PYRODINIUM BAHAMENSE VAR. *BAHAMENSE* CYSTS AS A DINOFLAGELLATE POPULATION AND DEPOSITION PROXY IN PUERTO MOSQUITO, VIEQUES, PUERTO RICO

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INTRODUCTION

Puerto Mosquito is a bioluminescent bay in Vieques, Puerto Rico occupied by the dinoflagellate *Pyrodinium bahamense* var. *bahamense*. This study is intended to analyze the validity of using dinoflagellate cysts as a proxy for population and depositional information, as determined by assessing relative contributions of carbon sources for the study bay using an organic carbon $\delