinocysts. It is the opening where the proptoplast escapes during excystment. The second is the arrangement of processes on cysts. Fensome et al. (1993) defined a process as a *"a structure which arises from an external surface and is columnar or spine-like. Processes may be simple or intricately branched or interconnected."* The arrangement of those processes can be used to deduce the thecal tabulation on the vegetative cell that earlier formed the cyst. Different morphological groups of cysts can be distinguished based upon the length of the processes: proximate, chorate and proximochorate cysts (Fig. 2.5). Proximate cysts form a layer of material directly within the theca. In this case, the shape and the size of the cyst resemble the morphology of the motile cell. Their external surfaces may be smooth or less than 10% of the shortest diameter of the main body. The cysts are called proximochorate when the processes measure between 10 and 30% of the body diameter. The cysts are termed chorate when the length of the processes exceeds 30% of the central body diameter.

Other characteristics are the cyst wall morphology and composition, the tabulation pattern, which is the cyst equivalent to the thecal tabulation, and the shape of the body and the processes. Modern living dinoflagellates are commonly classified based on the thecal morphology when visible (including the shape and primarily tabulation) and physiology.

The fossil record of dinoflagellates is perhaps more selective than for other groups of organisms. Only a minority of the living species form cysts that can withstand taphonomic processes and fossilize.. Many dinoflagellates species live without leaving any fossilizable trace.

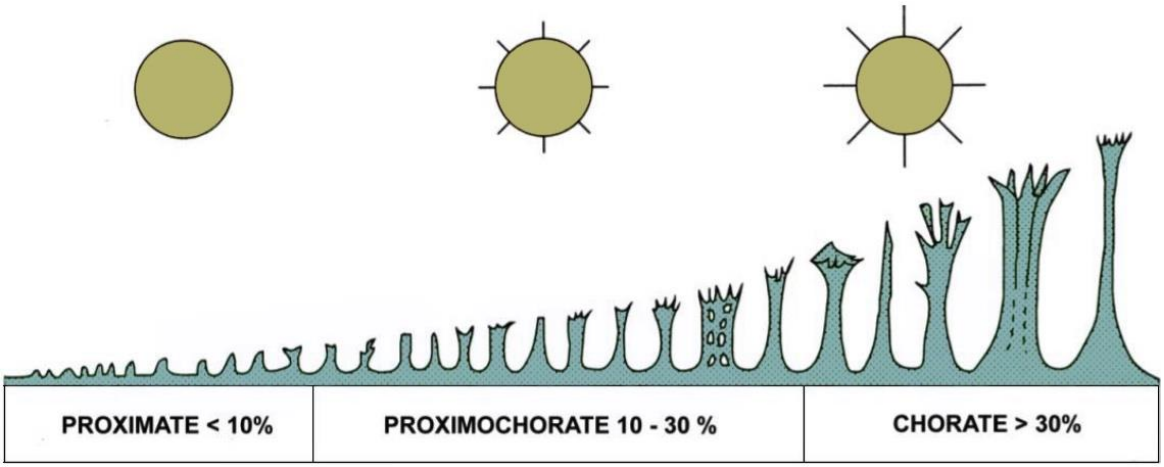


Fig. 2.5. Different cyst types in function of the length of the processes relative to the diameter of the cyst. Modified from Fensome et al. (1996), originally from Sarjeant (1982).

2.5 Ecology and Paleoecology of dinoflagellates

2.5.1 Introduction

Dinoflagellates inhabit most types of aquatic environments. They are recorded from lakes to open ocean, and occur at all latitudes in the world, from the equator to the poles. Together with diatoms and coccolithophores, they are responsible for the vast majority of primary production (de Vernal and Marret, 2007).

2.5.2 Ecology

Dinocysts occur most frequently in marine sediments, although there are some reports of freshwater cyst occurrences in Cretaceous to Quaternary deposits (e.g., Bourrelly, 1970; Harland and Sharp, 1980; Tappan, 1980). Meroplanktonic forms are marine cyst-producing species, which have a neritic habit. They are found in the shelf and upper slope sediments and are the most abundant in fine-grained sedimentary rocks such as shales, silt and sandstones. This may be due to the fact that the depositional behaviour of dinocysts follows that of silt particles, and that their abundance increases in sediments with higher mud contents (Lewis, 1988).

Fossil dinocysts are mostly known from marine sediments and it is assumed that they are abundant along continental margins and neritic environments (estuaries, shelves and slopes). Dinocysts are found from the Mesozoic until today and therefore widely used for biostratigraphy and paleoenvironmental reconstructions. Dinoflagellates even dwell in a wide range of oceanic conditions and therefore allow researchers to study the full oceanic environment (near shore, epicontinental seas, estuaries..). The dinocyst concentrations in sediments decrease significantly offshore, especially in low productivity ocean gyre areas (Marret, 1994). Thus, these dinocyst assemblages seem to be more useful in continental margins and are in studies regarded as a complementary proxy to calcareous dinocysts and planktonic foraminifera.

Many studies have documented the geographical distribution of modern dinocysts depositions on the sea floor (Wall et al. 1977). Datasets for the North Atlantic, Arctic Oceans, circum-Antarctic

Ocean, low latitudes of the Atlantic Ocean, the eastern and western Pacific Ocean margins are now available **(**e.g., synthesis by Rochon et al., 1999; de Vernal et al., 2001, 2005; Marret & Zonneveld, 2003, Zonneveld et al., 2013). These datasets are used to search for relationships between distribution of dinocyst assemblages and parameters like salinity, sea-ice cover and productivity or eutrophication **(**de Vernal & Marret, 2007**).** The number of these studies increased significantly during the last two decades **(**Taylor, 1987**).**

2.5.3 Cysts as environmental indicators

2.5.3.1 Temperature

Dinoflagellates are adapted to a broad range of temperatures, although dinoflagellate species diversity is highest in intertropical areas and high numbers of species are observed in polar environments. The relationship between the environment and the ability to encyst is difficult to assess (Graham and Wilcox, 2000).
de Vernal & Marret (2007) mentioned that the existence of a relation between sea surface temperature (SST) and dinocyst assemblages is unquestionable, and that the relationship probably is season-dependant. Therefore the dinocyst assemblages are associated with summer SST's.

2.5.3.2 Coast/oceanic signal

Wall et al. (1977) illustrated the zonation of oceanic and neritic taxa with regard to the distance to the shore. Species composition of assemblages and their relative abundances have been used to indicate sea level. Some taxa have a very specific distribution in the oceanic domain. Oceanic environments are often totally dominated by species of *Impagedinium* and *Nematosphaeropsis.* Dinocysts are also relatively abundant in estuarine environments as they are often characterized by stratified waters and a strong salinity gradient. For example, the number of *Polysphaeridium zoharyi* increases in neritic or estuarine environments (de Vernal and Marret, 2007).

2.5.3.3 Salinity

The majority of dinocysts are recorded in freshwater to hypersaline environments. Only a fraction is restricted to a certain salinity range. There are some typical examples of oceanic taxa, such as some *Impagidinium* species, which are only found in fully saline conditions. But on the other hand there are also brackish environments, such as the Baltic Sea and the Black Sea, whose assemblages are characterized by a high morphological variability (de Vernal and Marret, 2007).

There are some dinocyst species that develop different morphologies depending of the salinity conditions. Lewis and Hallet (1997) and., Mertens et al. (2009a) documented that *Lingulodindium machaerophorum* develops shorter processes in low salinity conditions.

2.5.3.4 Upwelling

Dinocyst assemblages consisting of mainly heterotrophic taxa reflect the trophic character of the upper water mass (de Vernal and Marret, 2007). In high productivity areas these heterotrophic dinocysts are the most common. High numbers of heterotrophic cysts are thought to reflect the fact that heterotrophic dinoflagellates feed on blooming diatoms, and also by the fact that diatoms outcompete autotrophic dinoflagellates in upwelling situations (Dale and Dale, 2002).

2.5.3.5 Eutrophication

Eutrophication stands for the increase in mineral nutrients in phosphates and nitrates in the environment (Encyclopædia Britannica Online, 2016). This is caused by an increase of organic material in the aquatic environment and influences the composition of the dinocyst assemblage. When human activity is the cause of the increase of eutrophication (i.e. land run-off from household wastewater or fertilizers in rivers) the process is called cultural eutrophication.

There are several studies dealing with dinocysts and eutrophication and/or human-induced impact in fjords, harbours or bays (Thorsen & Dale, 1997; Dale, Thorsen, and Fjellsa, 1999; Matsuoka, 1999; Dale & Dale, 2002; Pospelova et al., 2002). The increased abundance of *L. machaerophorum* in sediments has been used as indicator of eutrophication in fjords and lochs, in association with increased levels of nutrients due to human activity. It is not always clear that the trophic character reflected by the dinocysts is actually human-induced or is derived from natural variations. This requires further investigation (de Vernal and Marret, 2007).

2.5.4.6 Sea Ice cover

In polar environments dinocysts can be used as sea-ice indicators (de Vernal and Marret, 2007). There are only two species (*Polarella glacialis* and *Peridiniella catenata*) that survive in sea-ice environments, but there are a few taxa that are known in seasonal sea-ice sediment areas (e.g., Arctic *Polykrikos* sp. and *Selenopemphix antarctica*). Other taxa are tolerant to sea-ice cover and have a high abundance in sea-ice environments (*Operculodinium centrocarpum* and *Brigantedinium* spp.).

2.5.4 Feeding strategies

Dinoflagellate feeding strategies are very distinct. The majority is phototrophic and together with diatoms and coccoliths they are considered as important primary producers. Phototrophic dinoflagellates use light as source of energy and CO_2 as a carbon source. They are termed as photoautotrophs when light is utilized as the only energy source and if they do not require other organic micronutrient supplements such as vitamins. When this latter is not the case, they are called auxotrophs.

The other dinoflagellates are heterotrophic or mixotrophic. Heterotrophic dinoflagellates feed on other organisms or on dissolved organic substances. They do not have chloroplasts and use nutrients synthesized by other organisms. The macronutrients are taken up in various ways. Resorption, not to be confused with osmotrophy, is the uptake by nutrients by the direct passage

through the plasma membrane. The other technique is endocytosis, which is the digestion by the inclusion by the cell membrane. Mixotrophic dinoflagellates are in the first place photosynthetically active, but in addition they are heterotrophs (Schnepf & Elbrächter, 1992).

3. CHESAPEAKE BAY

"Nutrient pollution poses the greatest of all recognized threats to Chesapeake Bay."
L. Eugene Cronin (Baltimore Sun. March 22. 1967)

3.1 Geographical setting

Chesapeake Bay is the largest estuary in the United States and the third largest in the world (Cronin et al., 2000; Fig. 3.1). The surface area is about 11.100 km^2 and the Bay is elongated, with more than 300 km in length and with an average width of 20 km. With a watershed of 166.000 km^2, the Bay drains large portions of the states of Maryland, Virginia, Washington, Pennsylvania and New York and parts of West-Virginia and Delaware (Cronin et al., 2000). According to the Alliance for the Chesapeake Bay, 48 major rivers and around 100 small tributaries feed the Bay in a dendritic drainage pattern. The Susquehanna, Potomac, James, Rappahannock and the Patuxent are the major river sources of freshwater along the western shore. The Choptank and Nanticoke are the rivers along the eastern shore (Schubel and Pritchard, 1987). The average depth is around 8 m due to the shallow terraces, but a deep axial channel, a remnant of the old river valley, reaches 54 m of depth in the centre of the Bay (Colman and Hobbs III, 1988).

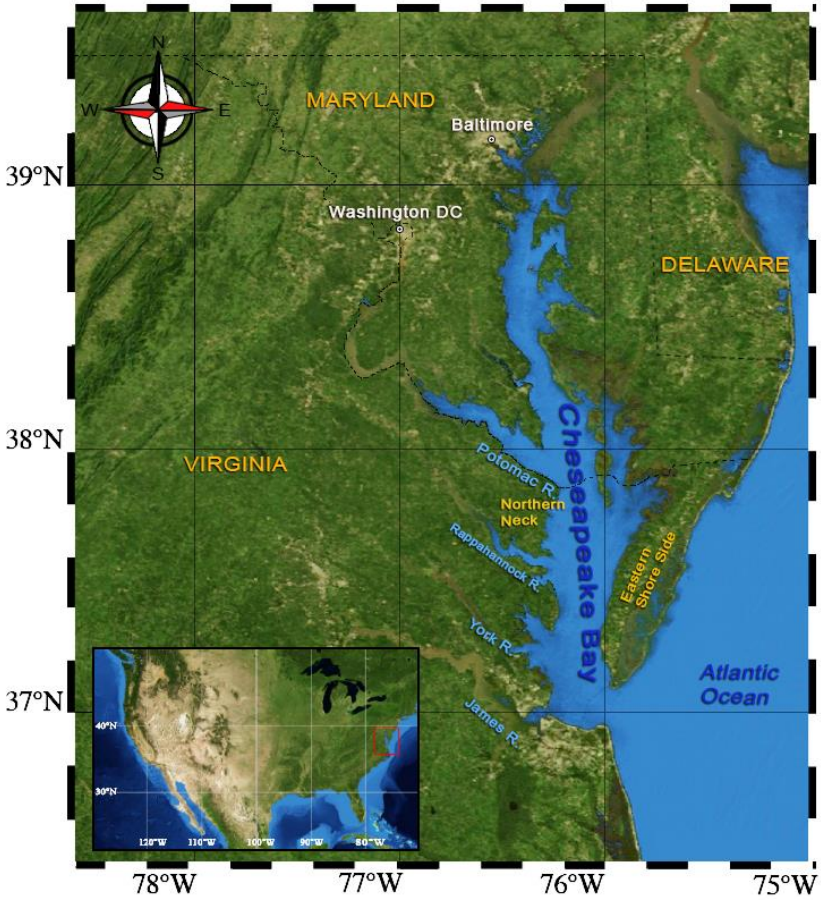


Fig. 3.1. Map showing location of the study area (red frame inset) with major rivers (identicated with R.) and States. Images retrieved from Global Mapper (LANDSAT image).

3.2 Geological history

In the late Eocene, around 35 million years ago, a bolide impacted the eastern shore of North America. This so-called Chesapeake Bay Impact crater at least partly determined the future location of the Bay (e.g. Poag, 1997).

The formation of the modern Chesapeake Bay estuary started in the Pleistocene (reference). The Susquenhanna River, the main river of the basin, extended and retreated during respectively warmer interglacial (glaciers retreat) and colder glacial (glaciers advance) episodes. During the last three glaciations, three different paleochannels were formed (Fig. 3.2): the Exmore Channel from 500,000 to 300,000 years ago and the Eastville Channel around 150.000 years ago (Colman et al., 1990). Then, relatively late compared with the development of other coastal-plain estuaries (e.g. Hudson River, Narragansett Bay, Coos estuary), Chesapeake Bay is formed during later stages of retreat of continental ice sheets of the last glaciation, from 18 ka onwards. This is the Cape Charles Channel, which has evolved into the modern channel. Streams from the west started carving and formed rivers that then submerged with the Susquenhanna River, creating drowned river valleys (Hobbs, 2004).

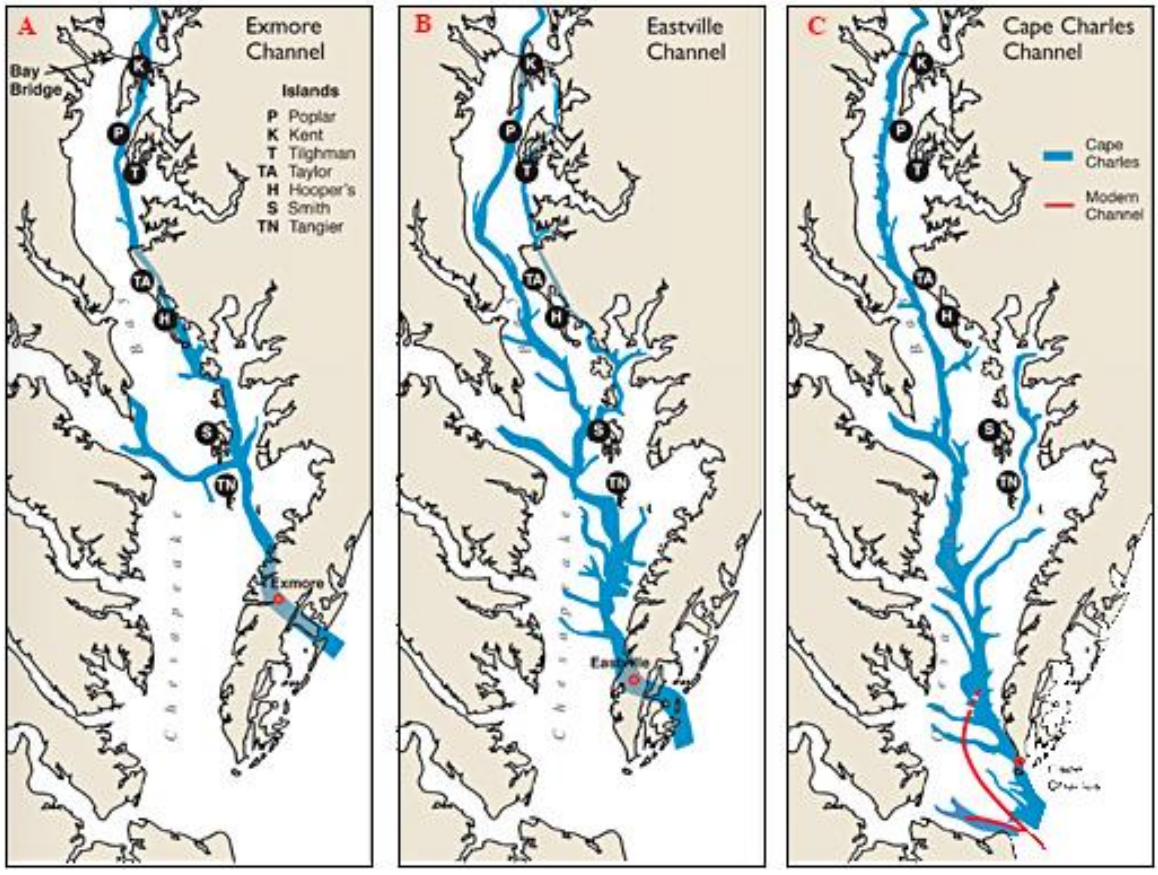


Fig. 3.2. Evolution from the Chesapeake Channel by three ancient channels. A) 300,000-500,000 years ago; B) 150,000 years ago; C) 18,000 years ago to the Present. From Fincham after Colman et al. (1990).

The modern Chesapeake Bay was formed during the Early-Holocene sea level rise, after the *"8.2 ka event".* This event is identified as a cold event in climate records in the North Atlantic region (Bratton et al., 2002). Between 8.2 and 7.4 ka due to warming up, a catastrophic release of meltwater

from the glacial Lake Agassiz flowed into the Atlantic Ocean, although the exact timing differs from region to region (Heil, 2008). The ocean then flooded the paleoriver valley and gave birth to Chesapeake Bay (Colman and Mixom, 1988). In this story the Delmarva Peninsula, which is nowadays the shore slide in the eastern, played an important role. This peninsula has prograded by delta development during the highest sea level position (Colman and Mixon, 1988). Hobbs (2004) confirmed this formation mechanism. The Delmarva Peninsula incrementally extended southwards as the major barrier spit, which resulted in the enclosing of what was to become Chesapeake Bay. Proof of this development can be found in the shoal deposits on the northern side of the mouth of the Bay, which were deposited during progradation. In MIS 5 or 7 the peninsula finally reached its modern configuration. Sea level rise declined about 6,000 years ago and the bay took its present configuration about 3,000 years ago (Hobbs, 2004).

The geological history of the Bay is represented in the cliffs that are present in the embayment, especially in the Calvert Cliffs. Fluvial-deltaic Cretaceous sediments of the Potomac Formation are covered with Cenozoic marine deposits. The Paleocene and Eocene epochs are represented by shallow shelf deposits consisting of glauconitic, silty sands. Miocene units are shelf deposits consisting of diatomaceous silts and silty, shelly sands. The Miocene beds form the thickest suite of the section in central Maryland (Powars et al., 2015). Figure 3.3 shows a geological map of the area.

Holocene sedimentation is mainly controlled by the Susquehanna River. The deposits are graded and finer, due to accumulation at the head of the peninsula during the last flooding (Langland and Cronin, 2003). Another large input of sediments comes from the Atlantic Ocean, near the mouth of the bay. However, the action of the Susquehanna River causes a net southwards sediment transport. An additional influx is that of reworked sediments through the action of coastal erosion caused by storms (Newell et al., 2004). The substrate consists mostly of unconsolidated sediments such as sand and gravel. Sediment eroded from higher up the watersheds is mainly sand, silt and clay (Brush, 1984).

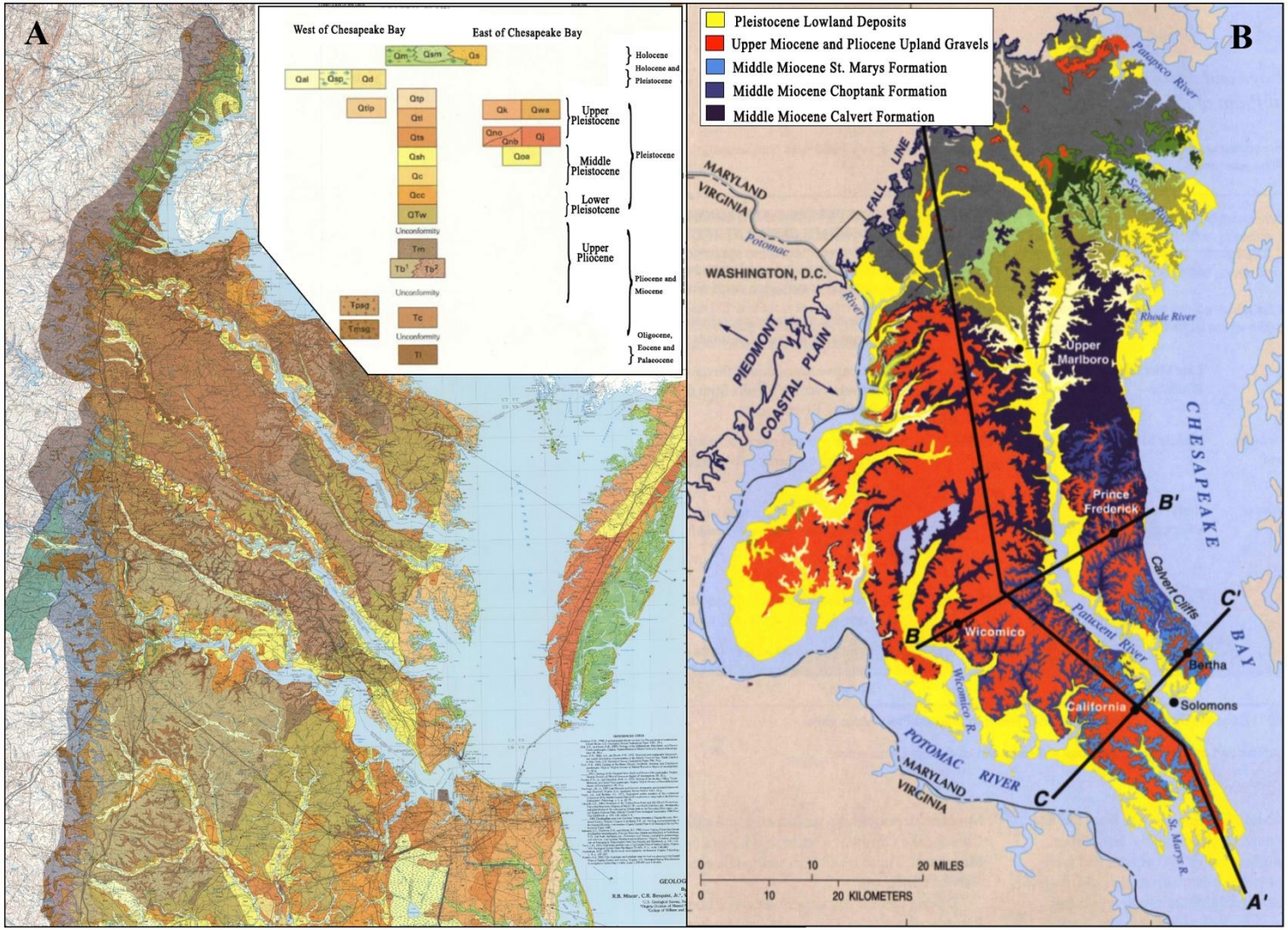


Fig. 3.3. Surficial geologica map of A) Virginia and B) Maryland coastal plain. A) Little adaptation from US Geological Survey. B) From Powars et al. (2015) Modified from Cleaves et al. (1968).

3.3 Modern estuarine conditions

The Chesapeake Bay estuary has a strong north-to-south salinity gradient, forming three zones: oligohaline (0-6 psu), mesohaline (6-18 psu) and polyhaline (18-30 psu) (Uruquhart et al, 2013). Freshwater flows into the estuary mainly from 48 major rivers and many additional tributaries. The main source of freshwater is the Susquehanna River, which accounts for approximately 45% of freshwater input into the Bay (Baird and Ulanowicz, 1989). The Chesapeake Bay is a partially mixed estuary, meaning that it is characterized by relatively large freshwater input with density-driven circulation in a two-layer structure. This consists of a fresh seaward-flowing upper layer and a saline-return flow below (Xu et al., 2002).

Cronin et al. (2003) examined the seasonal variability of the temperature from 1949 to 2000. The annual sea-surface temperature ranges from 1-2°C in winter tot 25-26°C in summer. Due to rapid shifts of spring temperature, the water temperature is 2°C higher now than during the 17th century. This global trend of climate warming will potentially cause harm to the bay, for example on the phytoplankton populations. Both warming and higher CO_2 promotes the growth of harmful algae (Reece et al., 2012). The trend today (especially since the last half of the 20th century) is that, temperatures within the Chesapeake Bay have been increasing. This is coincident with observed SST trends at regional, hemispheric, and global scale (Preston, 2004).

The majority of nitrogen and phosphate comes from sewage treatment plants and polluted runoff from agriculture. Nitrogen and phosphate average annual yields were estimated recently (Ator and Denver, 2015) at respectively 10.5 and 1.1 kg per hectare. The highest flux of total nitrogen and phosphate is found in the Susquehanna River, followed by the Potomac, James and Rappahannock (Ator et al., 2011). Due to this input of nutrients, Chesapeake Bay is considered to be eutrophic, while some tributaries are characterized by mesotrophic or even hypertrophic conditions (Boynton et al., 1995).

3.4 The Chesapeake Bay Ecosystem

A complex relationship exists among the living organisms and the environment of the Chesapeake Bay watershed. Even the smallest creature plays a vital role in the story of the Bay. Dinoflagellates are important members of the phytoplankton and represent a major part of food webs (Taylor, 1987). Many dinoflagellates store energy captured from sunlight through photosynthesis. As many other organisms are dependent upon this energy, dinoflagellates are found at the base of food chains (Spector, 1984). Besides phytoplankton and a large number of other groups of plants and animals in the water, birds, mammals and fish occupy the forests and wetlands in the watershed. Crabs, underwater grasses, clams, oysters and many other groups are also part of this ecosystem. This biodiversity creates a unique ecosystem, according to the Sea Grant Maryland. The Bay is still one of the most valuable natural treasures (Chesapeake Bay Program, 1982), supplying millions of pounds of seafood and functioning as a major hub for shipping and commerce (Chesapeake Bay Program, 2004). Today, the Bay's ecological balance considered to be threatened (Chesapeake Bay Program, 2008). Human activity directly affects the Bay by adding waste, consuming resources and changing the character of the land, water and air around it (U.S. Environmental Protection Agency, 2004). In the next paragraphs, we bring some aspects of this together.

3.4.1 Population growth

First settlement in Chesapeake was on a small island in the James River by European colonists in 1607. It was a strategic site against a potential Spanish attack. In following years, 30 to 40,000 Native Americans settled in Chesapeake Bay region. Maryland was colonized since 1634, resulting in growing settlement and the need for labour. Slaves were brought to the region to build the first harbours for trade. In 1670 the region around the Bay reached already a population of 41.000 (Smithsonian National Museum of History, 2009).

During the 17th and 18th century, continuing population growth resulted in more land clearance, thus more deforestation, which was noticed by the increase in ragweed pollen in sediments (Brush and Davis, 1984). An increase in sedimentation rates is documented in the geological record. (0.04 cm·yr^{-1} before settlement versus 0.25 cm·yr^{-1} after; Brush, 1984). The high sedimentation rates are related to increased shoreline erosion, slumping, climatic or anthropogenic influences. This latter was interpreted as land use change, mainly agricultural. Here it should be noted that more than 20% of the watershed must be cleared before there is sufficient erosion to cause an increase in sedimentation in the tributary (Brush, 1989). Agricultural land use change was also visible in the diatom species abundance and diversity records (Cooper and Brush, 1993). Since the 19th century, fertilizers were heavily applied and nutrients were enriched in the sediments (Cooper and Brush, 1993). After World War II, industrialization and urbanization caused a peak in nutrients and sewage discharge into the bay. Over the last 50 years, eutrophication and pollution was reflected in the diatom record (Cooper and Brush, 1993). This decrease parallels an overall decrease in diversity of larger organisms in Chesapeake Bay. All these changes are related to human influence (Cooper and Brush, 1993).

The US Geological Survey aims to maintain and even improve the current water quality conditions, but is challenged by the population growth of the last decades (Fig. 3.4) (Chesapeake Bay Program, 2008). The Federation for America Immigration Reform (FAIR) predicts a population growth of 150.000 people every year (Ruark, 2010). In 2015, 18 million people inhabited the Chesapeake Bay watershed and in 2050 this number will exceed 23 million. This increase is partly due to global population growth (10.5 billion expected by 2050, Ruark, 2010) and partly due to migration towards the Bay. For example, Maryland experienced the highest immigration-related population growth (98% of the state's population growth between 2000 and 2009). Immigration is the major cause of population increase since 1970 (Ruark, 2010).

The relation between overpopulation and ecological deterioration of the Bay is clear (Chesapeake Bay Foundation, 2012). Increased population means more traffic, more construction, fertilizers, pesticides, detergents and industrial and household waste in the Bay.

Year	Population (in Ma)
1950	8,383
1960	10,01
1970	11,765
1980	12,75
1990	14,233
2000	15,699
2005	16,535
2010	17,36
2013	17,875
2014	17,986
2020	*18,858*
2030	*20,256*
2040	*21,422*

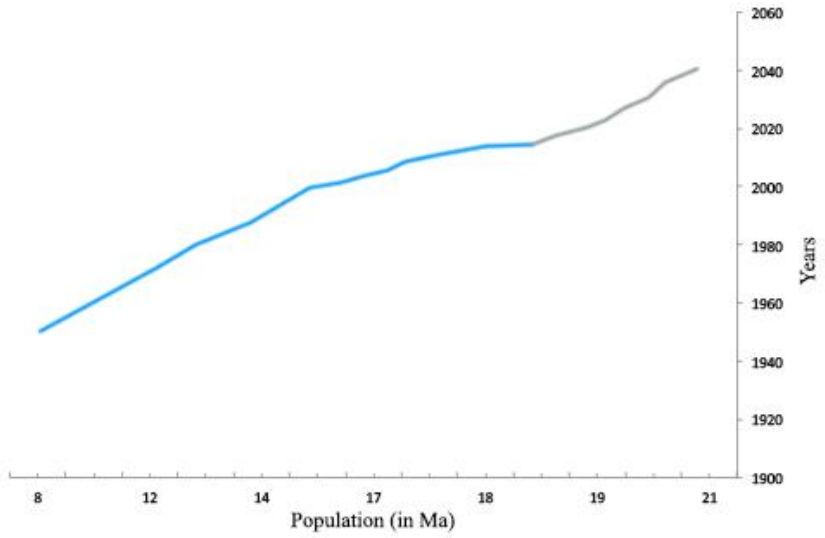


Fig. 3.4. Population growth in Chesapeake Bay (Left: Table with the figures; Right: Graphic. The grey coloured part indicates the interpolated values (numbers based on CBPO 2015).

3.4.2 Eutrophication

Neilson and Cronin (1981) were the first to document eutrophication in the Chesapeake Bay. They found that nutrient loading into Chesapeake Bay had been the cause of serious and extensive damage to the estuary and related this issue with population increase. More recent, Boesch et al. (2000, 2001) intensively investigated eutrophication and health of the Chesapeake ecosystem. Since the 20th century the public opinion, scientific research and politics expressed their concerns about the state of the estuary because of its jeopardizing social and economical importance to the region, denouncing problems like overharvesting in fisheries, industrial and toxic pollution, and land clearance (Davidson et al., 1997). As a consequence of its proximity to Washington, D.C., the Bay has been the subject of much political and scientific attention since the early 1960s, but it was not clear until the last quarter of the 20th century whether eutrophication had degraded the ecosystem of the Bay (Malone et al., 1993).

In 1987 a commitment was made to reduce the sources of nitrogen (N) and phosphor (P) by 40% by the year 2000 (CBP, 2000). Despite the fact that the Chesapeake Bay has been more extensively studied than any other coastal ecosystem – since 1985 the Chesapeake Bay Program (CBP) has monitored the water quality and living resources – the 1987 commitment was not fulfilled because the causes and effects of eutrophication were poorly understood (Boesch, 2000). In 2010 the federal Chesapeake Bay Program established 102 commitments about the health of the Bay and its living resources (Blankenship, 2011). The Chesapeake Bay Program 2010 was signed with the general goal to restore the Bay water quality, enhance and protect the finfish, shellfish and other living resources, also their habitats and ecological relationships to sustain all fisheries and provide for a balanced ecosystem. In the subcategory water quality protection, which can be monitored by means of the phytoplankton (e.g. dinoflagellates), the commitment is to continue and maintain the 40 % nutrient reduction goal in the Bay and its tidal tributaries. As such, the water quality to support all aquatic living resources of the Bay and to protect human health would be achieved and maintained (Blankenship, 2011).

In order to represent this deterioration and to express the health of the Bay to the non-scientific audience, the Chesapeake Bay Foundation (1998) produced an index of *State of the Bay*. This composite index is based on 12 factors, each scored on a total of 100, where the 100 represents the pristine conditions at the time of arrival of European colonists (Boesch, 2000). To give an idea about the evolution: The State of the Bay Index was 28 in 2000 and 32 in 2014, showing the initiated turnaround (Chesapeake Bay Foundation, 2014).

3.4.3 Anoxia, phytoplankton blooms and fish kill

A consequence of eutrophication is the depletion of dissolved oxygen in the water column by the decomposition of organic matter, added to or produced within the ecosystem (Boesch et al., 2001) Two types are recognized: anoxia (total lack of oxygen) and hypoxia (dissolved oxygen concentrations lower than those required by organisms). Many authors (e.g. Officer et al., 1984; Taft et al., 1980) discussed the problems of anoxia (O_2 < 0.2 mg l^{-1}) and hypoxia (O_2 > 2.0 mg l^{-1}) in Chesapeake Bay, which is, because to its nature, rather susceptible to the development of hypoxic conditions. Seasonal hypoxia was already reported in the mid 1930s (Newcombe and Horen, 1938).

Natural hypoxia happens because floating aquatic plants and phytoplankton are releasing oxygen during photosynthesis in the photic zone. Surface water is nearly saturated with oxygen most of the year, while deep bottom waters range from saturated to anoxic. During winter, the activity of organisms is relatively low. Till then, conditions (little salinity or temperature stratification) for mixing were good. This resulted in plentiful oxygen throughout the water column. During spring and summer, there is increased microbial activity and thus intensified stratification, which reduces the vertical mixing, with low levels of dissolved oxygen in the deep water as result.

When nourished with nutrients (mainly nitrogen and phosphate) due to eutrophication, dense populations of harmful algae blooms (HABs) can develop (Seitz et al., 2009). During summer, when oxygen level is lower due to natural stratification, phytoplankton remains sink to the bottom. They rot anaerobically, after using all the oxygen, and create low-oxygen zones that are hostile to clams,

worms and other bottom-dwelling invertebrates, which are the main foods for crabs. These zones are called ‘dead zones’ (Diaz and Rosenberg, 2008). To illustrate: the dead zones in Chesapeake Bay kill or prevent the growth of about 75,000 metric tons of clams and worms every year. This means 60 million blue crabs a year, which will not be harvested (Chesapeake Bay Foundation, 2008).

3.4.4 Economical and health consequences

Many harmful algal blooms (HABs) have significant economic impacts. Blooms of toxic or harmful algae represent a threat to human health and fisheries resources throughout the United States and the world (Anderson et al., 2000). Chesapeake Bay is known for its blue crab (*Callinectes sapidus*), which has declined in population over the past few years due to the combination of overfishing, poor water quality (nitrogen and phosphate pollution) and dead zones. In the last two causes, algal blooms are involved. Nowadays, the crab population is only 40% of its population in 1970. The Virginia Institute of Marine Sciences estimated a consequent loss of $640 million in revenue in 2007 (Chesapeake Bay Foundation, 2008). The industry of the American oyster, *Crassotrea virginica*, is suffering the same problem (Chesapeake Bay Foundation, 2012).

Besides economic impacts, there are also health issues. Blooms of *Pfiesteria piscicida* (a HAB species) occurred already in almost all Chesapeake Bay tributaries (Blazer et al., 2010). In 1997 for example, several tributaries were closed to recreation and fishing (Bowman, 1997) because of human health risk, hence again an economic impact.

3.4.5 A hypertrophic river: The Potomac

As it is clear from different reports (King, 1970; Jaworski, 1990; Chesapeake Bay Foundation, 2014; Potomac Conservancy, 2016), the Potomac is one of the most affected rivers of Chesapeake Bay in terms of eutrophication. Jaworski (1981) compared nutrient loadings in the Chesapeake Bay and found that the Potomac had hypertrophic conditions over multiple years during the seventies and eighties. In 2012, the Potomac River was still recognized as America’s most endangered Rivers (America’s Most Endangered Rivers®, 2012). In more recent years, the Potomac River was not listed in this top 10 anymore, confirming the effectiveness of different action programs (e.g. Chesapeake Bay Program and Clean Water Act). This is also visible in the State of the Nation’s River 2011 to 2016, were the Potomac evolved from a D to a B- based on wastewater, nutrients, biology (fish species), habitat (land use, urbanization) and people (recreation for example). The purpose is to get an A+ in 2025. This rate can be more visually represented as fishable and swimmable (Potomac Conservancy, 2016).

Hypertrophic state index is defined here with water quality parameters, as proposed by Bricker et al. (2003). This includes a.o. chlorophyll *a* values > 60 µm/l), a Secchi disk depth (a measure of light transparency in the water) < 1m, occurrence of toxic algae, high nitrogen and phosphate concentration (≥ 1 mg·l^{-1} and ≥ 0.1 mg·l^{-1} respectively) and anoxia or hypoxia occurrences. As can be seen in Figure 19 of Bricker et al. (1999), the Potomac is, based on a part of these parameters, the most eutrophicated river in Chesapeake Bay.

4.1 Sample collection

Samples were collected by the Virginia Health Department during campaigns in 2013 and 2014 (Fig. 4.1, Table 3). The Shellfish Sanitation staff used a Petite Ponar grab sampler to retrieve the sediment. The uppermost two centimetres of these sediments were placed in plastic tubes of 15 and 50 cc. The samples were transported on ice, stored in a cold-room at 4°C in the dark. Afterwards, they were shipped to Montreal before they were sent to Belgium. Unfortunately some of the tubes were damaged during the transport to Montreal.

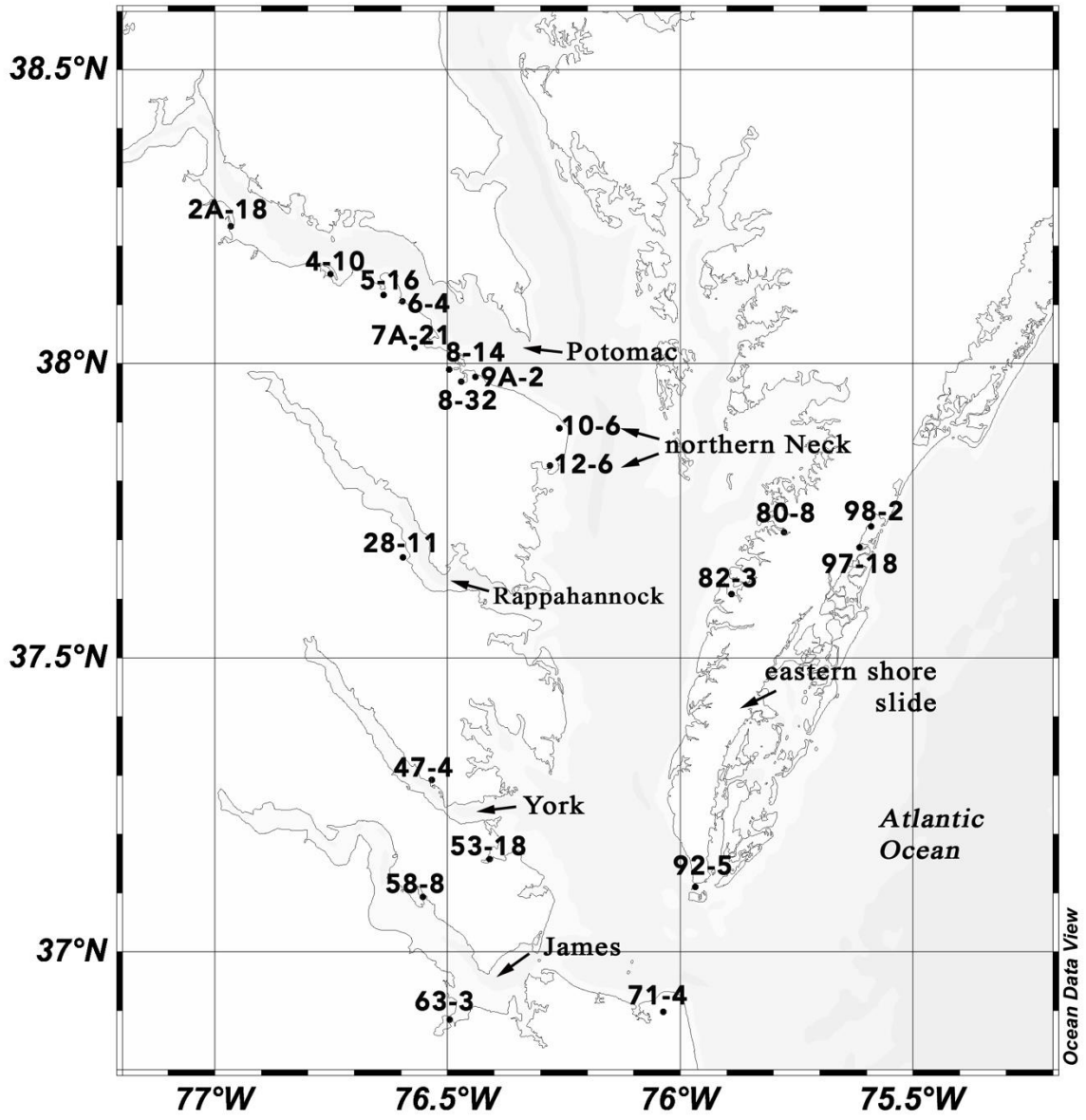


Fig. 4.1. Chesapeake Bay showing location of the surface sediment samples together with direction of the main rivers.

4.2 Sample preparation

The methodology, hereafter described, is largely conform with that of Price et al. (2016). The sediments were oven dried at 60°C for 24 hours and weighted. One to twenty-six cc was poured into plastic cups and treated with 50 to 100 mL of 20% hydrochloric acid (HCl) in order to remove carbonates. The solution was rinsed through decantation with distilled water until neutral. One *Lycopodium* clavatum tablet (9666 ± 2,2% *Lycopodium* spores, batch #3862) was added to the cups for the calculation of the absolute abundance (Benninghoff, 1962). Afterwards it was washed through a 150 µm metal sieve and a 10 µm nylon filter (except for the first set of samples (47-4, 53-18, 58-8, 63-3 and 71-4), which were filtered on a 20 µm mesh) to eliminate the coarser and finer fractions. Siliceous material was removed using 50 – 100 mL hydrofluoric acid (HF**,** 40%) for four days. After several decantations of the solution, a new treatment with 20% HCl was applied before the final sieving. The remaining residue was sonicated in an ultrasonic bath (Branson™, operating at 40 kHz) for two minutes and again sieved on a 10 µm mesh. This step liberates loose material that was still mechanically stuck by previous treatments. Immediately thereafter the remaining residue was scraped with a microspatula one or two times. Then the residue was mounted in glycerol gelatine jelly on a glass slide and covered slip. Afterwards the edges of the cover slip were sealed with transparent nail varnish to prevent the slides from drying. The slides were labelled with the name of the project and the sample number. The remaining concentrated residue was then stored in small 25 mL tubes. All slides, tubes and samples are stored in the collection of the Research Unit Palaeontology of Ghent University, Belgium.

The acid treatment was repeated for samples that still contained a considerable amount of sediment grains (mostly white large quartz grains) after the first treatment. Sample 2A-18 and 8-14 were treated differently after the acid treatment. The samples were placed in a large beaker and were agitated into suspension. The material in suspension was then discharged in another cup. The white minerals sank quickly to the bottom and were not carried to the new cup. This is a rapid, but less accurate, method than the alternative of using hot HF (Mertens et al., 2009b).

Table 1. Surface sediment samples. Collection data (location, region, sediment type and sample date)

Site ID	Latitude (°N)	Longitude (°W)	Location	Region	Sediment type	Sample Date
2A-18	38,2341	-76,966	Monroe Bay	Potomac River	Sand	14-07-2014
4-10	38,1523	-76,7521	Monroe Bay	Potomac River	Sandy gravel	14-07-2014
5-16	38,1173	-76,6381	Lower Mahodoc Creek	Potomac River	Very sandy	16-07-2014
6-4	38,106	-76,5978	Jackson Creek	Potomac River	Sandy gravel	16-07-2014
7A-21	38,0276	-76,571	Yeocomico River	Potomac River	Sandy gravel	16-07-2014
8-14	37,9899	-76,497	Coan River	Potomac River	Very sandy	08-07-2014
8A-32	37,9696	-76,4708	Glebe Creek	Potomac River	Sand	08-07-2014
9A-2	37,978	-76,4409	Cod Creek	Potomac River	Sand	08-07-2014
10-6	37,8901	-76,26	Little Wicomico River	Northern Neck	Sandy gravel	01-07-2014
12-6	37,8275	-76,281	Cockrell Creek	Northern Neck	Silt	03-07-2013
28-11	37,6712	-76,5966	Lagrange Creek	Rappahannock River	Sand	29-07-2013
47-4	37,2927	-76,5347	Timberneck Creek	York River	Silt	10-07-2013
53-18	37,1584	-76,4102	Poquoson River	York River	Silt	17-07-2013
58-8	37,0942	-76,5529	Warwick River	James River	Sand	17-07-2013
63-3	36,8855	-76,4958	Nansemond River	James River	Silt	16-07-2013
71-4	36,8985	-76,0368	Broad Bay	South Shore CB	Silt	31-07-2013
80-8	37,7137	-75,7781	Onancock Creek	Eastern Shore Side	Mud	18-07-2013
82-3	37,6086	-75,8906	Nandua Creek	Eastern Shore Side	Silty sand	24-07-2013
92-5	37,1111	-75,9684	Fisherman Island Inlet	Eastern Shore Side	Sandy silt	31-07-2013
97-18	37,6883	-75,6153	Folly Creek	Eastern Shore Side	Mud silty	25-07-2013
98-2	37,7238	-75,5911	Metomkin Bay	Eastern Shore Side	Silt	25-07-2013

4.3 Light microscopy

The microscopic work was carried out with a Zeiss Imager A1 transmitted light microscope at the Research Unit of Palaeontology at the University of Ghent. Lenses with magnifications of 20×, 40× and 100× were used in combination with 10× magnifying oculars to obtain magnifications of 200×, 400× and 1000× respectively. The slides were scanned exploratory with a 200× magnification and counted systematically with the 400× magnification. For detailed photomicrographs and identification the 1000× magnification with immersion oil was used. Photomicrographs were made with with a Zeiss Axiocam MRc5 camera.

4.4 Palynological analysis

4.4.1 Dinocysts counting methodology

For this study 21 sites over a total of 37 slides, were investigated for their palynological content. The palynomorphs were counted in non-overlapping parallel traverses, starting from the centre of the slide. According to Mertens et al. (2009b), counting of 300 dinocysts is sufficient for obtaining a reliable measure of the diversities and absolute abundances in Quaternary studies.

In a few slides 300 cysts were reached after counting a couple of traverses. The unscanned remainder of the slide was then further scanned qualitatively for rare species.

4.4.2 Dinoflagellate nomenclature

In order to apprehend the recent dinocyst morphology and taxonomy, half of the sample slides were fully scanned before counting and most of present specimens were photographed and evaluated. A catalogue with photomicrographs of identified species was made according to the recognized nomenclature. The taxonomical nomenclature is based predominantly on Rochon et al. (1999). Rossignol (1962), Reid (1974), Pospelova et al. (2002), Mertens et al. (2012), Sarai et al. (2013) were also consulted for detailed descriptions. Cysts of *Polykrikos kofoidii and P. schwartzii* were identified according to Matsuoka et al. (2009). A list of the counted dinocysts and their biological name or thecal equivalent is provided in table 2. A detailed taxonomy is found in the Appendix (A) with species names and authors.

Cysts were identified to the species level as much as possible. Some of the dinocysts taxa were grouped together, based on morphological characteristics. For example the genus *Spiniferites* formed a group of *Spiniferites* spp., unless a specific determination was possible. *Alexandrium* spp, *Dubridinium* spp. and *Achomosphaera* spp. were also grouped together. For some specimens, identification to species level was not possible. In these cases, Bengtson's (1988) rules for open nomenclature were applied:

> "**cf.**" indicates an uncertain or provisional identification, mostly due to poor preservation (e.g. *Operculodinium* cf *israelianum*).
>
> "**sp.**" or "**sp. indet**" indicates that for a certain specimen no specific identification could be assigned (e.g. Type sp. Indet. 1)
>
> "**spp.**" is used when a specimen could only be determined to genus level, and forms a group of the same genus (e.g. *Spiniferites* spp.).

Some cysts were grouped because of generic or specific identification difficulties. The group of round brown cysts (RBC) probably holds some *Brigantedinium* spp., but because of the absence of an archeopyle or unfavourable orientation during counting, an unambiguous identification to the genus *Brigantedinium* was not possible. The group of Spiny Brown Cysts (SBC) contains most probable species belonging to the genera *Echinidinium* spp. and *Islandinium*. Because of the dirt clogged to the cyst, more specifically the processes, they could not be easily characterized and the

archeopyle was often not well observed. A special remark must be made for the cyst of *Archaeperidinium minutum*. During counting it was noticed that certain *Echinidinium* spp. also were counted as cysts of *A. minutum*. Also *Islandinium brevispinosum* was counted and is thus also part of the cysts of *A. minutum* cf. group. Due to this difficult identification, interpretation of *A. minutum* is difficult and must be critically considered. Cysts of *Biecheleria* were also observed. Although the average body diameter of 20 – 30 µm (Moestrup et al., 2009) exceeds the size of the nylon filter gaps, Price and Pospelova (2011) measured cysts as small as 8 µm. Besides the fact that cysts of *Biecheleria* could pass through the filter during the sample preparation, this palynomorph was not yet confirmed as a dinocyst. Warns et al. (2012) and Takahasi et al. (2014) solved this problem and described different species of *Biechelaria* as dinoflagellates. The cysts of *Biecheleria* were counted, but were not involved in the interpretation or other analyses.

Table 2. Taxonomic citation of dinocysts used in this study. Motile equivalents are taken from Head (1996), Rochon et al. (1999), Pospelova and Head (2002), Pospelova et al. (2002) and Pospelova and Kim (2010).

	Cyst based taxonomy (paleontological name)	Motile stage-based taxonomy (biological name)	HABs species
Autotrophic			
Gonyaulacaceae	*Achomosphaera* spp.	*Gonyaulax spinifera complex*	-
	Cyst of …	*Alexandrium/Scrippsiella* spp.	yes
	Ataxiodinium choane	*Gonyaulax spinifera complex*	-
	Lingulodinium machaerophorum	*Lingulodinium polyedrum*	yes
	Nematosphaeropsis labyrinthus	*Gonyaulax spinifera complex*	-
	Operculodinium centrocarpum sensu Wall and Dale (1966)	*Protoceratium reticulatum*	yes
	Operculodinium israelianum	*? Protoceratium reticulatem*	yes
	Cyst of … (sensu Wall and Dale, 1968)	*Protoperidinium minutum*	-
	Polysphaeridium zoharyi	*Pyrodinium bahamense*	yes
	Spiniferites spp.	*Gonyaulax spinifera complex*	-
	Spiniferites mirabilis	*Gonyaulax spinifera complex*	-
	Spiniferites bentorii	*Gonyaulax digitalis*	-
	Spiniferites pachydermus	*Gonyaulax spinifera complex*	-
	Spiniferjtes delicatus	*Gonyaulax spinifera complex*	-
	Spiniferites ramosus	*Gonyaulax spinifera complex*	-
Protoperidiniaceae	Cyst of …	*Pentapharsodinium dalei*	-
Heterotrophic	Cyst of …	*Gymnodinium nolleri*	yes
Protoperidiniaceae	Cyst of …	*Archaerperidinium minutum cf.*	-
	Echinidinium aculeatum	*Protoperidinium* group	-
	Lejeunecysta paratenella	*Protoperidinium* group	-
	Cyst of …	*Protoperdinium nudum*	-
	Quinquecuspis concreta	*Protoperidinium leonis*	-
	Selenopemphix nephroides	*Protoperidinium subinerme*	-
	Selenopemphix quanta	*Protoperidinium conicum*	-
	Cyst of ...	*Protoperidinium stellatum*	-
	Trivantedinium applanatum	*Protoperidinium pentagonum*	-
	Votadinium calvum	*Protoperidinium oblungum*	-
	Votadinium spinosum	*Protoperidinium claudicans*	-
	Xandarodinium xanthum	*Protoperidinium divaricatum*	-
	Dubridinium spp.	*Diplopsalid group*	-
Diplosalidaceae	Cyst of …	*Polykrikos schwartzii* sensu Matsuoka et al., 2009	-
Polykrikaceae	Cyst of …	*Polykrikos kofoidii* sensu Matsuoka et al., 2009	-

4.4.3 Other palynomorphs

In addition to dinocysts, other marine and terrestrial palynomorphs were included in the counting. In every slide (bisaccate) pollen, microforaminiferal linings, different acritarchs (*Radiosperma corbiferum*, *Halodinium* spp*., Polysterias* and *Pseudoschizeae*), Cyst Type P (Reid and

John, 1978) and Tintinnid lorica type B (Price and Pospelova, 2011) were counted. Also different unknown types were counted and were descripted in the result chapter.

4.5 Environmental parameters

The precise location of the sample location was determined during field work. The salinity of that moment was also measured (summer season). To carry out multivariate analysis, extra environmental parameters were determined such as chlorphyll *a*, nitrogen, phosphate, temperature and salinity. It seemed interesting to get as well seasonally as annually measurements for salinity and other parameters due to the large variations between winter and summer, especially for temperature. These were mainly derived from Ocean Data View 4.7.4 for Mac (Schlitzer, 2016). The World Ocean Database was used as input for ODV. WOD13 (Boyer et al., 2013) contains almost 13 million temperature profiles and nearly 6 million salinity measurements. For the Chesapeake Bay region, some 1000 data points are represented in the database. The extrapolation is visually represented in figure 4.2.

4.6 Data analysis

4.6.1 Concentration calculation and estimated error

Concentrations of dinocysts were calculated for each sample, following the equation by Benninghoff (1962):

$$n = \frac{N \cdot T \cdot a}{w \cdot L}$$

in which
n: concentration = number of dinocysts per gram dried sediment
N: number of counted dinocysts
T: number of *Lycopodium clavatum* spores/tablet
a: number of tablets added to the sample
w: weight of dried sediment (g)
L: number of counted *Lycopodium clavatum* spores

As mentioned before, one *Lycopodium clavatum* tablet was added to each sample during maceration. One tablet (no. 3862) contains 9666 *Lycopodium* spores, with a standard deviation of 2,2% (213 spores). The error of the calculation of the absolute abundance is determined by the method of Stockmarr (1971). The total error (e) is the sum of e_1 (error of spores in the marker tablets or tablet calibration), e_2 (error on dinocysts counted) and e_3 (error on *Lycopodium clavatum* spores counted).

$$e = \sqrt{e_1^2 + e_2^2 + e_3^2} = 100 \cdot \sqrt{\left(\frac{\sigma_1}{r_1}\right)^2 + \left(\frac{\sigma_2}{r_2}\right)^2 + \left(\frac{\sigma_3}{r_3}\right)^2}$$

Where

$\sigma 1$: standard deviation on number of spores in marker tablets

$\sigma 2$: standard deviation on dinocysts counted = $\pm \sqrt{r_2}$

$\sigma 3$: standard deviation on *Lycopodium* spores counted = $\pm \sqrt{r_3}$

$r1$: number of spores in marker tablets

$r2$: number of dinocysts counted

$r3$: number of *Lycopodium* spores counted

4.6.2 Species richness and diversity (Shannon – Wiener index)

Taxon richness is a measure for the variety of species. This refers to the number of species in a sample. The species diversity is commonly expressed by the Shannon-Wiener diversity index H.

$$\mathrm{H}' = -\sum_{i=1}^{n} P_i \cdot \ln(P_i)$$

where P_i is the proportion of individuals belonging to the *i*th species in the dataset of interest. The higher the ', the higher the diversity of species is in the dataset. The natural logarithm (ln) is applied to convert all the taxa less represented taxa in a percentage to increase their weight. These taxa often have more narrow ecological affinities and could be more diagnostic for environmental conditions (de Vernal and Giroux, 1991).

4.6.3 H/A ratio

The H/A ratio gives the ratio between heterotrophic dinoflagellates and autotrophic dinoflagellates. This ratio tends to increase with increasing nutrient enrichment. It is especially interesting for estuarine systems, where cultural eutrophication often happens (Pospelova and Kim, 2010). It is calculated as:

$$\frac{H}{A} = \frac{nH}{nH + nA}$$

in which

nH: Heterotrophic dinocysts.

nA: Autotrophic dinocysts.

In contrast to the H/A-ratio, the "P/G index" (Versteegh, 1994) was not used in this study. The P/G ratio is the ratio of Peridiniales to Gonyaulacales and is approximately corresponding with the H/A-ratio, but does not involve the heterotrophic families Polykrikaceae and Diplopsalidaceae. The H/A-ratio was here the proper tool to interpret the assemblage. The P/G curve of Harland (1973), which uses number of Protoperidinioid and Gonyaulacoid taxa instead of the specimens of a certain taxon, was also calculated, but gave no unambiguously result for this study and was not discussed furthermore.

4.6.4 Multivariate analysis

With multivariate analysis, the relationship between environmental data (e.g. temperature, salinity) and the dinocyst assemblages can be scrutinized. Following Pospelova et al. (2004) and Patterson et al (2011), who studied comparable study areas, we used direct gradient analysis. First statistics were tested in Past3 (Hammer et al., 2001), which is free software for scientific data analysis, including functions for multivariate statistics and ecological analysis. Principal Component Analysis and Detrended Correspondence Analysis (DCA) were used to test the nature of variability of the dinocyst assemblage and to quantify trends in the cyst distribution.

Other statistical analyses, with environmental data, were carried out using CANOCO 5.0 for Windows 9 (ter Braak and Smilauer, 2012). The relationship between dinocyst distributions and environmental parameters was assessed by species scores and their ordination patterns. Both Redundancy Analysis (RDA) and Canonical Correspondence Analysis (CCA) were also carried out. These are both constrained correspondence analyses based on the Eigenvalue analysis method applied to a matrix of dinocyst data (samples-by-taxa) and to a matrix of environmental data (samples-by-parameters). The analyses identified patterns of distribution and influence among species and environmental variables. Forward selection was used to identify the variables that could explain the greatest amount of variance in the species data set. The significance of each environmental variable was tested by the significance of the first axis using Monte Carlo testing. This permutation test tests the distribution, which is based on the number of environmental parameters, correlation structure and species abundances for example (Leps and Smilauer, 2003).

The arrows on the ordination plot point in the direction of maximum variation and the length of the arrows show the relative importance of each variable. The greater the angle between two environmental arrows, the less likely that they are related to each other. More details about interpretation of the ordination can be found in ter Braak and Prentice (1988).

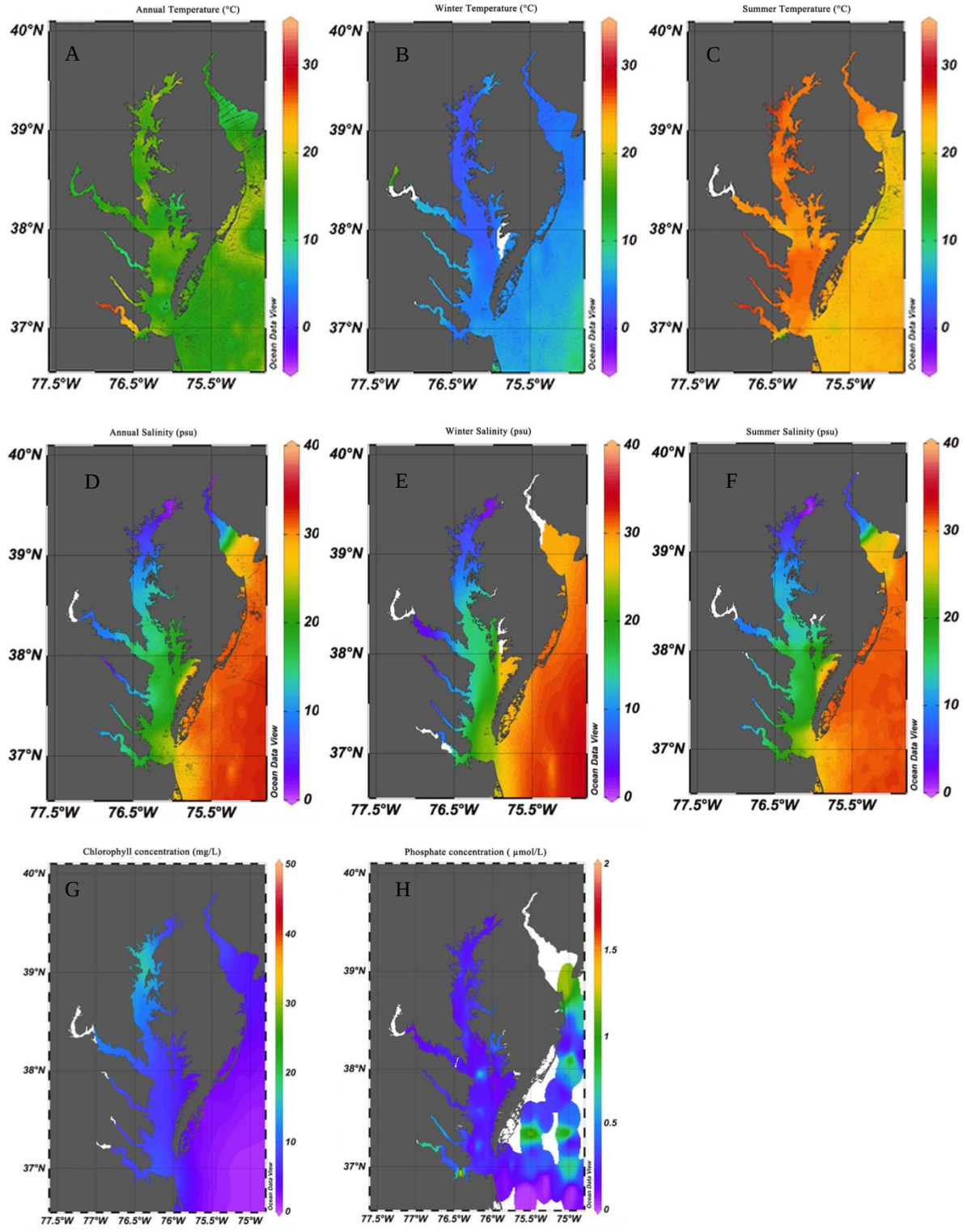


Fig. 4.2. Plots of environmental parameters, generated with ODV. A) Annual temperature (°C); B) Winter temperature (°C); C) Summer temperature (°C); D) Annual salinity (psu); E) Winter salinity (psu); F) Summer salinity (psu); G) chorophyll *a* concentration (mg/L) and H) Phosphate concentration (µmol/L)

5.1 Dinocysts

5.1.1 Total dinocysts concentrations

An average of 265 dinocysts (minimum 23 in sample 6-4 and maximum 342 in sample 4-10 and 47-4) was counted in the 21 samples (listed in table 3 per their location). The counts are included in the appendix (B). The 300 dinocysts threshold was not reached for every sample after counting all available residues. Sites 6-4 and 8-32 even did not yield a total of 100 dinocysts in the slide. Microphotographs of selected dinocysts are depicted in plates 1, 2, 3, 4 and 5. In some samples, reworked pre-Quaternary dinocysts were encountered (Plate 6).

The total dinocyst concentration per gram of dry weight of sediments (cysts g^{-1}) ranged from 5 to 135,900 cysts g^{-1} (in samples 6-4 and 71-4, respectively), averaging 16,000 cysts g^{-1}. The highest values of cyst concentrations were observed in the South Shore of the Bay (site 71-4; 135,900 cysts g^{-1}) and in the Eastern Shore Side (site 80-8; 108,000 cysts g^{-1}). The lowest cyst concentration was found in sites in the Potomac River (Fig. 5.1A). The total error was also calculated. According to De Schepper (2006) samples with an error > 20% are not considered as statistically reliable. The average error for these samples was 11.2%. The total error is controlled mainly by the error on the *Lycopodium* spore count. Sites 6-4, 71-4 and 80-8 has the largest error (21.1; 21.7 and 23,75 %), showing less statistically reliability. Less than 25 *Lycopodium* spores were counted in these last two samples, which created a higher error. Sample 6-4 had the lowest number of dinocysts, but on the contrary, almost the highest amount of Lycopodium spores were counted, resulting in a large total error. The average *dinocyst / Lycopodium* ratio is circa 2.5. A ratio of 10 or higher, usually gives an error higher than 15% (De Schepper, 2006), which is only the case for sites 71-4 and 80-8.

Table 3. Different zones in Chesapeake Bay with the sampled sites.

Zones	Sites
The Potomac River	2A-18, 4-10, 5-16, 6-4, 7A-21, 8-14, 8-32, 9A-2
The Northern Neck	10-6, 12-6
Rappahannock River	28-11
York and James River	47-4, 53-18, 58-8, 63-3, 71-4
The Eastern Shore Side	80-8, 82-3
Atlantic Ocean	92-3, 97-18, 98-2

5.1.2 Diversity

As presented in the materials and methods chapter, two different ways were used to calculate the diversity: taxa richness and the Shannon-Wiener index. In total, 42 cyst taxa were identified in the 21 samples, of which 29 to species level. The number of taxa for an individual site (taxa richness) varied from 6 to 27, with an average of 15.57. Low values of taxon richness were observed generally in the Potomac River. Sites located at the Eastern Shore Side and Atlantic Ocean of Chesapeake Bay had the highest species richness (Fig. 5.1B).

The Shannon-Wiener index followed generally the same pattern as the taxa richness (R^2 = 0,57207). Sites with the highest taxa richness have the highest SW index. Site 98-2 has the highest SW index (2.41), while the lowest (0.42) is encountered at site 5-16. The highest taxa richness was observed in 92-5, but this site had a lower SW index compared to the other samples. Remarkable is site 63-3, which has a high taxa richness number (18), but a low SW index (1.33). The opposite is recorded in site 6-4, with a low taxa number (8) and relative high SW index of 1.57.

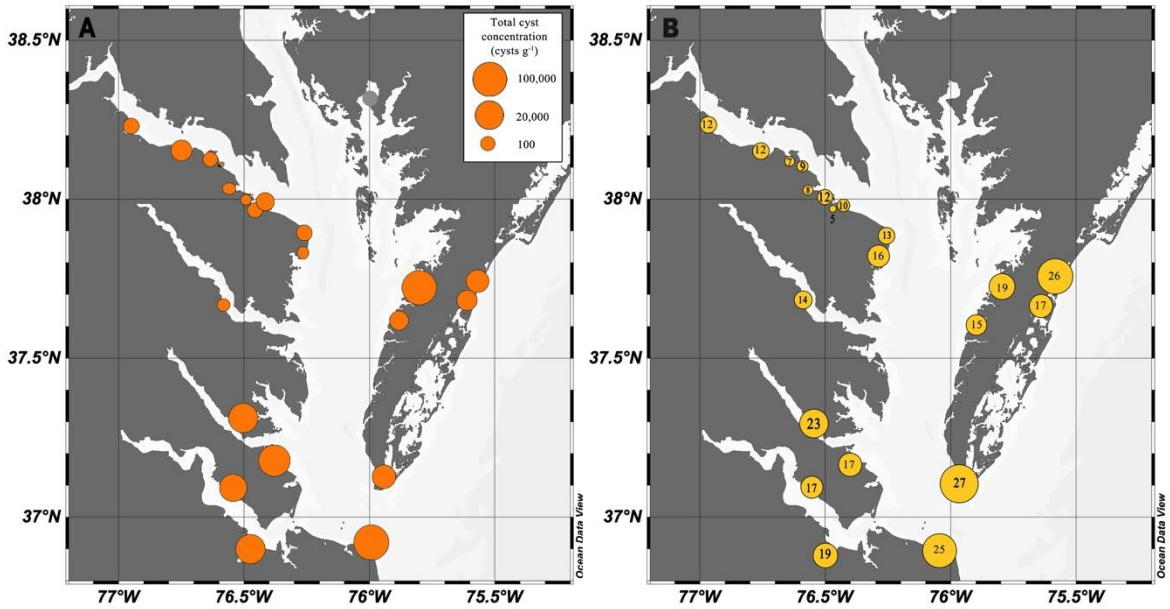


Fig. 5.1. Distribution of total dinocyst concentration (A) and dinocyst taxa richness (B) in surface sediments of Chesapeake Bay.

5.1.3 Dinocyst assemblages

The composition of the dinocyst assemblages is given in Figure 5.2. The assemblages are dominated by *Spiniferites* spp, cysts of *Alexandrium* spp, *Operculodinium centrocarpum*, Round Brown Cysts and *Polysphaeridium zoharyi*. Here a list is presented with all observed species in the assemblage. Descriptions are found in chapter 8, taxonomy. The types are described in section 5.1.3.

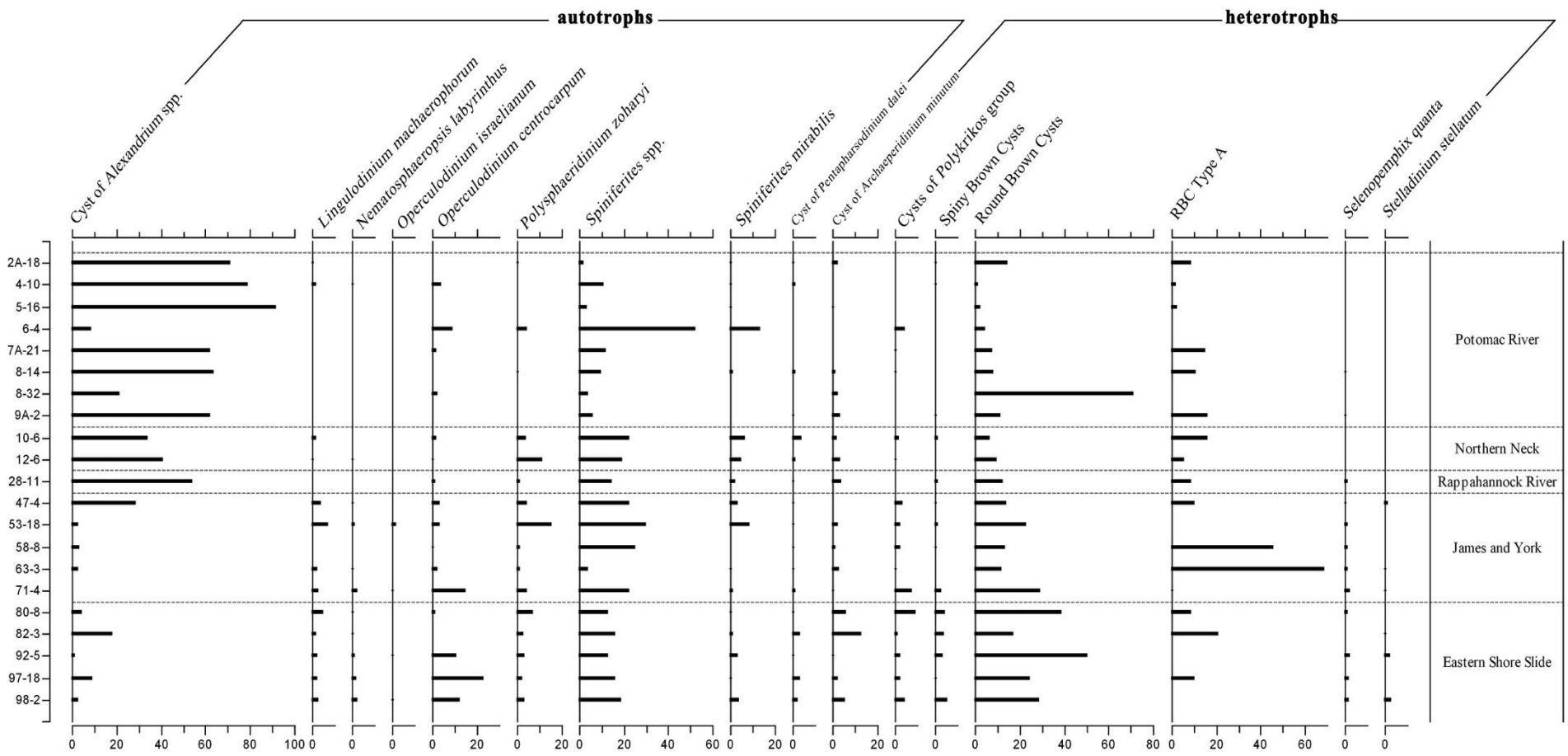


Fig. 5.2. Relative abundance of dinocyst taxa that contribute more than 3% to the cyst assemblage. Ordered from north to south and divided in subcategories according to their location. Figure made by C2 (version 1.7.6), a paleoenvironmental visualization tool (Juggings, 2007).

The relative abundance of Cysts of *Alexandrium* spp. varied between 0 to 91.4%. Especially the Potomac River was characterized by large relative abundances. Apart from sites 6-4 and 8-32, which had low cyst counts, all sites in the Potomac Rover had relative abundances above 60%. The southern sites show low abundances, varying from 0 (71-4) to 20% (82-3), with an average of 6%. The Northern Neck sites and the one from the Rappahannock River site showed again higher values, 33 to 54 %.

The Round Brown Cysts (RBC) were ubiquitous in the studied sites. The relative abundance varied from 2.6 to 79.7%. The highest relative abundances were recorded in the southern sites (especially site 63-3, 58-8 and 92-5). The York River (47-4) and sites at Atlantic coast show intermediate abundances (23 to 47%). The Potomac River is characterized by low abundances (2.6 to 27%), with the exception of site 8A-32 (71%). More specifically, RBC Type A is part of the RBC group. Largest relative abundance of this type were found at site 63-3, in the James River, followed by 58-8, somewhat more north, in the York. It is noteworthy that sites at the Atlantic Ocean were marked with very low abundances (0 to 10%). The sites at the Potomac River also showed low relative abundances for RBC type A (0 to 15%).

Spiniferites spp. was recorded in every sample, ranging from 2 to 52%. Highest relative abundances were noticed at the southern sites (12-30%) and the Northern Neck (22%). Site 63-3, which is also located south, is an exception with 3.5%. The Potomac River sites relative abundances varied from 2 to 12%, if sample 8A-32 is excluded. If we took all *Spiniferites* (*S. ramosus, S. bentorii, S. mirabilis* etc.) together with the *Spiniferites* spp. group, we did not observe remarkable differences with the above description of the relative abundances. *S. mirabilis* is common in the sites with abundances from 0 to 8.3%. Highest abundances appeared in the Northern Neck sites.

Operculodinium centrocarpum was relatively abundant in the Chesapeake Bay sites, especially in the sites near the Atlantic Ocean (samples 92-5, 98-2 and 97-18), where its relative abundance varies from 11 to 22%. *O. centrocarpum* is much less abundant in the Potomac River, with values from 0 to 4%. The York, the Rappahannock and the eastern shore of Chesapeake showed a variance from 0 to 3.5%.

Polysphaeridium zoharyi is less abundant than *O. centrocarpum*. The dinocyst did not occur in the Potomac River, except at 2 sites (2A-18 and 6-4). In the Northern Neck the dinocyst was present with relative abundances of (3.6 to 11.2%). The sites at the Atlantic Ocean coast showed values ranging from 2.3 to 4.1%. The highest abundance was found in sample 53-18 in the York River. The other sites showed intermediate abundances, from 1 to 6.6%.

Lingulodinium machaerophorum was not abundant in the Chesapeake region. In the Potomac River the dinocyst did not have a large distribution and in many sites the dinocyst was not present. *L. machaerophorum* varied in the sites near the Atlantic Ocean from 1.9 to 2.6%. The highest abundance was recorded in sites 53-18, 47-4 and 80-8, i.e. in the southern part of the Bay.

Less common dinocysts were those produced by the motile-defined genus *Polykrikos*. Cysts of *P. kofoidii, P. schwartzii and Polykrikos* indet. were combined together in relative abundances. The relative abundance of Cysts of *Polykrikos* spp. abundance varied from 0 (absent) up to 9% in site 80-8. The highest relative abundances were recorded in sites with the highest absolute dinocyst concentration, namely 71-4 and 80-8. Specifically, sites at the Atlantic Ocean had the highest abundances, from 2 to 7.6%. The Potomac River was characterized by the near absence of *Polykrikos*. Relative abundances up to 3% were recorded in the southern sites.

Cysts of *Archaeperidinium minutum* were common in the assemblage. The highest relative abundance was observed in sites 80-8 and 82-3 on the Eastern Shore Side. In the other parts of the Bay its abundance varied from 0 to 5%. *Achomosphaera* spp., Cyst of *Protoperidinium stellatum*, *Selenopemphix quanta*, *Nematosphaeropsis labyrinthus* and Cysts of *Pentapharsodinium dalei* were present in most sites, but never reached more than 4% of relative abundance of any assemblage. *Dubridinium spp.*, Cysts of *Gymnodinium nolleri,*, Cysts of *Protoperdinium minutum* sensu Wall and Dale (1968) and Cysts of *Protoperidinium nudum* were found in a few sites, but with a relative abundance maximum of 1% of the assemblage. *Trinovantedinium applanatum* (0-0.3%), *Votadinium spinosum* (0-0.3%), *Votadinium calvum* (0-0.6%), *Xandarodinium xanthum* (0-0.3%), *Lejeunecysta paratenella* (0-0.3%), *Selenopemphix nephroides* (0-0.7%), *Echinidinium aculeatum* (0-0.3%) and *Ataxodinium choane* (0 to 4.3%, in site 6-4) were very rare with only 1 to 5 specimens counted in the whole assemblage.

A general trend is observed when evaluating the absolute abundances per taxon. Most abundant species per site are found in almost every site. When comparing the absolute species abundances with the total dinocysts abundance, we see correlation with most species. For example: RBC (R^2 = 0,965); *Spiniferites* spp. (R^2 = 0,92) and *O. centrocarpum* (R^2 = 0,62). *Alexandrium* spp. is not correlated (R^2 = 0,16 with a significance of 0,075).

5.1.3 Unidentified species or acritarchs

Our dinocysts study of surface sediments revealed the presence of dinocysts not previously recorded in Chesapeake Bay, or were not yet identified (Plates 2 and 5). Probably not all these types are resting cysts of dinoflagellates, but especially Type indet 3 was already observed in coastal waters (Mertens, pers. comm.). This species was not yet descripted or depicted. In this study, five types are found and will be discussed below:

RBC Type A

Observations: This cyst is brown and has a round ambitus. Typically it contains a endospore with cell content in the inner part of the cyst. A membrane surrounds the cyst wall, which is visible through little pleats on the smooth surface. The wall is thick and sometimes the cysts are dented antapical. An archeopyle was never observed. This cyst could ne labelled as a type thanks to these morphological characteristics. Measurements: minimum 26 (average: 27.85) maximum 29 μm (n = 10).

Type Spiniferites A

Observations: This *Spiniferites* type has the basic morphology of a *Spiniferites ramosus*, i.e. a spherical body without apical boss and a smooth wall. The difference between *S. ramosus* and this type are the length of the processes, which are only 4-7 μm (5,52 μm average, n=5). This morphological characteristic is not observed on other *Spiniferites* and therefore we can label this species as a type. Cyst diameter is minimum 30,41 μm, average 32.28 μm and maximum 36,68 μm.

Type indet 1

Observations: Chorate cyst with delicate and fine reticulate ornamentations. The reticulate pattern forms crests over the entire surface. The crests go over into fine but wrinkled aculeate processes. The wall is thick and is coarsely granular. An archeopyle is not observed, but the cysts seem to split up along the cingulum. Observed cyst length is between 28 and 36 μm, based on 3 measurements.

Type indet 2

Observations: The cyst is dark brown and could be related to RBC, but it has processes. It is characterized by very small and many processes which are hairy. The wall, which is difficult to observe due to the large amount of processes, is granulate and thin. The cyst displays an opening, probably an archeopyle, and the operculum is still attached. Cyst diameters are: 28 (29.3) 30 μm (n= 5).

Type indet 3

Observations: This cyst is not counted as a dinocyst due to its unknown biological affinity. The cyst body is elongated and has two major apical and antapical extentions. Sometimes these resemble chalices of flowers with extra processes. The wall is very thick and is characterized by vacuoles. A couple of these cysts have cell content. Cysts were measured (length: 25 (30.62) 35 μm (n=10)).

Type indet 4

Observations: The cyst has a similar morphology of the cyst *Operculodinium longispingerum* (Matsuoka, 1983). The round chorate cyst is characterized by many short processes. These processes are located in equidistant concentric lines around the almost round opening, which could represent the archeopyle. The double wall structure is observed in the opening. The wall is microgranulate and thick and the processes are solid. Processes are to 4 μm in length, while the cyst diameter is about 25 μm (based on one measurement).

5.1.4 H/A-ratio

The ratio of cysts produced respectively by autotrophic and heterotrophic dinoflagellates varied from site to site. The relative abundance of heterotrophic taxa ranges from 3% to 87% (average 41%). The H/A-ratio calculated from all samples is given in Figure 5.3. The highest H/A-ratios were recorded at site 63-3 and other high values are recorded in samples from the Eastern Shore Side. Lowest ratios are observed in the Potomac River, where the H/A-ratio varies from 3% to 31%, excluding site 8A-32. In the Northern Neck H/A-ratios increase to 30% and in the southern sites, H/A-ratios of 35-67% are observed. Samples at the Atlantic coast vary between 44 and 65%.

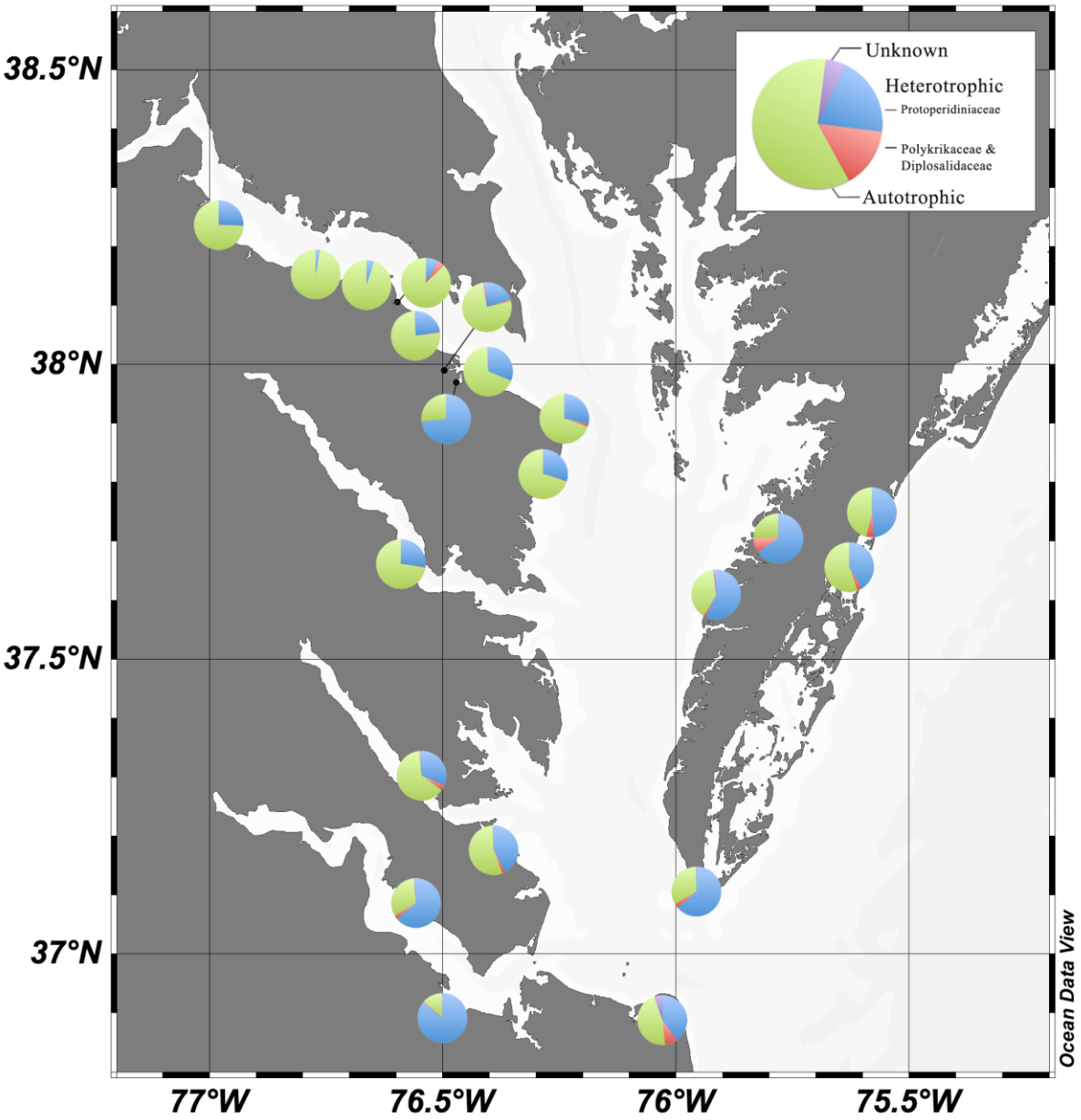


Fig. 5.3. Relative abundance (%) of cysts of heretotrophic (Protoperidiniaceae, Polykrikaceae and Dipsalidaceae) and autotrophic dinoflagellates in assemblages from Chesapeake Bay.

5.1.5 Algae blooms

Alexandrium spp. was encountered in almost every sample. During and after counting, we have noted that this group consists not only of *Alexandrium* spp. but a minority can also be identified as *Scrippsiella* spp. and *Prorocentrum minumum.* At least there are three groups to distinguish, i) a small type with sometimes little processes, which is *Scrippsiella* spp.; ii) a group with round cysts around 40 µm, which is identified as *Alexandrium* spp; and iii) a heart shaped group with a central ring, *Prorocentrum minimum*. *Scrippsiella* spp. and *P. minimum* were not counted, but were taking into account for further interpretation and research. Photomicrographs of these different cysts types are given in plates 1 and 2.

5.1.6 Other palynomorphs

Microforamiferal linings are recorded at every site. Their distributional pattern is positively correlated with the dinocyst concentration (R^2 = 0,667). The foram linings reach up to ~8000 linings g^{-1} in sites with the highest dinocyst concentrations, while the average concentration is 580 linings g^{-1}.

Tintinnid lorica type B (according to Price and Pospelova, 2011) also showed this positive correlation (R^2 = 0,932) with dinocyst concentration. The relative abundances of palynomorphs were calculated on the total amount of palynomorphs without dinocysts. High relative abundances were observed in the samples on the Atlantic Ocean coast (98-2, 92-5, 71-4, 97-18). Sites at the Potomac River had much less ciliate cysts in their assemblages, varying from 0 to 45%.

Highest absolute abundances of total pollen (bissacate and non-bissacate) were recorded in sites with the largest dinocysts concentration (71-4 and 80-8) with respectively 758,000 and 2,508,325 pollen g^{-1}. The Potomac River sites had the smallest amount of pollen concentrations of 55 to 2150 pollen per gram. The southern sites also had high abundances of pollen, ranging from 10,000 to 467,000 pollen per gram. The Northern Neck samples held less pollen: 470 pollen g^{-1} for 10-6 and 2150 pollen g^{-1} for 12-6. The pollen abundances did not correspond with the dinocysts concentrations (R^2 = 0,031). All southern sites were characterized by the dominance of bissacate pollen, in contrast to the Potomac River sites, where non-bisaccate pollen were more dominant.

Other palynomorphs (listed in the Appendix) showed one general trend. Largest absolute abundances were recorded in the southern samples, and accord with the dinocyst concentrations. *Radiosperma corbiferum* was found in almost every sample, while *Halodinium* spp. and *Polysterias*, Cyst Type P (Reid and John, 1978) and *Pseudoschizaea*, all acritarchs or ciliate cysts, were seldom found in samples from the Potomac River.

5.1.7 Reworked dinocysts

As mentioned above, reworked dinocysts (see Plate 6) were observed in seven different sites (2A-18, 4-10, 6-4, 7-21, 12-6, 47-4 and 97-18). Most dinocysts were decribed by de Verteuil and Norris (1996) and identified by Prof. Dr. S. Louwye. Sites 4-10 and 97-18 had the most diverse reworked species. The species have a Cenozoic age, from the Oligocene, the Miocene into the Pliocene. *Cleistosphaeridium placantha*, *Dapsilidinium pseudocollegerum*, *Reticulatosphaera actinocoronata* and reworked *Spiniferites* spp. were found in sample 4-10. Only one reworked *Spiniferites* spp. was found in samples 6-4 and 12-6. In sites 7-21 and 47-4 respectively a reworked *Operculodinium centrocarpum* and *Operculodinium israelianum* was found. Site 97-18 had the most reworked species with a.o. *Heteraulacacysta campanula,* and *Spiniferites perforates.*

5.2 Environmental parameters

The results of the extrapolations in ODV are shown in table 4. There were not enough available measurements of nitrate to extrapolate the nitrate concentration in the Chesapeake Bay area. Furthermore, we were able to extrapolate the values for chlorophyll *a* from the database because there were enough measurements available in the Bay. This was not the case for the rivers. For this reason, the weighed-average gridding in ODV became too high for the x- and y-axis and gave unreliable extrapolated values.

Some variables do not vary much across the sample sites. The average phosphate concentration is around 0.3 µmol/L, but for site 63-3 an aberrantly high value of 0.95 µmol/L is retrieved. Annual chlorophyll *a* shows a trend from high (11.78 mg/L) to low (2.78 mg/L) from north (Potomac River samples) to south (Atlantic Ocean samples), with an average of 7.92 mg/L.

The difference between winter and summer temperature is large, but the site differences are less per temperature parameter. The winter temperature varies from 4.02 to 7.24 °C with an average of 5.52°C. The highest temperatures are recorded in the Potomac River. The summer temperatures vary from 22.52 to 26.56 °C with an average of 24.67°C. There is more contrast in the annual temperature for the samples with variations from 14.13 to 23.14 °C with an average of 16.08 °C. Highest temperatures occur in the James (58-8) and the York (47-4) rivers.

The winter, summer and annual salinity do not show much variation. Only in winter, low salinities (2 to 10 psu) in the Potomac River are distinct. The average winter, summer and annual salinity are, respectively, 15.28, 19.10 and 18.35 psu. In summer, the salinity in the southern sites reaches 31 psu, which is close to the winter (29 psu) and annual (30 psu) salinity.

Site ID	Coordinate Lat (N)	Long (W)	Phosphate (µmol/L)	Chlorophyll (mg/L)	Temperature winter (°C)	Temperature summer (°C)	Temperature annual (°C)	Salinity winter (psu)	Salinity summer (psu)	Salinity annual (°C)
2A-18	38,2341	-76,966	0,16	11,78	7,24	25,15	14,13	2,99	9,47	9,13
4-10	38,1523	-76,7521	0,19	10,93	7,08	25,32	14,88	4,72	11,36	9,32
5-16	38,1173	-76,6381	0,23	10,35	6,98	25,18	15,49	7,44	13,10	12,47
6-4	38,106	-76,5978	0,23	10,13	7,02	25,11	15,26	8,50	13,53	13,42
7A-21	38,0276	-76,571	0,23	9,59	6,72	24,69	14,96	9,05	15,04	12,55
8-14	37,9899	-76,497	0,22	9,15	5,38	24,23	15,47	11,16	15,92	14,48
8A-32	37,9696	-76,4708	0,22	8,97	4,82	24,09	15,62	11,80	16,19	14,87
9A-2	37,978	-76,4409	0,21	8,92	4,01	24,08	15,76	12,55	16,29	15,10
10-6	37,8901	-76,26	0,23	7,94	3,33	23,01	16,99	14,53	16,60	17,04
12-6	37,8275	-76,281	0,22	7,84	4,12	23,47	17,18	14,13	16,79	17,38
28-11	37,6712	-76,5966	0,25	8,29	6,97	26,56	10,53	9,26	13,90	10,89
47-4	37,2927	-76,5347	0,44	8,29	5,73	25,65	18,93	10,70	15,01	17,90
53-18	37,1584	-76,4102	0,28	8,59	4,18	25,69	16,55	15,30	18,07	19,03
58-8	37,0942	-76,5529	0,38	8,30	5,82	25,82	23,14	11,43	15,80	15,17
63-3	36,8855	-76,4958	0,95	8,17	4,02	22,52	16,95	15,49	18,05	17,19
71-4	36,8985	-76,0368	0,15	8,23	5,94	22,82	15,64	23,57	25,74	23,92
80-8	37,7137	-75,7781	0,16	3,71	5,51	25,26	15,85	28,73	31,00	30,24
82-3	37,6086	-75,8906	0,19	5,01	4,86	25,36	16,27	26,81	30,00	28,59
92-5	37,1111	-75,9684	0,24	6,54	5,19	23,84	14,85	24,40	26,86	25,29
97-18	37,6883	-75,6153	-	2,78	5,46	25,09	16,42	29,04	31,23	30,63
98-2	37,7238	-75,5911	-	2,78	5,51	25,17	16,74	29,20	31,24	30,72

Table 4. Sampling locations (latitude, longitude) together with environmental parameters: phosphate concentration (µmol/L); chlorophyll *a* concentration (mg/L); winter, summer and annual temperature (°C) and the winter, summer and annual salinity (psu), extrapolated and extracted from Ocean Data View.

5.3 Multivariate analysis

The dataset was simplified before data analysis input. Some taxa were grouped: All Spiniferites species and Spiniferites spp. were combined as "*Spiniferites* group", RBC together with RBC Type A as "RBC Group". *Polykrikos* group consists of Cysts of *P. kofoidii*, *P. schwartzii* and *Polykrikos* indet. and *Selenopemphix quanta* was grouped together with Cysts of *Protoperidinium nudum*. All less abundant species (<2%) were not taken into account in the multivariate analysis. Afterwards the relative abundances were rescaled to 100%. Sites 6-4 and 8-32 were also excluded from this simplified dataset by not reaching 300 dinocysts, respectively 23 and 51 counts. Counting 300 cysts can provide up to 98% confidence (Germerad et al., 1968). These low counts are insufficient and thus not representative.

Principal Component Analysis (PCA) was carried out on the relative abundances of dinocysts. The results of PCA show that the first ordination axis (PCA1) explains 81,2% and the second axis (PCA2) 13,6% of variance (Fig. 5.4). If they were included, the PCA1 and PCA2 variance was much lower and gave blurred results. The positive PCA1-axis shows a cluster of sites from the Potomac River. Then a cluster from the Northern Neck sites (10-6 and 12-6) acts as the transition towards the Atlantic Ocean sites. Sites 63-3 and 53-18 are positioned as two extremes at the PCA2-axis. The other southern samples form together a cluster. There is less significant variance between the sites on the negative x-axis, because the sites vary more according the y-axis (PCA2), which is known by less variance.

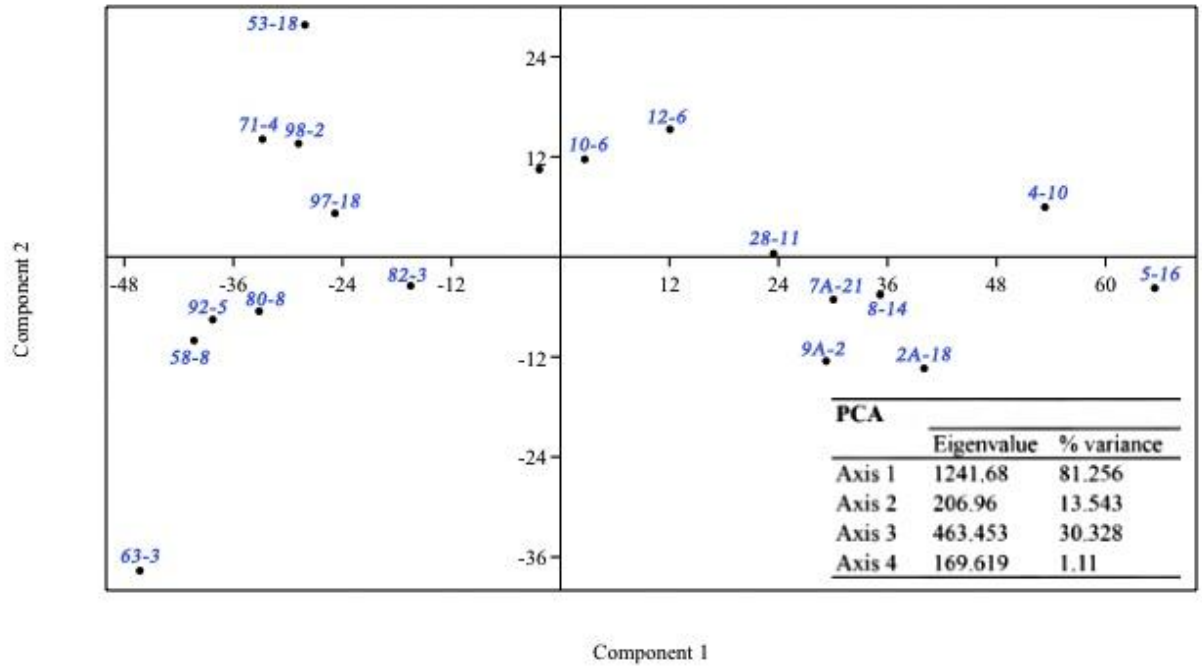
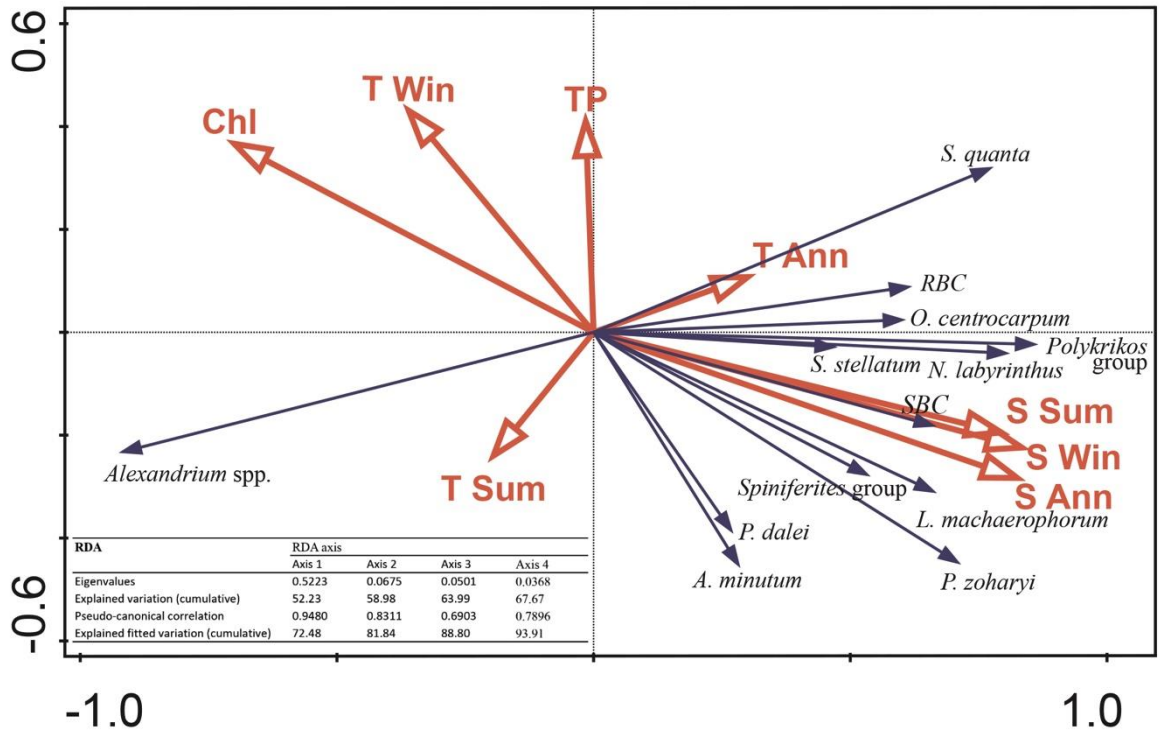


Fig. 5.4. PCA biplot showing the sample ordination, eigenvalues of the PCA analysis and corresponding variance.

Redundancy Analysis (RDA) was executed on the relative abundances of the dinocysts together with the environmental data. The results of RDA show that the first ordination axis (RDA1) explains 52.2% and the second axis (RDA2) 6,7% of the variance. The first four axes are statistically significant (P=0.002). This indicates a relationship exists between species and these environmental

parameters. The RDA ordination diagram is shown in Figure 5.5. The first and second axes are significantly and positively correlated with chlorophyll *a*. All the salinity parameters point towards high positive RDA1 values and high negative RDA2 values. TP follows the positive RDA2. The best fit for the first axis is from *Alexandrium* spp. (91%), *Polykrikos* (85%) and *Nematosphaeropsis labyrinthus* (80%). All species, except *Alexandrium* spp., plot on the positive RDA1 axis, following the winter, summer and annual salinity and to a lesser extent, the annual temperature. *Alexandrium* spp. plots on both negative axes and follows preferentially the summer temperature and the chlorophyll *a* concentration. The winter temperature and TP seems to have no correlation at all.



RDA	RDA axis			
	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.5223	0.0675	0.0501	0.0368
Explained variation (cumulative)	52.23	58.98	63.99	67.67
Pseudo-canonical correlation	0.9480	0.8311	0.6903	0.7896
Explained fitted variation (cumulative)	72.48	81.84	88.80	93.91

Fig. 5.5. RDA results of Chesapeake Bay sites showing ordination of species and environmental parameters on axes 1 and 2 (respectively 52.2 and 6.7 %). The following abbreviations of the environmental parameters were used: Chl – annual chlorophyll *a*; T Win – temperature in winter; T Sum – temperature in summer; T Ann – annual temperature; S Win – salinity in winter; S Sum – salinity in summer; S Ann – annual salinity; and TP – total phosphate concentration.

CCA was carried out on the same dataset and gave a similar topography of the ordination plot from RDA. Due to the lower, and thus lower variance (43% and 5%), the results of this analysis are not further explained nor used. One of the small differences is that the annual temperature plots stronger with some species. The other species follow the other environmental parameters, like the RDA analysis.

Detrended Correspondence Analysis was carried out on the relative abundances together with environmental data. The result is an ordination diagram (Figure 5.6) with the species, samples and environmental data in one plot. This triplot explains 51% of the variance in the first axis (DCA1) and 14% in the second (DCA2). Especially the annual, winter and summer salinity show high negative correlation (-0.72) with axis 1. Chlorophyll *a* shows the highest positive correlation (0.589) with the

first axis. The samples from the Potomac form a distinct group in the positive quadrants of the DCA1 axis, which fits the best for winter temperature and chlorophyll *a*. *Alexandrium* spp. is also plotted on this side of the diagram. All other species are plotted more in the centre, showing less to none correlation with the samples. Sites at the Atlantic Ocean form another group, in the first quadrant (negative DCA1 and positive DCA2 axis), but are not correlated with an environmental variable. The southern sites (63-3, 53-18 and 58-8) also form a group in the centre of the plot, correlating with the annual temperature. Smaller, but also distinct groups, are the Northern Neck sites in the middle of the plot and the Eastern Shore Side sites at negative position in the second axis. Site 28-11 does not belong to a group, but could tentatively be placed in the group with the Northern Neck sites.

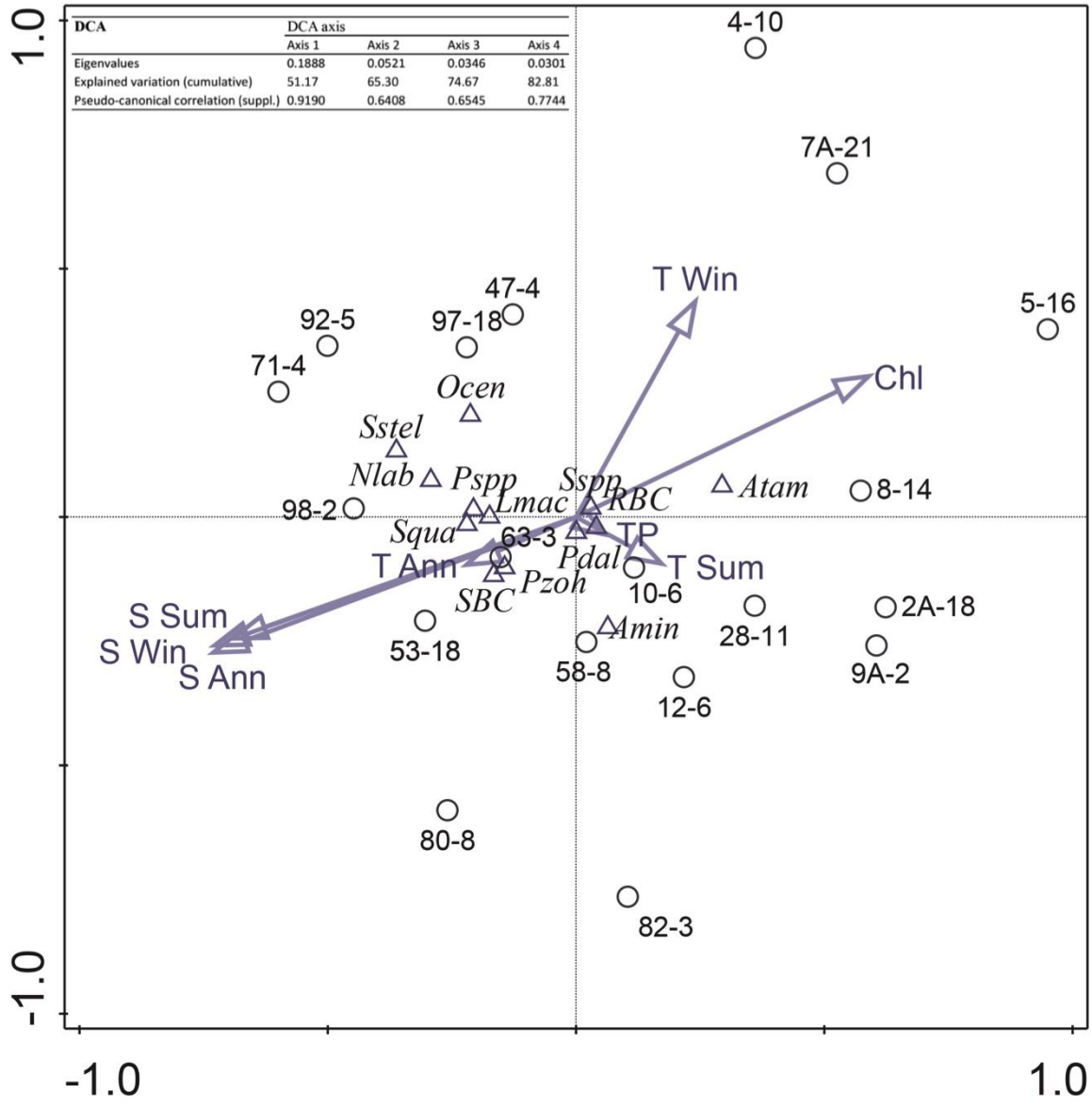


Fig. 5.6. Ordination diagram generated from DCA, showing species and sample distribution and the direction of environmental parameters. Eigenvalues and explained variation are given in left upper corner. *Atam* = *Alexandrium* spp.; *Amin* = Cyst of *Archaeperidinium minitum* cf.; *Ocen* = *Operculodinium centrocarpum; Sstel* = Cyst of *Protoperidinium stellatum; Nlab* = *Nematosphaeropsis labyrinthus; Pspp* = *Polykrikos* group*; Sspp* = *Spiniferites* group; RBC = Round Brown Cysts + Type RBC A; *Lmac* = *Lingulodinium machaerophorum; Pzoh* = *Polysphaeridium zoharyi; Squa* = *Selenopemphix quanta* and Cyst of *Protoperidinium nudum*; SBC = Spiny Brown Cysts and *Pdal* = Cyst of *Pentapharsodinium dalei.*

6. DISCUSSION

"We are concerned about Alexandrium monilatum because it has been reported toxic to fish, and has been related to a variety of fish kills…throughout the world. We are certainly on the lookout for it!"
- Dr. Harold Marshall (Daily Bay, August 20 2009)

6.1 General observations

The upper centimetres of the sediments (i.e. surface sediments) represent a certain number of depositional years, which can be approximately 10 years in estuaries (Pospelova et al., 2005), but it is clear that this number depends largely on the sedimentation rate. Some papers (e.g. Rodriguez and Kuehl, 2013; Sanford & Halka, 1993) showed erosion in Chesapeake Bay, where older layers could be at the surface, but we assume here that the surface sediments are modern (i.e. deposited during the last 10 years). Langland and Cronin (2003, Table 6.1) report sedimentation rates in the Chesapeake Bay varying between a minimum 0.1 cm y^{-1} to maximum 19 cm y^{-1} sediments, but most of the rate numbers range between 0.5 and 1 cm yr^{-1}. This implies that the upper 2 or 4 cm represents surface sediments, and as such the cyst assemblage represents an average of the past 5 to 10 years of deposition.

Few publications have dealt with dinocyst assemblages of Chesapeake Bay. A palynological study by Verardo (1999) recorded similar cyst assemblages in the upper centimetres of the cores studied. Seaborn and Marshall (2008) also studied surface assemblages in Chesapeake Bay, and recorded assemblages comparable to our recorded assemblages. Except that they found cysts of two bloom-forming species, i.e. *Heterocapsa triquetra* and *Cochlodinium polykrikoides.* They used a different method to extract the cysts and did not publish images of the dinocysts in their paper, which complicates comparison.

Absolute abundances correspond principally with the lithology of the sediments. The highest concentrations of dinocysts were recorded in sediments with high contents of clay, silt and organic matter. This is probably due to the fact that the dinocyst settling behaviour is comparable with that of fine sediment particles and that their abundance increases with higher mud contents in sediments (Dale, 1983). We see that the Potomac River sedimentation is very sandy and many of sites in this river contain small pebbles. In those exceptional cases of sandy lithology with small pebbles the absolute abundances are extremely low (5 to 50 cysts g^{-1}). At the sandy sites, the absolute abundance increases to 100-300 cysts per gram of sediment. Almost all southern sites have a silty sediment composition. The highest abundances were observed in samples from sites 71-4 and 80-8 where sediments consist grey to black fine mud.

Beside mechanical settling behaviour of dinocysts, primary production and selective preservation of cysts also potentially influence the absolute abundances. Zonneveld et al. (1997) described preservation differences between species of dinocysts. Especially the *Protoperidinium* species are considered sensitive to oxidation. The Potomac River is known for its dead zones (Kemp

et al., 2005), which is good for preservation. It seems that the selective preservation does not play a role in the observed assemblage there.

Earlier studies by Pospelova et al. (2002, 2005) linked increasing amplitude in the temporal and spatial fluctuation of total cyst abundances as a strong indicator of stressed environments. In the New Bedford Harbor the total abundance of cysts varied from 6,000–12,000 cyst g^{-1} (in 16-19th centuries) to 2,600–23,000 g^{-1} (20th century), indicating stressed environments in the 20th century (Pospelova et al., 2002). We noticed that the total cysts abundances in the Potomac River samples varied with a factor 200 (5 to 1,000 cysts g^{-1}), which is much larger than cyst abundances of the southern samples, being a factor of 14 (10,000 to 140,000 cysts g^{-1}). The huge cyst fluctuations in the Potomac River could be explained by a stressed environment, where blooms of *Alexandrium* spp. and *Scrippsiella* spp. are frequent and affect cyst concentrations/fluxes, which leads to high temporal fluctuations in cyst abundances. It seems due to the special conditions in this environment, the normal assemblage is replaced by autotrophs. But the main role, besides these differences in primary productivity, is that this large range of fluctuations in surface sediments is a reflection of the large differences in sediment accumulation rates from site to site (Pospelova et al., 2005).

6.2 Dinocyst assemblages

6.2.1 Composition of assemblages

The dinocysts observed in the surface sediments form a diverse assemblage. Most of the cysts belong to widely distributed, neritic, cold to temperate taxa (Dale, 1996). *Spiniferites* spp. dominates the cyst assemblages. The distribution of *Spiniferites* spp. generally reflects the North Atlantic circulation pattern (Harland, 1983). *Spiniferites* spp. is also commonly found in coastal and estuarine systems (Dale, 1996). Pospelova and Chmura (1998) found that this group shows the greatest tolerance to extreme environmental conditions, e.g. low salinities (5 to 15 psu).

Operculodinium centrocarpum is also one of the most abundant dinocyst types occurring in Chesapeake Bay. Its cyst distribution matches closely with that of the Gulf Stream and the North Atlantic drift (Harland, 1983). It is regarded as a cosmopolitan species with high relative abundances in all environments (Zonneveld et al., 2013). However, during the past 1,000 years the abundance of this species has decreased in Chesapeake Bay (Verardo, 1999).

Nematosphaeropsis labyrinthus is also an oceanic species, with a very few exceptions (Zonneveld et al., 2013). We found a trend in the distribution of both *N. labyrinthus* and *O. centrocarpum*, namely that they are the most abundant in the southern samples (near the Atlantic Ocean) and reduce inward, with the lowest abundances in the Potomac (northern Chesapeake Bay and farthest away from the Atlantic Ocean. This reflect the preferentially behaviour of these species in oceanic environment.

6.2.2 Species not recorded here

Dinocyst assemblages of the estuarine environment of Chesapeake Bay are characterized by the absence of open oceanic taxa such as *Impagidinium* species and *Pyxidinopsis reticulata*, which are common in Pacific and Atlantic margins (Radi et al., 2007).

Seaborn and Marshall (2008) identified cysts of *Heterocapsa triquetra* as the most common cysts in Chesapeake Bay tributaries (60-80% of assemblages in surface sediments of the York and James River), but did not provide a description or illustration. The Chesapeake Bay Monitoring Program even measured in the summer of 2007 5,734,865 *H. triquetra* cells per litre in the York River. *H. triquetra* is a common bloom producer in these rivers. The only description of the cysts of *H. triquetra* has no iconography, but two drawings of process-bearing cysts (Braarud and Pappas, 1951). It is even not sure if the dinoflagellate *H. triquetra* makes resting cysts. Probably the only resting stage is a short temporary cyst stage. This temporary cyst stage was described later, but resting cysts are not yet unambiguously identified (Olli, 2004). The description of the cyst by Olli (2004) differs as a matter of fact from original description of Braarud and Pappas (1951). These latter authors described a spiny cyst with a smooth wall, while Olli (2004) described an ovoid to cylindrical cyst with cell content but without spines. We were unable to identify cysts as described in those papers. Marshall et al. (2005) later recorded the presence of *H. triquetra* in the phytoplankton. Seaborn and Marshall (2008) furthermore used a sample processing method differing from the palynological technique used in our study. It is possible that due to our acid treatment cysts of *H. triquetra* were destroyed and thus cannot be observed in surface sediments.

A similar problem applies to the Cyst of *Cochlodinium polykrikoides.* The dinoflagellate is extensively observed in the phytoplankton of Chesapeake Bay (Marshall et al., 2005; Mulholland et al., 2009; Egerton, pers. comm.). In the James, the Cheseapeake Bay Program monitored 260,480 cells of *C. polykrikoides* in 2008. Cysts of *C. polykrikoides* are observed by Seaborn and Marshall (2008) in the surface sediments of the Bay, but are not described or illustrated. Different descriptions have been put forward by several authors (Matsuoka, 1985; Matsuoka and Fukuyo, 2000; Pospelova and Kim, 2010; Tang and Gobler, 2012; Li et al., 2014). Only Li et al. (2014) established the motile stage-cyst relationship through an incubation experiment; however in our sites, cysts similar to those described by Li et al. (2014), were not observed and four different explanations for this can be put forward. First of all, is the cyst of Li et al. (2014) the cyst of *C. polykrikoides* or another *Cochlodinium* species? Second, does the dinoflagellate species in Chesapeake Bay correspond to *Cochlodinium polykrikoides* originally described by Margalef (1961) from the Caribbean? Third, would there be a taphonomical issue that the cysts of *C. polykrikoides* do not preserve well? The last explanation would be that the morphology of the cyst changes strongly with the environmental conditions in Chesapeake Bay. If we use the cyst of *C. polykrikoides* depicted by Pospelova and Kim (2010) for identification, than it is possible that the cyst of *C. polykrikoides* is present in the assemblage; however, these cysts resemble *Alexandrium* spp. cysts and we were unable to distinguish both.

6.2.3 Recycling specimens

There are concerns about the in situ production of two cyst species, namely *Polysphaeridium zoharyi* and *Operculodinium israelianum*. In Chesapeake Bay *Pyrodinium bahamense*, the motile

stage of *P. zoharyi*, has not been recorded in the plankton (J. Wolny, pers. comm., Mertens, pers. comm.). This dinoflagellate is however regionally observed in the plankton of the west coast of Florida in large blooms (Ishman et al., 1996; Philips et al., 2011). Possibly, the cysts of *P. bahamense* were transported northwards by the Gulf Stream, which has been suggested by Wall et al. (1977). Verardo (1999) also observed *P. zoharyi* cysts in surface sediments and older sediments of Chesapeake Bay. Marshall et al. (2005) described *P. bahamense* as present in the phytoplankton, but showed no iconography. Seaborn and Marshall (2008) then have both observed *P. zoharyi* as cyst and motile dinoflagellate, but as already remarked, their macertation methodology differs from ours and the cysts were not incubated. In addition some reworked, pre-Quaternary *P. zoharyi* were observed. Even considering the large abundance of *P. zoharyi* in the assemblage, *P. bahamense* is a subtropical to tropical species (Zonneveld et al., 2013) and cysts of *P. bahamense* are probably transported from the coast of Florida. Motile dinoflagellates also could be transported. For example, *Karenia* brevis red tides occur in the West Florida shelf and could be transported due to the Gulfstream as far north as North Carolina (Wolny et al., 2015). The motile dinoflagellate *P. bahamense* however is too fragile to withstand transportation (J. Wolny, pers. comm.).

The same situation applies to *Operculodinium israelianum*. Wall et al. (1977) suggested that this cyst is recycled from elsewhere. *O. israelianum* is present in the south of Chesapeake Bay, around the Pamlico Sound. The cysts also occur in small bays in the Caribbean region, around the Bermuda and Bahama Islands. It is uncertain whether the motile stages occurs in the modern plankton of Chesapeake Bay, largely because the motile affinity has not yet been revealed, but probably it is the cyst of a *Protoceratium* species (Zonneveld and Pospelova, 2015). Also, some reworked *O. israelianum* are observed, probably from Cenozoic sediments or even Quaternary (Wall et al., 1977). As long as the motile affinity is not yet recorded in the phytoplankton, there can be no confirmation whether the species is exotic or not. Germination experiments are needed to further resolve this by providing a cyst-motile stage relationship for this species.

6.3 Eutrophication

6.3.1 Species richness and populations

Nutrient enrichment may have positive effects on biodiversity of dinocysts and diatoms, but passing a threshold (due to overpopulation or urbanization) will potentially cause detrimental effects (Pospelova et al., 2002). Biodiversity or species richness of dinocysts also increases when an ecosystem is shifting from an oligotrophic to mesotrophic condition (Pospelova et al., 2002). When the estuary becomes hypertrophic, however, diversity declines.

Dinoflagellate populations increase with the level of nitrogen loading in estuaries (Evgenidou et al., 1999). A similar pattern is observed for diatoms, but proportionally, the increase for diatoms is much larger as they constitute a larger part of the phytoplankton (Evgenidou et al., 1999). Southern samples have the largest populations, which we assumed as eutrophication. Smallest populations are found in the Potomac River, suggesting hypertrophic conditions.

6.3.2 Individual species indicators

Taxa respond differently to water quality or changes in environment. Cysts of *Polykrikos schwartzii* and *P. kofoidii* increase in abundance with increasing nutrient enrichment. When analysing their spatial distribution, it is observed that these species are absent from the most polluted sites. This may indicate that these are suppressed in hypotrophic (hypereutrophic) and highly polluted environments, as was found by Pospelova et al. (2005) in the Buzzards Bay, Massachusetts. *Dubridinium* spp. was rarely identified and did not give a decisive signal due to its low abundance; even this taxon is commonly seen with nutrient enrichment (Pospelova et al., 2005). This species is in all probability underrepresented in the assemblage and cysts could be counted as RBC.

Lingulodinium machaerophorum, *Operculodinium israelianum* and *Selenopemphix quanta* decline when conditions in embayments are changing from oligotrophic to mesotrophic (Pospelova et al., 2002). These species were absent or had very low abundances (<4%) in most cyst assemblages of our study. As it concerns a spatial study, we could not see a temporal decline in species abundances. But, low abundances do not really contradict a temporal study, since probably all sites studied are characterized by mesotrophic to eutrophic conditions (Pospelova et al., 2005).

6.3.3 Heterotrophic versus autotrophic dinoflagellates

Increased abundance of cysts of heterotrophic dinoflagellates, and Polykrikaceae and Diplopsalidaceae in particular, are interpreted as a signal of nutrient enrichment of embayment waters (Matsuoka, 1999; Matsuoka et al., 2003; Pospelova et al., 2002; Pospelova et al., 2005; Pospelova and Kim, 2010). As such, the H/A-ratio could be used as an indicator of eutrophication in estuarine regions. Here, the H/A-ratio can be equated to heterotroph/autotroph ratio due to the low abundances of Polykrikaceae and Diplosalidaceae. Sites 63-3 and 80-8 have the highest proportions of cysts produced by heterotrophic dinoflagellates. Sites 71-4 and 80-8 have the highest numbers of Polykrikaceae and Diplosalidaceae. We interpret this as a sign of eutrophication. An increase of heterotrophs is usually seen when going from meso- to eutrophic conditions, but when the system passes the threshold to the highly eutrophic (hypertrophic) conditions the dinoflagellates react differently. Dinocyst diversity declines, autotrophs dominate and cyst total fluxes fluctuate greatly (Pospelova, pers. comm.).

Site 63-3 brings two additional elements into the story about eutrophication. First, as one can see in Table 4, Chapter 4, levels of phosphate were here the highest of all samples. This site is located in the Nansemond River, an inlet of the James River in the southern part of Chesapeake Bay, close to the cities of Norfolk and Chesapeake. According to the Nansemond River Report (NRPA, 2014), levels of phosphate far exceed the threshold of 0.05mg/mL Passing the threshold causes algal blooms and eutrophication, which has been demonstrated. This huge amount of phosphate can probably be explained through the huge amount of wastewater that the Nansemond has to treat (10-50 millions gallons a day). This means also that 10 to 50 million gallons of treated water returns into the water with the possibility that not all the phosphate is filtered out from the water (USEPA, 2010).

The sites at the Potomac River are characterized by low heterotrophs/autotrophs ratios, suggesting there are predominantly autotrophic dinoflagellates in the plankton. As already stated, the

Potomac is one of the most affected rivers of Chesapeake Bay in terms of eutrophication. The heterotroph/autotroph ratio does not correlate to the eutrophication recorded in the study area. This is related to the higher number of blooms of autotrophic species. The Potomac River is the most polluted river in the Bay, probably even considered as a hypertrophic river, where blooms of autotrophic species are abundant due to the stressed environment. As cyst assemblages represent multiple years, changes in trophic status could happen during that period. It was already mentioned that the trophic status in Chesapeake Bay has ameliorated during the last couple of years. That does not mean that the water quality of the Bay was previously ameliorating. The result here is that the assemblage represents a global hypertrophic (i.e. a bad environment) condition of approximately the last 10 years (2003-2014), even that the Potomac nowadays is just eutrophic.

6.4 Cyst bed hotspots

The dinoflagellate *Alexandrium monilatum* is a common HAB (harmful algal bloom) species that historically causes red tides (see cover picture) along the southern Atlantic, with a recent expansion into the mid-Atlantic region (Virginia Institute of Marine Science, 2015). It has no known human health impacts, but it is monitored because of concerns in hatcheries and restoration programs due to the production of the toxin *goniodomin A* (Hsia et al., 2005), a toxin that leads to lethal fish and shellfish poisoning (The National Centres for Coastal Science, 2015).

In Chesapeake Bay these blooms cause mass mortalities of oyster larvae (*Crassostrea virginica*), that are cultured for human consumption (Reese et al., 2012). Blooms of *Alexandrium monilatum* will consequently have an economical impact. Future blooms will spatially expand because toxic organisms shift up towards the north due to climate change and warming (Reese et al., 2012). Hence the importance of characterizing the blooms and their impacts.

The Virginia Department of Health (VDH) is primarily interested in *Alexandrium* cysts in order to localise bloom initiation areas or to identify the cyst bed hotspots. These cysts of *A. monilatum* form seedbeds that, upon excystment, may initiate new blooms (Anderson and Wall, 1978).

The blooms appear to be spreading from a relatively small area, namely the mouth of the York River, to a much larger area throughout the entire lower Bay and extending into the Atlantic Ocean. *A. monilatum* is observed since 2007 in the region and has bloomed almost annually in southern Chesapeake Bay (Reese et al., 2012). A commonly expressed explanation by marine biologists is that the species was brought in with the ballast water ships (Hallegraeff and Bolch, 1991). The hypothesis of expansion is that the blooms spread from cyst deposition, which act the year prior as a widening cyst bed for excystment in the following seasons (T. Egerton, pers. comm.). *Cochlodinium polykrikoides* (Mulholland et al., 2009) shows a similar pattern but, as stated above, the cysts of this species was not observed during this study.

Our study could help to localise these bloom initiation areas that could potentially be subject to remediation or at the least monitoring at a higher frequency as sentinels for a larger regional bloom. Cysts of *Alexandrium* spp. (including cysts of *Alexandrium monilatum*, but not exclusively cysts of *A. monilatum*) are recorded almost in every site, conforming the importance of *Alexandrium* species in the phytoplankton. Blooms are probably abundant here due to the unstable or eutrophic environments (Anderson et al., 2002; Seitz et al., 2009). These nuisance and harmful blooms (due to their toxin) of dinoflagellates may be caused by high nitrogen loading and high organic matter (Paerl, 1988). Given the spatial resolution of our study, it is not possible from our results to exactly locate the cyst bed hotspots or to determine its true spatial extent. However, some preliminary indications can be put forward. Considering the absolute concentrations of *Alexandrium* spp., the site with the highest concentration is site 47-4 in the York River. This corresponds with the observations of Pease et al. (2015a,b).

6.5 Multivariate analysis

Through PCA on the simplified dinocyst relative abundance data set it was possible to identify spatial clusters of sites. All sites of the Potomac clustered together due to the strong relative abundance of *Alexandrium* spp. Other clusters are less discernible, due to the more diverse assemblages. Northern Neck sites are also characterized by *Alexandrium* spp. and plot closer to the Potomac sites. The southern sites (York and James River) resemble the Eastern Shore Side sites, but have small variations in their assemblages, which is why they do not form clearly separate clusters.

Quantitative analysis shows which environmental factors control the distribution of dinocysts in the Chesapeake Bay. RDA shows that the assemblage is mostly influenced by winter and annual salinity and in a lesser degree by the annual temperature. Especially *Selenopemphix quanta* and the *Polykrikos* group are influenced by the annual temperature. *Alexandrium* spp. is the only taxon that is influenced by summer temperature. As *Alexandrium* spp. is a bloom forming dinoflagellate it is prevalent in Chesapeake Bay in the late summer months, usually July to September, ant this explains the relation. Normally *Alexandrium* will be produced more during summer months (when samples are taken) than in winter months, but the samples are time averaged. *Alexandrium* spp. is also influenced by the chlorophyll *a* concentration, which is linked to the productivity of cysts and blooms. Unfortunately winter temperature and total phosphate (TP) seems to have no observable impact on the dinocyst assemblages. The extrapolations of TP were made with a few measurements and show probably not the real phosphate concentrations. Interpretation of sewage as main role in eutrophication is not possible and is, as above, based on qualitative data. This RDA plot reflects the environmental preferences (temperature and salinity) of dinocyst taxa from studies in estuaries (Pospelova et al., 2002; 2004).

Pospelova et al. (2005) found that water temperature and salinity did not control the distribution of dinocysts. In fact it was the availability of nutrients that has been put forward as the main controlling factor on the dinocyst assemblage. In our study it was not possible to extract more environmental data (nitrogen, total organic carbon, chemical oxygen demand, summer and winter chlorophyll *a*). With those extra and accurate (i.e. correctly measured) variables, significant relations could possibly be found. In addition many species were placed in higher taxa, obscuring the signal.

The DCA reveals a clustering comparable to the PCA. Here, different areas are better resolved. The Potomac River sites cluster together with winter temperature and chlorophyll *a*. Cyst of *Alexandrium* spp. is again the closest species for these sites due to its the large abundance. *Operculodinium centrocarpum* is the most abundant in the Atlantic Ocean sites due to its cosmopolitan character and proportional increase at the neritic-oceanic boundaries (Wall et al., 1977). The other sites can be regarded as intermediate between the two extreme groups and function as an ecological transition from north (Potomac) to south and Eastern Shore Side. Annual and summer temperature and phosphate seem to have no distinct effect on the assemblages.

7. CONCLUSIONS

The study of the dinocysts from Chesapeake Bay provides information about the ecology and the environment. In the first place, the dinocyst taxonomy of the Bay is outlined from modern surface sediments, and will serve as a basis for further dinocyst studies in Chesapeake Bay. Secondly, this dissertation demonstrated that the spatial distribution of dinocysts reflects the polluted environment. Species richness, absolute abundances, fluctuations in abundances and relative abundances were related to environmental gradients and can therefore be used as an indicator for environmental changes (e.g. pollution and eutrophication) or global ecology (e.g. salinity and temperature).

The assemblages are dominated by *Spiniferites* spp, cysts of *Alexandrium/Scrippsiella* spp, *Operculodinium centrocarpum* sensu Wall and Dale 1966, Round Brown Cysts and *Polysphaeridium zoharyi*. Site 71-4 in the southern part of the Bay, was characterized by the highest dinocyst concentrations (135,000 cysts g^{-1}). The sites located by the Potomac River had the lowest dinocyst assemblages, probably diluted by terrigenous input. The largest taxa richness occurred in all the southern sites (17-27 taxa richness) while in the Potomac River only 6 to 12 species were determined.

Our study confirms that the heterotrophic/autotrophic ratio (H/A-ratio) is most indicative for environmental conditions. The H/A-ratio generally was high, compared to studies from Pospelova et al. (2002, 2004, 2005, 2010), in the Chesapeake Bay area. We assume that this is a signal for eutrophication. The southern sites all were characterized by high H/A-ratios. Especially site 63-3 in the James River, with 86,9% heterotrophic species in the assemblage. This site is known as one of the most polluted sites in Chesapeake Bay due to its point sources of wastewaters and high phosphorus levels. Sites in the Potomac River on the other hand were characterized by low H/A-ratio's, indicating high abundances of autotrophic dinoflagellates. This signal, together with the signals of low dinocyst concentration and low taxa richness was interpreted as a hypertrophic status.

Cyst of *Alexandrium/Scrippsiella* spp. was the most abundant taxon in Chesapeake Bay. This is the result of the harmful algal blooms that return every summer in Chesapeake Bay. The highest abundances were recorded in the neighbourhood of site 47-4, in the York River. This is consistent with the findings by Reece et al. (2014) and Pease et al. (2015a,b). To restore the area, we then suggest sampling around site 47-4. It has to be stressed that there were some difficulties with the identification of *Alexandrium* spp. compared with other round hyaline cysts. Consequently, also *Scrippsiella* spp. was included in the group, potentially overestimating the impact of *Alexandrium* spp.

Additionally, *Alexandrium* spp. or *Scrippsiella* spp. were not the only blooms in recent years around or in the Bay. The potential cyst of *Prorocentrum minutum* was also recorded in the cyst assemblage, but in lower abundances. Cysts of *Cochlodinium polykrikoides* and *Heterocapsa triquetra* were not found in the cyst assemblage, although the motile stage is abundant in the plankton and extensively described literature. The absence of these cyst species can have multiple, perhaps not mutually exclusive, explanations. This can be due to preservation or a different morphology of the

cysts in the Bay, and hampering comparison with the existing literature. Another possibility is that there are no cysts formed under certain specific conditions. This problem is not yet solved.

The multivariate analysis was of limited applicability due to the poor characterization of environmental variables from the World Ocean Atlas (2013). It was possible to identify different spatial clusters of sites from Chesapeake Bay using a PCA. It was much more difficult to discern controlling environmental parameters with RDA or DCA. These suggest that salinity and temperature are the most important factors influencing the assemblage.

This dissertation provides recent results that can be used for understanding the ecology of dinocysts in estuaries. There are not yet universally accepted variables to identify the relationship between dinocysts and eutrophication, but the heterotrophic/autotrophic ratio is here proposed as the most indicative proxy for eutrophication. However, ecology of dinocysts in estuarine systems remains complex and needs further research, which will be explained in the outlook.

8. OUTLOOK

As the primarily interest of the Virginia Health Department is to localise the seed beds in order to dredge them and restore the Bay, it is appropriate to take some more samples in the neighbourhood of the sites where we found the highest numbers of Cysts of *Alexandrium* spp. The cooperation between biologists (non-destructive method and species-specific primers) and palaeontologists (palynological method) would be very helpful to localise the seed beds more precisely.

Furthermore, several cysts of bloom forming dinoflagellates are not yet known. To resolve this, cyst incubation experiments need to be conducted to infer cyst-motile stage relations. Here it would clarify the problems with *Cochlodinium polykrikoides* and *Heterocapsa triquetra*: whether, and, if, under which conditions, they are cyst-producing and/or have a variable morphology in Chesapeake Bay relative to other sites where they were originally described. Thus, we suggest to apply germination experiments in order to establish the cyst-theca relationship in Chesapeake Bay.

To understand better the dinocyst ecology in Chesapeake Bay, it would be important to study more surface samples. As all the sites in this study are at the mouth of rivers or inlets in Chesapeake Bay, we suggest sampling the central part of the Bay as well and more river transects (for example: from source to mouth of the James River, on regular distances).

To assess the spatial environmental variations through Chesapeake Bay, nutrient concentrations must be better scrutinized. As the assemblage represents multiple years, a time average of nutrient concentrations should be established. This is not possible if there are not enough measurements every year or season. A common (but expensive) added value is to carry out sediment trap studies (e.g. Pospelova et al., 2010; Price and Pospelova, 2011) where the ecological and environmental conditions under which dinocysts are produced during a specific time period are recorded.

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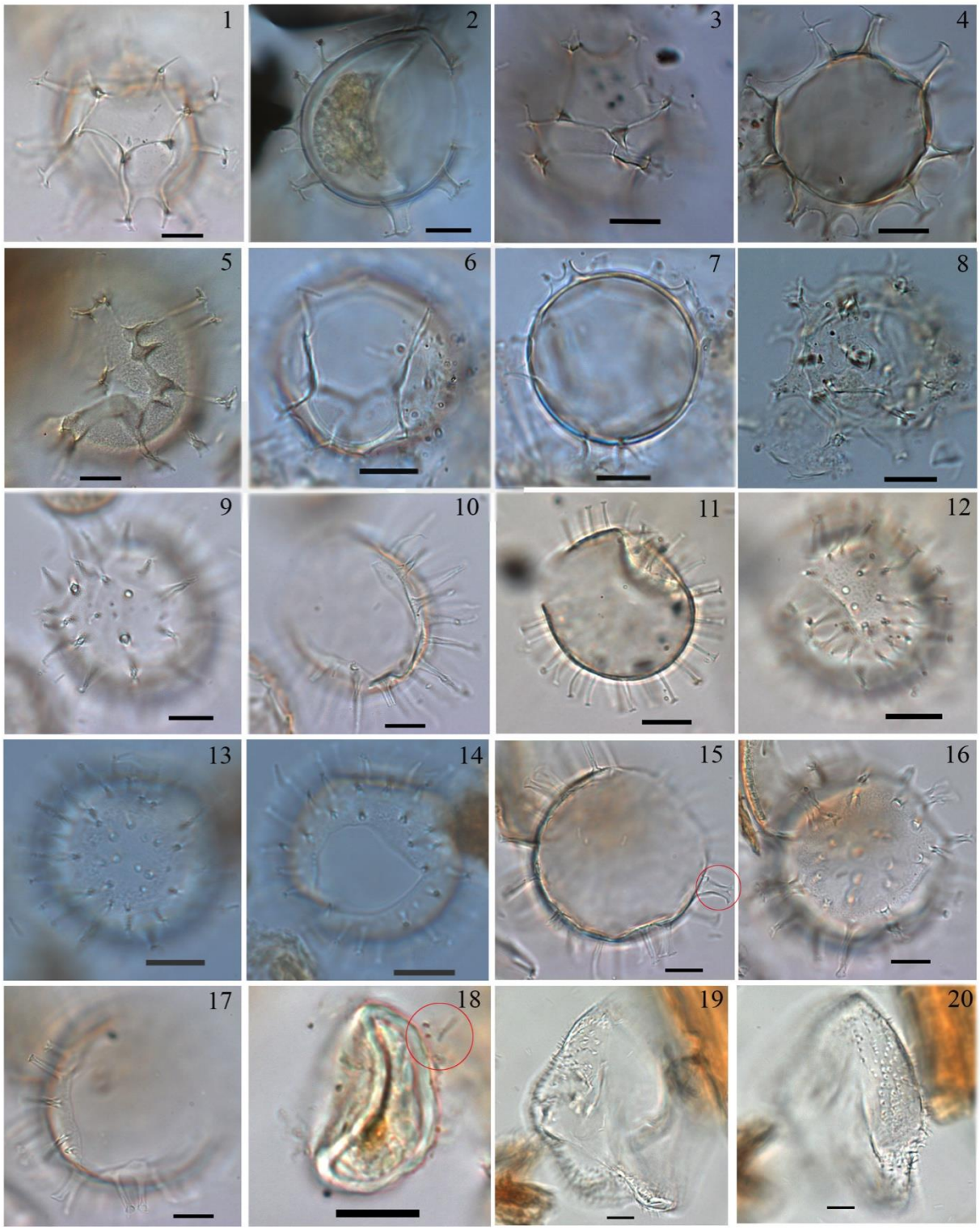

Plate I. Bright-field photomicrographs. **1.** Single specimen of *Spiniferites ramosus* with smooth wall, 92-5B, optical section **2.** *Spiniferites bentorii* with distinct pear shape, 9-2B, optical section. **3-4.** *Spiniferites mirabilis* with antapical flange, 53-18A, upper focus and optical section. **5.** *Spiniferites delicatus*, 63-3A, optical section. **6-7.** Single specimen of *Spiniferites* Type 1 with distinct reduced processes, 53-18B, upper focus and optical section. **8.** *Achomosphaera* spp., without crests 53-18A, optical section. **9-10.** Single specimen of *Lingulodinium machaerophorum* showing long spines with striations at the base and large archeopyle composed of several precingular plates, 63-3A, upper focus and optical section. **11-14.** Two specimen of *Operculodinium centrocarpum* sensu Wall and Dale (1966), with distinct capitate processes and fibruous outer wall, 71-4A, different focus, archeopyle visible (no. 14). **15-17.** Single specimen of *Polysphaeridium zoharyi*, with distinct capitate, broad processes, which can be fused (red circle), 98-2B, optical sections. **18.** Cyst of *Scrippsiella* spp., with processes (red circle) 4-10A, orientation uncertain. **19-20.** *Trinovantedinium applanatum*, transparant, peridinoid in shape and beset with made short processes, 98-2A, optical sections. Scale bars: 10 µm.

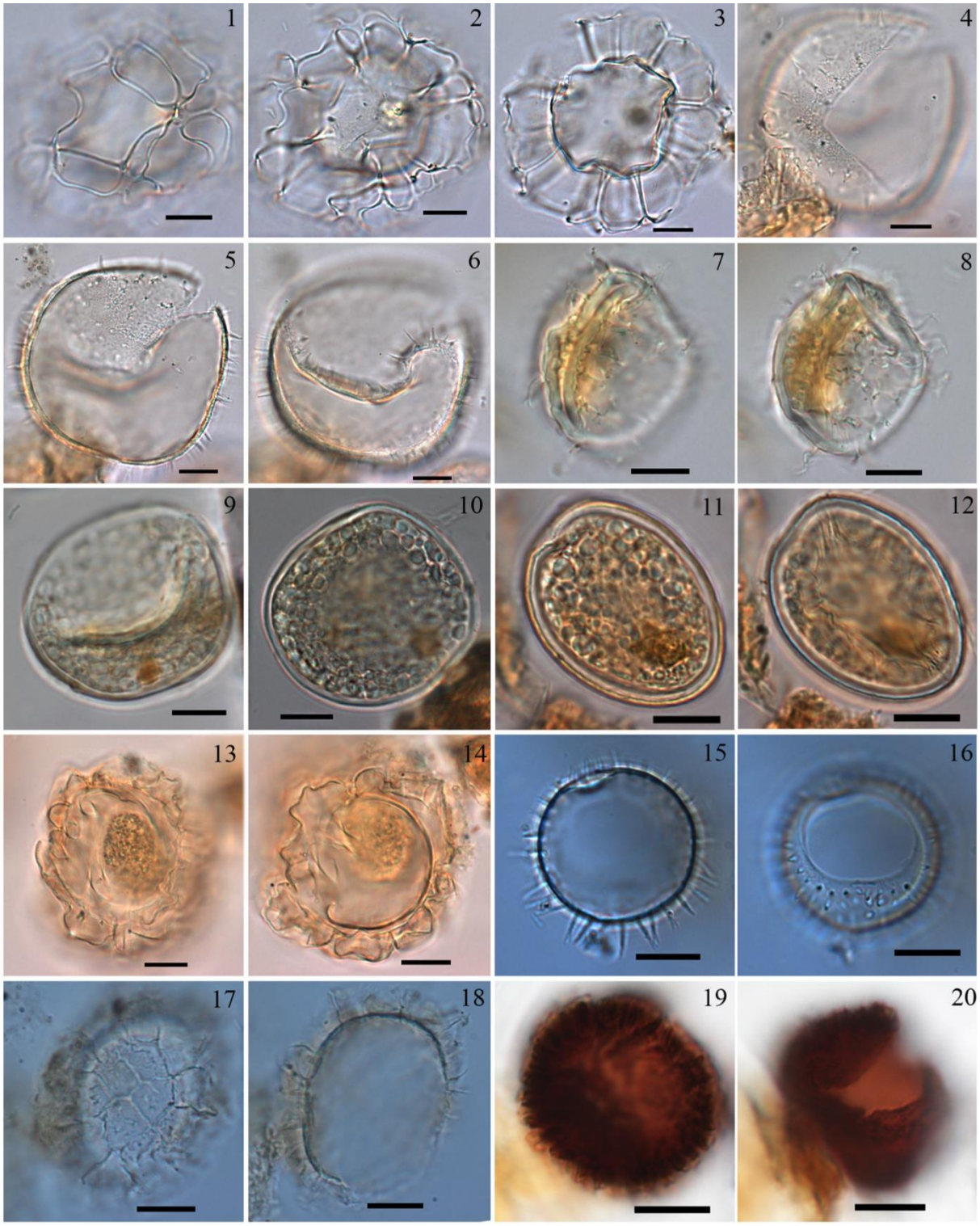

Plate II. Bright-field photomicrographs. **1-3.** Single specimen of *Nematosphaeropsis labyrinthus* with distinct pairs of trabeculae connecting distal ends of the processes, 63-3A, high to mid-focus. **4-6.** Single specimen of *Operculodinium israelianum* with many short capitate processes with striations at the base and an archeopyle corresponding to 3", 53-18B. **7-8.** Cyst of *Pentapharsodinium dalei* with cell contents present and distinct processes (branched or unbranched) on one specimen, optical sections. 4-10A. **9-10.** Two specimens of Cyst of *Alexandrium* spp. with cell contents and red body, 4-10A. **11-12.** Possible Cyst of *Prorocentrum minimum* with cell contents and distinct prorocentroid shape, 4-10A. **13-14.** Single specimen of *Ataxodinium choane* with distinctly undulating outer wall, 6-4A, optical sections. **15-16.** Single specimen Type indet 3, with fine tips and distinct archeopyle (cf. *Operculodinium longispinigerum),* 98-2A, optical sections. **17-18.** Single specimen of Type indet. 1, 71-4A, distinct crests and granulate surface. **19-20.** Single specimen of Type indet. 2, with distinct hairy processes, 97-18C. Scale bars: 10 µm.

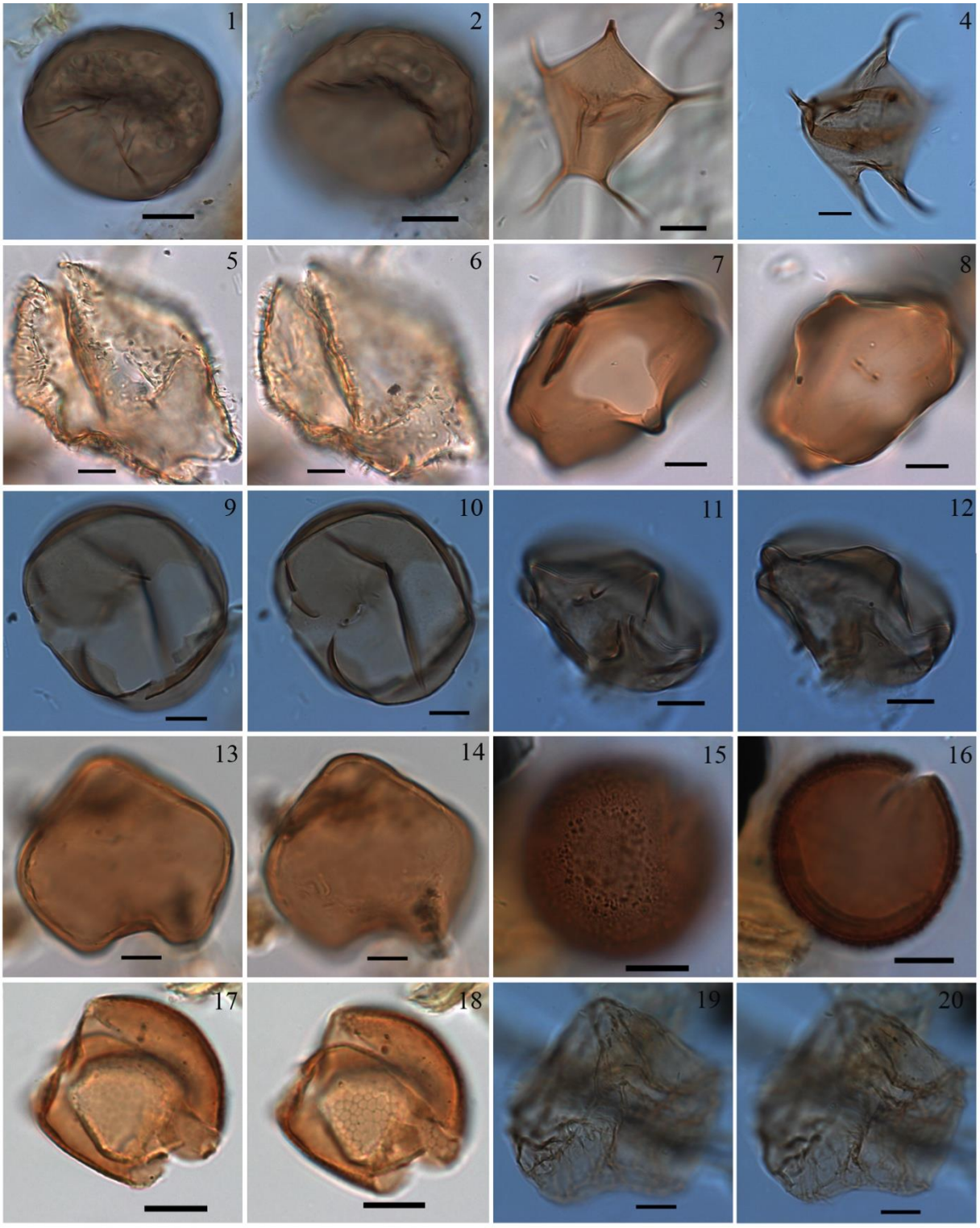

Plate III. Bright-field photomicrographs. **1-2.** Single specimen of Round Brown Cyst with cell contents, 9-2A. optical sections. **3-4.** Two specimen of Cyst of *Protoperidinium stellatum* with distinct peridinioid shape and long solid horns, 98-2A and 98-2B, optical sections. **5-6.** Possible *Votadinium spinosum*, 92-5B, upper focus. **7-8.** One specimen of *Quinquecuspis concreta* with dorsal intercalary archeopyle, 92-5B, 92-5A. **9-12.** Two specimen of *Selenopemphix nephroides* with dorsal archeopyle and flagellar scars, 92-5B, multiple optical sections. **13-14.** Single specimen of *Votadinium calvum* with large operculum still in place, 98-2A. **15-16.** Single specimen of *Dubridinium spp.*, 82-3A. **17-18.** Single specimen of microreticulate cyst of *Gymnodinium* nolleri/microreticulatem, 98-2A. **19-20.** Single specimen of *Lejeunecysta paratenella* sensu Harland 1977 with typical wall ornamentation, 98-2B. Scale bars: 10 µm.

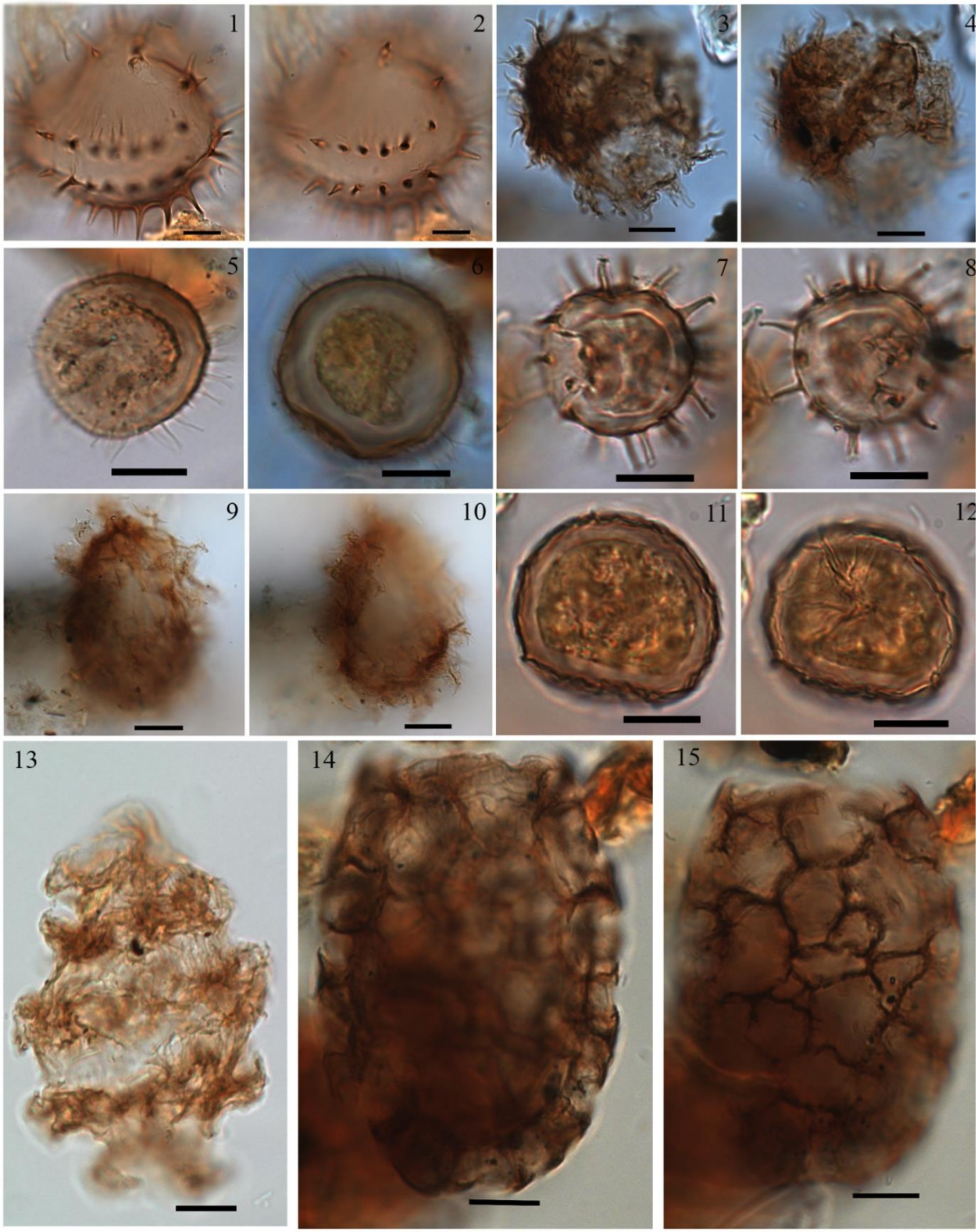

Plate IV. Bright-field photomicrographs. **1-2.** Single specimen of *Selenopemphix quanta*, 58-8B. **3-4.** Single specimen of *Xandarodinium xanthum*, 63-3A. **5-6.** Two specimen of Cyst of *Archaeperidinium minutum* cf. with small size and distinct fine processes, 98-2B and 9-2A (with cell content). **7-8.** Single specimen of *Echinidium aculeatum*, 63-3A. **9-10.** Single specimen of *Protoperidinium minutum* sensu Wall and Dale (1968) with distinct processes, 47-4A. **11-12.** Single specimen of RBC Type A with distinct folding, the external membrane and cell content , 63-3A. **13.** Cyst of *Polykrikos schwartzii* sensu Matsuoka et al., (2009) with rows of processes, 98-2B. **14-15.** Single specimen of Cyst of *Polykrikos kofoidii* sensu Matsuoka et al., (2009) with distinct connected processes, 71-4A. Scale bars: 10 µm.

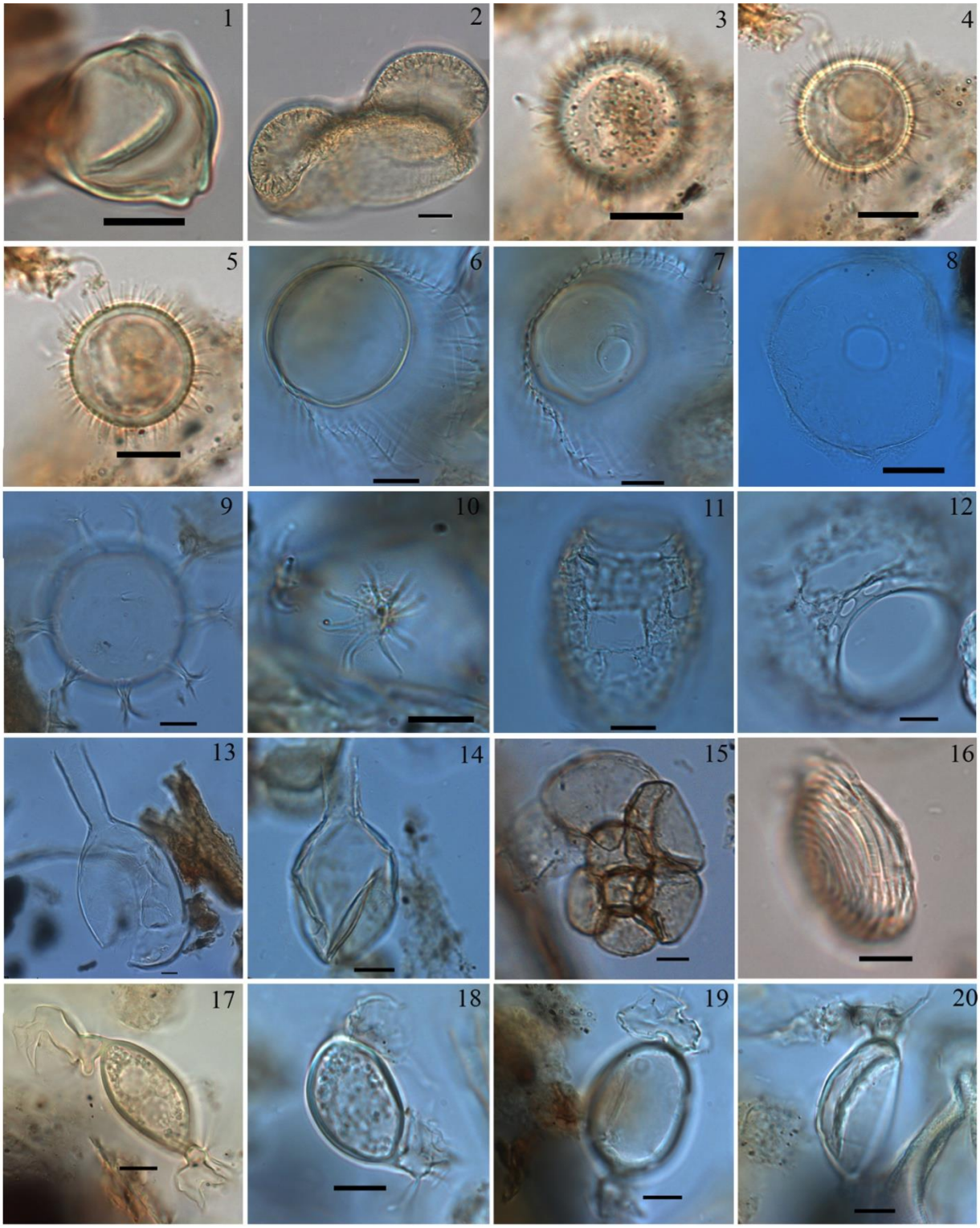

Plate V. Bright-field photomicrographs of other palynomorphs. **1.** Non-bissacate pollen of *Corylus*, 98-2A. **2.** Bisaccate pollen, 98-2A. **3-5.** Single specimen of Cyst of *Biecheleria* spp. with solid processes and capitate distal ends, 71-4A, optical sections. **6-7.** *Radiosperma corbiferum*, 82-3A. **8.** *Halodinium minor*, 98-2A. **9-10.** Single specimen of *Polyasterias problematica*, 8-14A and 80-8B. **11-12.** Tintinnid lorica type B sensu Price and Pospelova (2011), 98-2A and 92-5A. **13-14.** Cyst Type P (Reid and John, 1978), 71-4A and 80-8A. **15.** Trochospiral microforaminiferal organic lining, 5-16. **16.** *Pseudochizaea* sp., 8-14B. **17-20.** Type indet 3 with distinct processes on both sides of the ovoid body, 12-6A and 82-3A. Scale bars: 10 µm.

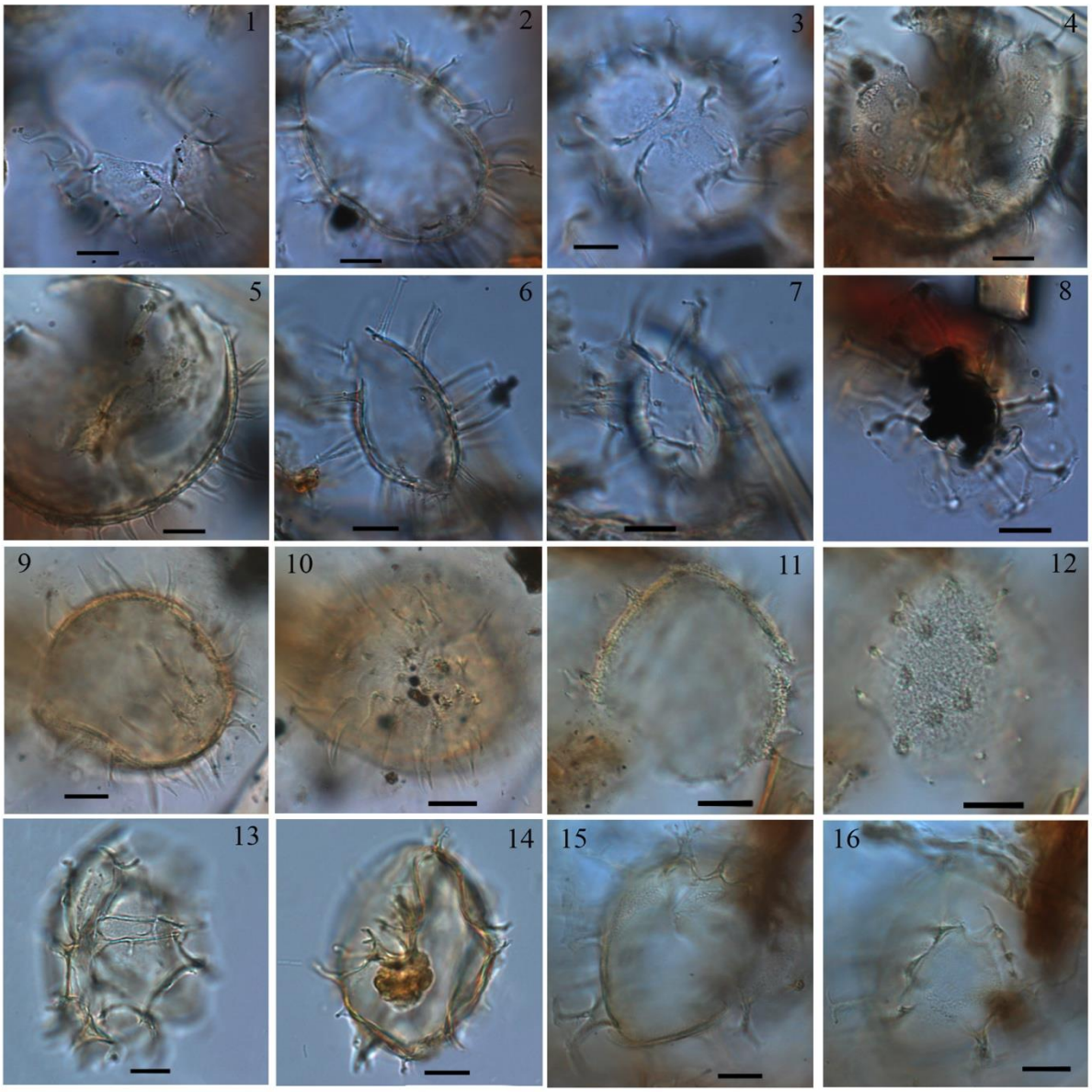

Plate VI. Bright field photomicrographs of reworked specimens. **1-3.** Single specimen of *Cleistosphaeridium placantha*, with distinct round crests on surface, 4-10A, optical sections and upper focus. **4-5.** Single specimen of *Lingulodinium machaerophorum*, 4-10A, optical sections. **6-7.** Single specimen of *Dapsilidinium pseudocollegerum* with distinct large bifurcate processes, 4-10A, optical section and upper focus. **8.** *Reticulatosphaera actinocoronata*, with distinct large processes and small spherical central body, 4-10A, upper focus. **9-10.** Single specimen of *Operculodinium centrocarpum*, 7-21A. **11-12.** Single specimen of *Operculodinium israelianum*, 47-4A. **13-14.** Single specimen of *Spiniferites* spp., 97-18C. **15-16.** Single specimen of *Spiniferites perforatus* with distinct bubbles in processes, 97-18D, different focuses. Scale bars: 10 μm.

Appendix

Appendix A: Taxonomy

Appendix B: Counts

Appendix C: Relative Abundances

Appendix D: Absolute Abundances

Division DINOFLAGELLATA (Bütschli 1885) Fensome et al. 1993a
Class DINOPHYCEAE Pascher 1914
Subclass GYMNODINIPHYCIDAE Fensome et al. 1993a
Order GYMNODINIALES Apstein 1909 Suborder GYMNODINIIINEAE (Autonym)
Family POLYKRIKACEAE Kofoid & Swezy 1921
Genus *Polykrikos* Bütschli 1873

Cyst of *Polykrikos kofoidii* Chatton 1914 sensu Matsuoka et al., 2009

Observations. The cyst is elongated and pale to dark brown in color. The outer wall layer formed the reticulate ornaments. These ornaments formed different rows on the surface, which are connected. The reticulations become smaller in the middle than on the polar sides. The diameter and shape of the reticulations was variable. The cyst length ranges from minimum 55 µm (average: 65 µm) to maximum 77 µm (n=5).

Cyst of *Polykrikos schwartzii* Bütschli 1873 sensu Matsuoka et al., 2009

Observations. The cyst was proximochorate and elongated in shape, with medium to dark brown color. The cyst is characterized by five rows of separated processes. The processes are hollow and cylindrical to infundibular and are separated. The archeopyle is described by Matsuoka et al. (2009) as apical, but was not observed in this study. The cyst length is 55 (60) 65 µm (n=2). Process length varies between 7 and 10 µm.

Family GYMNODINIACEAE (Bergh 1881a) Lankester 1885

Genus *Gymnodinium* Stein, 1878

Cyst of *Gymnodinium nolleri/microreticulatum* Ellegaard and Moestrup, 1999

Observations. Spherical cysts, sometimes dorso-ventrally compressed, resulting in a heart-shaped cyst. The cysts are reddish brown in color. Cyst surface is characterized by many four- to seven-sided polygons in small dimensions (~0.5 – 1.5 µm). Around the sulcus, finer polygons form rows, which are regular in size and shape. We have not seen the number of paravesicle rows in the paracingulum (Ribeiro et al., 2012), thus is not possible to identify to species-level *nolleri* or *microreticulatum.* Measured cysts (n=5) were 30 (34) 40 µm in length.

Subclass PERIDINIPHYCIDAE Fensome et al. 1993a
Order GONYAULACALES Taylor 1980
Suborder GONYAULACINEAE (Autonym)
Family GONYAULACACEAE Lindemann 1928
Subfamily CRIBROPERIDINIOIDEAE Fensome et al. 1993a

Genus *Lingulodinium* Wall 1967 emend. Dodge 1989

Lingulodinium machaerophorum (Deflandre & Cookson 1955) Wall 1967

Observations. Spherical central body with heavily granulate wall surface. The cyst is often broken. The processes are hollow and closed distally. They are known by the distinct circular base and terminate as a pointed tip. Processes have a smooth surface, but also small spinules towards the top of the process. There is also much variability in process length and morphology. Archeopyle was never observed and is considered not important for identification. Cyst diameter is 36.4 (40) 44 μm (n=7) in measured specimens.

Genus *Operculodinium* Wall 1967 emend. Matsuoka et al. 1997

Operculodinium centrocarpum sensu Wall & Dale 1966

Observations. The cyst is spherical and has numerous slender processes, which are usually intratabular in position. The cyst wall is fibrillar with very fine and faintly visible fibrils. The processes are hollow with conical bases. The surface of the processes is smooth and the processes may broaden a bit at their base. The tips are capitate. Cyst diameter ranges from 31 (36) 43 μm (n=10). Process length varies between 6 and 12 μm.

Operculodinium israelianum Rossignol 1962 emend. Wall 1967

Observations. Large chorate, subspherical to ovoid cysts. No tabulation visible, but the surface is very fibrilar to spongeous. The archeopyle is hexagonal, precingular (type P). The processes are as in *O. centrocarpum* sensu Wall and Dale 1968, but shorter. The processes have a large base and are concave; quickly narrowing with a capitate ending. Wall 1967 documented much smaller processes (3 to 6 μm) than Rossignol (6 to 10 μm). Cyst diameter varies from 45 (48) 51 μm (n=3), while most processes have a length of about 5 μm.

Subfamily GONYAULACOIDEAE

Genus *Ataxiodinium* Reid 1974

Ataxiodinium choane Reid 1974

Observations. The central body is ovoid to egg shaped. The outer layer forms a large crenulate velum around the central body. It is irregular and characterized by funnel-shaped depressions. Archeopyle is formed by the loss of a single precingular plate, but is not observed in this study. Other tabulation is not present. Based on one single specimen, the cyst body length was 29 μm.

Genus *Nematosphaeropsis* Deflandre & Cookson 1955 emend. Wrenn 1988

Nematosphaeropsis labyrinthus (Ostenfeld 1903) Reid 1974

Observations. The cyst has an ovoid central body, sometimes with a slight apical protuberance. The surface of the body is smooth. Processes are exclusively gonal and have hollow, slender shafts with distal trifurcations. These distal ends are connected by parallel pairs of fine trabeculae, reflecting the plate sutures. The distal ends of multiple processes are however unbranched. Central body length is 40 (42) 45 μm (n=4) with a process height of 12 to 18 μm.

Genus *Spiniferites* Mantell 1860

Spiniferites bentorii (Rossignol 1964) Wall & Dale 1970

Observations. The central body is spherical to ovoid, pear shaped. The main characteristic is the pronounced apical boss. The processes are gonal and occasionally intergonal. The bases of the processes could be fenestrate. The processes have a variable morphology, sometimes slender but usually short and stumpy. The wall is marked with low crests/ridges. The cysts are between 30 (30.5) 34 μm (n=4) in length.

Spiniferites mirabilis (Rossignol 1964) Sarjeant 1970

Observations. As most of the spiniferate cysts, S. *mirablis* has a broadly ovoid to spherical central body. The surface of the wall is little microgranulate. Processes are both gonal and intergonal and intergonal processes are distally furcate with recurved bifurcate tips. Sutural crests are very low except at the antapex where they form a broad and conspicuous flange between the antapical processes. The length without processes is 38 (42) 45 μm (n=10). The antapical flange 10 to 16 μm.

Spiniferites ramosus (Ehrenberg 1838) Mantell 1854 sensu Rochon et al. 1999

Observations. This is the most morphologically simple *Spiniferites*; ovoid to spherical central body without apical boss. Smooth wall surface. Exclusively gonal processes; they are long, hollow, and have long distal furcations with trifurcate tips. Sutural crests are low. Length of the cyst amounts to 33 μm and the processes are around 10 μm in length (n=2).

Spiniferites delicatus Reid 1974

Observations. Ovoid to spherical central body with microgranular to microreticulate surface. The processes are membranous and connect the gonal processes and resemble skeletal rods. The sutural crests are relatively high. The processes and septa are also known by faintly granular surfaces. The archeopyle is precingular (type P), loss of plate 3''. Central body length varies from 45 (48.5) 52 μm (n=2). Process length 14 to 18 μm.

Subfamily PYRODINIOIDEAE Fensome et al. 1993b.

Genus *Polysphaeridium* Davey and Williams, 1966, emend. Bujak et al., 1980

Polysphaeridium zoharyi (Rossignol 1962) Bujak et al. 1980

Observations. The cyst is round with fibrous cyst wall. Processes are numerous, hollow and distally open. The base of the processes could be weakly striated. Some processes are fused at their bases and the others are distally bifurcate. Archeopyle is epicystal and often splits the cyst into two parts. Cyst diameter ranges from 45 (47) 50 μm (n=10), with an average process length of 9 to 12 μm.

Suborder GONIODOMINEAE Fensome et al. 1993a
Family GONIODOMACEAE Lindemann 1928
Subfamily HELGOLANDINIOIDEAE Fensome et al. 1993a

Genus *Alexandrium* Halim 1960 emend. Balech 1990

cf. *Alexandrium tamarense* (Lebour 1925) Balech 1985-type cysts
Cyst of *Alexandrium* spp.

Observations. Cysts have an elongate cylindrical central body with rounded ends. The outer wall surface is smooth, but due to the membranous, the outer surface is often wrinkled. The outer wall is loosely-attached (i.e. a membrane) to the central body. The majority of these cyst in the studied assemblages still contained cell contents, such as lipied bodies and red bodies surrounded by an endospore. All cyst diameters of *Alexandrium* spp., are around 40 μm (n=10).

Order PERIDINIALES Haeckel 1894
Suborder PERIDINIINEAE (Autonym)
Family PERIDINIACEAE Ehrenberg 1831

Genus Peridinium Ehrenberg 1831

Cyst of *Peridinium minutum* Kofoid 1907 sensu Wall and Dale 1968
Observations. The cysts are brown and spherical (ovoid in our views). The cyst is characterized by short hollow processes with circular bases and flat-topped distal extremities. These extremities have moistly three pointed spines at the top. The archeopyle is not visible. Processes 7 to 9 μm with a cyst length of 35 (36) 37 μm (n=2).

Subfamily CALCIODINELLOIDEAE Fensome et al. 1993a
Genus *Pentapharsodinium* Indelicato & Loeblich III 1986 emend. Montresor et al. 1993

Cyst of *Pentapharsodinium dalei* Indelicato & Loeblich III 1986

Observations. Cysts of *P. dalei* are small, colorless with spherical central body. The processes are solid and may be branched or unbranched. Both types are usually present on a single specimen. Processes may branch at any point along their length. The branches may be very broad proximally. The outer wall surface is smooth. Cell content was also observed. Cyst length varies between 21 (24) and 32 μm (n=5). Processes are very short, between 4 and 6 μm.

Family PROTOPERIDINIACEAE Fensome et al., 1993
Subfamily PROTOPERIDINIOIDEAE Balech, 1988

Genus *Protoperidinium* Bergh 1881

Cyst of *Protoperidinium nudum* (Meunier 1919) Balech 1974?

Observations. The cysts are perinoid, spiny and brown in color. They are sometimes grouped with *Selenopemphix quanta*. The cysts differentiate from *S. quanta* in weakly polar compression. Also the spines are aligned along the whole cysts surface, where the spines in *S. quanta* are only aligned along cingular margins. Cyst width: 45 (50) 55 μm (n=2) with spines of 8 to 10 μm.

Genus *Quinquecuspis* Harland 1977

Quinquecuspis concreta (Reid 1977) Harland 1977

Observations. The cysts are pentagonal, dorso-ventrally compressed with distinct developed apical and antapical horns. The antapical horns, separated by an antapical depression, are rounded and could be irregular thickened at their top. The equatorial girdle, which is wide, excavated, characterizes cyst and is marked by a semicontinuous ridge as thickening of the wall. The surface is smooth or roughened and may show faint longitudinal lineations and low folds. Archeopyle intercalary formed by loss of plate 2a. Cyst width around 55 μm (n=2).

Genus *Selenopemphix* Benedek 1972 emend. Head 1993

Selenopemphix nephroides Benedek 1972 emend. Bujak in Bujak et al. 1980

Observations. The cysts are light brown in color and the wall is smooth. They are always polar compressed with an ovoidal to reniform outline in polar view. The apical and antapical horns are very low and rounded. The cingulum is strongly indented, resulting in flange-like margins in polar view. Archeopyle hexa-intercalary and formed by loss of the 2a plate. Measured cyst width is 54 μm, based on single specimen.

Selenopemphix quanta (Bradford 1975) Matsuoka 1985

Observations. Polar compressed cysts, circular to slightly reniform in polar view. The wall is light to medium brown and smooth. Processes are hollow at the base, otherwise solid. The tips are sharp or sometimes blunt. Processes occur along the cingular margins and with variable density in rows on epi- and hypocyst, but do not occur in the sulcus. The archeopyle reflects the 2a plate but is not been observed. Cyst width: 48 (53) 60 μm (n=5), with spines of 5 to 10 μm.

Genus *Lejeunecysta* Artzner and Dörhöfer, 1978

Lejeunecysta paratenella (Benedek 1972) Artzner and Dörhöfer, 1978

Observations. The cysts are light brown to almost colorless. The shape is pentagonal and the width is larger than the length. The cyst wall fibreous but the cingulum is defined by low ridges. The antapical horns are visible as well apical granules, a thickened structure. Archeopyle was not observed. Measured on a single specimen, cyst length of 49 μm and width of 53 μm.

Genus *Stelladinium* Bradford 1975

Cyst of *Protoperidinium stellatum* (Wall in Wall & Dale 1968) Reid 1977

Observations. Discussion of cyst name can be found in Rochon et al., (1999). The cyst is pentagonal and often (in my case always) dorso-ventrally compressed. The main characteristics were five distinct horns that are positioned at the extremities of the cyst body. The epicyst is larger than the hypocyst. Archeopyle is large and corresponds to the 2a plate. Cyst length between 29 (31) 34 μm (n=3) with horns of 9 to 19 μm.

Genus *Trinovantedinium* Reid 1977 emend. de Verteuil & Norris 1992
Trinovantedinium applanatum (Bradford 1977) Bujak & Davies 1983

Observations. The cysts are pentagonal in shape and transparent (the only living congruentidiacean cyst type that is transparent) with an apical horn and two antapical lobes/horns. The cyst is dorso-ventrally compressed. The apical horn may have a short solid boss. Processes are very short, solid and have very fine tips. Processes occur over the whole cyst and also have an intratabular distribution. At the cingular margins they are aligned. Around the sulcus, there exist a large unornamented area. The archeopyle corresponds to the 2a plate One single specimen was 85 μm in length with processes of 4 μm.

Genus *Votadinium* Reid 1977

Votadinium calvum Reid 1977

Observations. The light to dark brown cyst is dorso-ventrally compressed, with a 'inverse heart shaped' (peridinioid) outline. The cyst is characterized by one apical horn and two antapical horns, separated by a shallow depression. The archeopyle extends over the apex, and corresponds to the 2a intercalary plate. Cyst has a smooth surface and the antapical horns may be equal in length. Cyst length varied from 30 to 45 μm (35 μm average, n=5).

Votadinium spinosum Reid 1977

Observations. The shape of the cyst is a inversely heart-shaped with two antapical horns that are rounded and separated by a shallow depression in dorsal view. The cyst is often dorso-ventrally compressed to the half of the width of the cyst body. The wall is smooth and thin and ornamented. Short little sold spines are distributed on the cyst wall and could be curved. Archeopyle is not observed. Cyst length of 65 μm based on one single specimen with small processes of maximum 4 μm.

Genus *Xandarodinium* Reid 1977

Xandarodinium xanthum Reid 1977

Observations. The cysts are light brown in color and ovoidal in shape. Processes are tabular in distribution and form extensions from the wall. The processes are hollow and develop complex flanges of multiple spines (i.e. multifurcate extensions). Some areas in ventral and anterior view don't have ornamentation. Archeopyle formed by loss of a single, apparently intercalary plate. Processes 7 to 9 μm with a cyst length of 35 – 37 μm (n = 2).

Genus *Dubridinium* Reid, 1977

Dubridinium spp. Reid 1977

Observations. The cysts are light to dark brown and lenticular in shape. There is often a slight dorso-ventral compression. The wall is thick and microgranulate. You have to see the cingular lists reflected on the cyst, but it is often not observed. Cyst diameter: 30 (32) 34 μm (n=3).

Genus *Archaeperidinium* Jörgensen 1912 emend. A. Yamaguchi, Hoppenrath, Pospelova, T. Horiguchi & B.S. Leander 2011

Archaeperidinium minutum cf Mertens, Yamaguchi, Kawami et Matsuoka sp. nov., 2012

Observations. The cysts of *A. minutum* are small in diameter, spherical and dark brown. Numerous hollow processes are covering the smooth wall. The processes had capitate distal ends, are hollow and distally closed. The processes have circular bases, which is seen in upper focal. Processes are straight, but can be curved sometimes. Archeopyle is not observed, but is described by Mertens et al. (2012) as theropylic. Range in this assemblage for diameter is between 20 to 28 μm. Processes length between 3 and 5 μm.

Subfamily Protoperidinioideae Balech, 1988
(or Subfamily Diplopsalioideae Abé, 1981)

Genus *Echinidinium* Zonneveld, 1997 ex Head et al.

"Echinidinium aculeatum" Zonneveld 1997 [invalid name]

Observations. The cyst is spherical tin shape, brown in color and processes are randomly distributed. The processes are hollow and smooth and are tapering towards their distal ends, with at least 2 open aculeate distal tips. The process bases are spherical. The archeopyle splits along a single suture. No tabulation is observed. Cyst diameter is 21 μm, based on measurement on one specimen and processes around 5 μm.

Sample site	Dry Weight (g)	L. Clavatum counted	No. Cysts/gram	Species Richness	Nos. Of counted cysts	*Achomosphaera* spp.	Cyst of *Alexandrium/Scrippsiella* spp.	Cyst of *Archaeperidinium minutum* cf	*Ataxodinium choane*	*Dubridinium* spp.	*Echinidinium aculeatum*	Cyst of *Gymnodinium nolleri/microreticulatum*	*Lejeunecysta paratenella*	*Lingulodinium machaerophorum*	*Nematosphaeropsis labyrinthus*	*Operculodinium centrocarpum* sensu Wall & Dale 1966	*Operculodinium israelianum*	*Operculodinium* cf *israelinianum*	Cyst of *Pentapharsodinium dalei*	Cyst of *Protoperidinium nudum*	*Polysphaeridium zoharyi*	Cyst of *Polykrikos schwartzii* sensu Matsuoka 2009
2A-18	20,71	734	184	12	289		205	6		1		1		2					1		1	
4-10	17,36	184	1035	12	342		269							5	1	13			3			
5-16	24,07	972	125	7	302		276	2														
6-4	24,6	1749	5	9	23		2		1							2					1	
7A-21	22,4	1701	37	8	146		90									2						
8-14	25,94	981	117	12	309		197	3		2									4		1	
8-32	11,51	1044	42	6	52		11	1								1						
9A-2	21,69	747	186	10	311		192	10											1			
10-6	27,21	808	49	13	112		38	2						2		2			4		4	
12-6	15,19	567	370	16	330		135	10						2	2	1			3		37	
28-11	14,33	1916	81	14	229		123	8		1		2				2			1		2	
47-4	2,05	91	17721	23	342	2	97					1		13	2	11			2		14	
53-18	0,68	121	38180	17	325	2	8	7						22	3	11	5		1		50	
58-8	2,84	107	9733	17	306		10	4		1						1			1	2	3	1
63-3	2,75	59	18766	19	315		8	8			1			6	2	6			2	3	4	
71-4	0,98	23	13594	25	317	4		1			1			8	7	47	2	1	3		13	1
80-8	1,41	19	3	21	301		12	18		1				14	2	3			1		20	1
82-3	11,51	495	568	16	335		60	43		1	1			5	1				10		8	
92-5	8,27	171	2098	27	307	1	4	1			1			6	4	33	1	1	1		9	1
97-18	8,28	356	846	17	258		23	6		2				5	4	59			8		6	
98-2	10,2	197	1535	26	319	3	8	17				1	1	8	7	39	1		6		10	3

Appendix B. Counts of dinocysts and other palynomorphs of Chesapeake Bay samples (part 1).

Sample site	Cyst of *Polykrikos kofoidii* sensu Matsuoka 2009	*Polykrikos* group ind.	Cyst of *Protoperidinium minutum* sensu Wall *Quinquecuspis concreta*	Round Brown Cyst	RBC Type A	*Selenopemphix nephroides*	*Selenopemphix quanta*	*Spiniferites* spp.	*Spiniferites mirabilis*	*Spiniferites bentorii*	*Spiniferites delicatus*	*Spiniferites ramosus*	*Spiniferites* Group Type 1	Spiny Brown cyst	Cyst of *Protoperidinium stellatum*	*Trivantedinium applanatum*	*Votadinium calvum*	*Votadinium spinosum*	*Xandarodinium xanthum*
2A-18				41	24			5	1					1					
4-10				4	5			37	1		2		1	1					
5-16				7	6			10	1										
6-4		1		1				12	3										
7A-21		1		11	22			17					2						
8-14		1		25	33		1	30	4										
8-32				37				2											
9A-2				35	49		1	18		1				2					
10-6		2		7	18			25	7					1					
12-6		2		32	18			64	16		1	5	2						
28-11				28	19		2	33	5					3					
47-4	1	11	3	1	47	35		2	77	10			2	1	3		1		1
53-18		7			74			3	98	27	2			3					
58-8		6		1	41	139		4	77				10	1					
63-3		2			36	215		3	11	1		1		4		1			
71-4	1	24	1		92	1	1	7	71	3			1	8	1			1	
80-8		27	2		116	25		3	39	1		1		2	12				1
82-3		3			57	69			54	3				12	1				
92-5	1	6	1	2	155		2	6	41	9			2	1	10	6		1	
97-18		6			63	26		4	42	2					1		1		
98-2		13	2	1	91			5	59	11	2			3	17	8	1	2		

Appendix B. Counts of dinocysts and other palynomorphs of Chesapeake Bay samples (part 2).

Sample site	Cyst of *Biecheleria*	Type indet 1	Type indet 2	Type indet 3	Pollen	Bisaccate pollen	*Radiosperma corbiferum*	*Halodinium*	*Polysterias*	Ciliate cysten	Cyst type P (Reid and John, 1978)	Foram Linings	*Pseudoschizaea*	Type indet 4
2A-18					2351	1028	10	3		8	1	9		5
4-10					320	153	2			4		2		1
5-16	2				729	385				19		350	3	2
6-4	2				142	103				1		10		3
7A-21	96		1		391	156		2		15		6		2
8-14	123		8		1769	2658	2	1		3		35	2	5
8-32	32				1212	562				15	1	47	3	1
9A-2	67		2		397	408	3	7		4		4		1
10-6	26				571	516	2				1	7		
12-6	44				2091	1228	1			12	2	9	2	17
28-11	12				6535	5167	28			73		11	3	7
47-4	2	1	4		1653	6905	33			1	4	12	2	6
53-18	3				1142	2834	6	1		1	8	10		4
58-8	6		4		1608	1971	25	1		4	12	4		5
63-3	1	1			715	1226	17	5		23	4	11		
71-4	4	17			764	1004	7	6	1	60	24	19	1	
80-8	33				3449	3503	6	7	1	3	15	2	2	
82-3	61	1	6		4775	5296	5	1	3	2	12	2	2	12
92-5	4				1617	551	3	5	1	532	1	36	1	4
97-18	18				6588	1778	2			64	1	25	1	
98-2	1			1	1619	566	2	8		157	5	52	1	

Appendix B. Counts of dinocysts and other palynomorphs of Chesapeake Bay samples (part 3).

Sample site	Nos. of counted cysts	*Achomosphaera* spp.	Cyst of *Alexandrium/Scrippsiella* spp.	Cyst of *Archaeperidinium minutum*	*Ataxodinium choane*	*Dubridinium* spp.	*Echinidinium aculeatum*	Cyst of *Gymnodinium nolleri/microreticulatum*	*Lejeunecysta paratenella*	*Lingulodinium machaerophorum*	*Nematosphaeropsis labyrinthus*	*Operculodinium centrocarpum* sensu Wall & Dale 1966	*Operculodinium israelianum*	*Operculodinium* cf *israelinianum*	Cyst of *Pentapharsodinium dalei*	Cyst of *Protoperidinium nudum*	*Polysphaeridium zoharyi*	Cyst of *Polykrikos schwartzii* sensu Matsuoka 2009	Cyst of *Polykrikos kofoidii* sensu Matsuoka 2009	*Polykrikos* group ind.	Cyst of *Protoperidinium minutum* sensu Wall and Dale, 1968	*Quinquecuspis concreta*	Round Brown Cyst	RBC Type A	*Selenopemphix nephroides*	*Selenopemphix quanta*	*Spiniferites* spp.	*Spiniferites mirabilis*	*Spiniferites bentorii*	*Spiniferites delicatus*	*Spiniferites ramosus*	*Spiniferites* Group Type 1	Spiny Brown cyst	Cyst of *Protoperidinium stellatum*	*Trivantedinium applanatum*	*Votadinium calvum*	*Votadinium spinosum*	*Votadinium spinosum*	*Xandarodinium xanthum*	Type indet 1	Type indet 2	Type indet 3	Different zones
2A-18	289	0,0	70,9	2,1	0,0	0,3	0,0	0,3	0,0	0,7	0,0	0,0	0,0	0,0	0,3	0,0	0,3	0,0	0,0	0,0	0,0	0,0	14,2	8,3	0,0	0,0	1,7	0,3	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
4-10	342	0,0	78,7	0,0	0,0	0,0	0,0	0,0	0,0	1,5	0,3	3,8	0,0	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	1,5	0,0	0,0	10,8	0,3	0,0	0,6	0,0	0,3	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
5-16	302	0,0	91,4	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,0	0,0	0,0	3,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
6-4	23	0,0	8,7	0,0	4,3	0,0	0,0	0,0	0,0	0,0	0,0	8,7	0,0	0,0	0,0	0,0	4,3	0,0	4,3	0,0	0,0	0,0	4,3	0,0	0,0	0,0	52,2	13,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
7A-21	146	0,0	61,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0	7,5	15,1	0,0	0,0	11,6	0,0	0,0	0,0	0,0	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,0	Potomac River	
8-14	309	0,0	63,8	1,0	0,0	0,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,3	0,0	0,3	0,0	0,3	0,0	0,0	0,0	8,1	10,7	0,0	0,3	9,7	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,6	0,0		
8-32	52	0,0	21,2	1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	71,2	0,0	0,0	0,0	3,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
9A-2	311	0,0	61,7	3,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	11,3	15,8	0,0	0,3	5,8	0,0	0,3	0,0	0,0	0,0	0,6	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,0		
10-6	112	0,0	33,9	1,8	0,0	0,0	0,0	0,0	0,0	1,8	0,0	1,8	0,0	0,0	3,6	0,0	3,6	0,0	1,8	0,0	0,0	0,0	6,3	16,1	0,0	0,0	22,3	6,3	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	Northern Neck	
12-6	330	0,0	40,9	3,0	0,0	0,0	0,0	0,0	0,0	0,6	0,6	0,3	0,0	0,0	0,9	0,0	11,2	0,0	0,6	0,0	4,3	0,0	9,7	5,5	0,0	0,0	19,1	4,8	0,0	0,3	1,5	0,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
28-11	229	0,0	53,7	3,5	0,0	0,4	0,0	0,9	0,0	0,0	0,0	0,9	0,0	0,0	0,4	0,0	0,9	0,0	0,0	0,0	0,0	0,0	12,2	8,3	0,0	0,9	14,4	2,2	0,0	0,0	0,0	0,0	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	Rappahannock	
47-4	342	0,6	28,4	0,0	0,0	0,0	0,0	0,3	0,0	3,8	0,6	3,2	0,0	0,0	0,6	0,0	4,1	0,3	3,2	0,0	0,9	0,3	13,7	10,2	0,0	0,6	22,5	2,9	0,0	0,0	0,0	0,6	0,3	0,9	0,0	0,3	0,0	0,3	0,3	1,2	0,0		
53-18	325	0,6	2,5	2,2	0,0	0,0	0,0	0,0	0,0	6,8	0,9	3,4	1,5	0,0	0,3	0,0	15,4	0,0	2,2	0,0	0,0	0,0	22,8	0,0	0,0	0,9	29,8	8,3	0,6	0,0	0,0	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
58-8	306	0,0	3,3	1,3	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,3	0,7	1,0	0,0	2,0	0,3	0,0	0,3	13,4	45,4	0,0	1,3	25,2	0,0	0,0	0,0	0,0	3,3	0,3	0,0	0,0	0,0	0,0	0,0	0,0	1,3	0,0	York and	
63-3	315	0,0	2,5	2,5	0,0	0,0	0,3	0,0	0,0	1,9	0,6	1,9	0,0	0,0	0,6	1,0	1,3	0,0	0,6	0,0	0,0	0,0	11,4	68,3	0,0	1,0	3,5	0,3	0,0	0,3	0,0	1,3	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	James River	
71-4	317	1,3	0,0	0,3	0,0	0,0	0,3	0,0	0,0	2,5	2,2	14,8	0,6	0,3	0,9	0,0	4,1	0,3	7,6	0,3	0,3	0,0	29,0	0,3	0,3	2,2	22,1	0,9	0,0	0,0	0,0	0,3	2,5	0,3	0,0	0,0	0,3	0,0	5,4	0,0	0,0		
80-8	301	0,0	4,0	6,0	0,0	0,3	0,0	0,0	0,0	4,7	0,7	1,0	0,0	0,0	0,3	0,0	6,6	0,0	9,0	0,3	0,7	0,0	38,5	8,3	0,0	1,0	13,0	0,3	0,0	0,3	0,0	0,7	4,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0		
82-3	335	0,0	17,9	12,8	0,0	0,3	0,3	0,0	0,0	1,5	0,3	0,0	0,0	0,0	3,0	0,0	2,4	0,0	0,9	0,0	0,0	0,0	17,0	20,6	0,0	0,0	16,1	0,9	0,0	0,0	0,0	0,0	3,6	0,3	0,0	0,0	0,0	0,0	0,0	1,8	0,0		
92-5	307	0,3	1,3	0,3	0,0	0,0	0,3	0,0	0,0	2,0	1,3	10,4	0,3	0,3	0,3	0,0	2,9	0,3	2,0	0,3	0,3	0,7	50,5	0,0	0,7	2,0	12,7	2,9	0,0	0,0	0,7	0,3	3,3	2,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	Eastern Shore	
97-18	258	0,0	8,9	2,3	0,0	0,8	0,0	0,0	0,0	1,9	1,6	22,9	0,0	0,0	3,1	0,0	2,3	0,0	2,3	0,0	0,0	0,0	24,4	10,1	0,0	1,6	15,9	0,8	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,4	0,0	0,0	0,0	0,0	0,0	Side	
98-2	319	0,9	2,5	5,3	0,0	0,0	0,0	0,3	0,3	2,5	2,2	11,9	0,3	0,0	1,9	0,0	3,1	0,0	4,1	0,9	0,6	0,3	28,5	0,0	0,0	1,6	18,5	3,4	0,6	0,0	0,0	0,9	5,3	2,5	0,3	0,6	0,0	0,0	0,0	0,0	0,3		

Appendix C. Relative abundance (%) of dinocysts in our samples. Sites are ordered according to their location (zone).

Sample site	No. Cysts/gram	*Achomosphaera* spp.	Cyst of *Alexandrium*/*Scrippsiella* spp.	Cyst of *Archaeperidinium minutum*	*Ataxodinium choane*	*Dubridinium* spp.	*Echinidinium aculeatum*	Cyst of *Gymnodinium nolleri*/*micrreticulatum*	*Lejeunecysta paratenella*	*Lingulodinium machaerophorum*	*Nematosphaeropsis labyrinthus*	*Operculodinium centrocarpum* sensu Wall & Dale 1966	*Operculodinium israelianum*	*Operculodinium cf israelinianum*	Cyst of *Pentapharsodinium dalei*	Cyst of *Protoperidinium nudum*	*Polysphaeridium zoharyi*	Cyst of *Polykrikos schwartzii* sensu Matsuoka 2009	Cyst of *Polykrikos kofoidii* sensu Matsuoka 2009	*Polykrikos* group ind.	Cyst of *Pronoperidinium minutum* sensu Wall and Dale, 1968	*Quinquecuspis concreta*	Round Brown Cyst	RBC Type A	*Selenopemphix nephroides*	*Selenopemphix quanta*	*Spiniferites* spp.	*Spiniferites mirabilis*	*Spiniferites bentorii*	*Spiniferites delicatus*	*Spiniferites ramosus*	*Spiniferites* Group Type 1	Spiny Brown cyst	Cyst of *Protoperidinium stellatum*	*Trivantedinium applanatum*	*Votadinium calvum*	*Votadinium spinosum*	*Xandarodinium xanthum*	Type indet 1	Type indet 2	Type indet 3	Different zones
2A-18	183,767	0	130	4	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0	26	15	0	0	3	1	0	0	0	0	1	0	0	0	0	0	0	0	0	
4-10	1034,92	0	814	0	0	0	0	0	0	15	3	39	0	0	9	0	0	0	0	0	0	0	12	15	0	0	112	3	0	6	0	3	3	0	0	0	0	0	0	0	0	
5-16	124,77	0	114	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
6-4	5,16713	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,2	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
7A-21	37,038	0	23	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	3	6	0	0	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	Potomac River
8-14	117,372	0	75	1	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	9	13	0	0	11	2	0	0	0	0	0	0	0	0	0	0	0	3	0	
8-32	41,8287	0	9	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	30	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
9A-2	185,536	0	115	6	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	21	29	0	1	11	0	1	0	0	0	1	0	0	0	0	0	0	1	0	
10-6	49,2408	0	17	1	0	0	0	0	0	1	0	1	0	0	2	0	0	0	1	0	0	0	3	8	0	0	11	3	0	0	0	0	0	0	0	0	0	0	0	0	0	Northern Neck
12-6	370,356	0	152	11	0	0	0	0	0	2	2	1	0	0	3	0	42	0	2	0	0	0	36	20	0	0	71	18	0	1	6	2	0	0	0	0	0	0	0	0	0	
28-11	80,6196	0	43	3	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	10	7	0	1	12	2	0	0	0	0	1	0	0	0	0	0	0	0	0	Rappahannock
47-4	17720,6	104	5026	0	0	0	0	52	0	674	104	570	0	0	104	0	725	52	570	0	155	52	2435	1814	0	104	3990	518	0	0	0	104	52	155	0	52	0	52	52	207	0	
53-18	38180	235	940	822	0	0	0	0	0	2584	352	1292	587	0	117	0	5874	0	822	0	0	0	8693	0	0	352	11395	3172	235	0	0	0	352	0	0	0	0	0	0	0	0	
58-8	9733,43	0	318	127	0	32	0	0	0	0	0	32	0	0	32	64	95	0	191	32	0	32	1304	4421	0	127	2449	0	0	0	0	318	32	0	0	0	0	0	127	0	York and
63-3	18766	0	477	477	0	0	60	0	0	357	119	357	0	0	119	179	238	0	119	0	0	0	2145	12809	0	179	655	60	0	60	0	238	0	60	0	0	0	60	0	0	James River
71-4	135942	1715	0	429	0	0	429	0	0	3431	3002	20155	858	429	1287	0	5575	429	10292	429	429	0	39453	429	429	3002	30019	1287	0	0	0	429	3431	429	0	0	429	0	7290	0	0	
80-8	108603	0	4330	6495	0	361	0	0	0	5051	722	1082	0	0	361	0	7216	0	9742	361	812	0	41854	9020	0	1082	14071	361	0	361	0	722	4330	0	0	0	0	271	0	0	0	
82-3	568,344	0	102	73	0	2	2	0	0	8	2	0	0	0	17	0	14	0	5	0	0	0	97	117	0	0	92	5	0	0	0	0	20	2	0	0	0	0	2	10	0	
92-5	2098,38	7	27	7	0	0	7	0	0	41	27	219	7	7	7	0	62	7	41	7	7	14	1059	0	14	41	267	62	0	0	14	7	68	41	0	0	7	0	0	0	0	Eastern Shore
97-18	846,031	0	75	20	0	7	0	0	0	16	13	193	0	0	26	0	20	0	20	0	0	0	207	85	0	13	134	7	0	0	0	0	3	0	0	3	0	0	0	0	0	Side
98-2	1534,51	14	38	82	0	0	0	5	5	38	34	183	5	0	29	0	48	0	63	14	10	5	438	0	0	24	284	53	10	0	0	14	82	38	5	10	0	0	0	0	5	

Appendix D. Absolute concentration of dinocysts (cysts per gram of dry sediments) from Chesapeake Bay sites. Sites are ordered according to their location (zone).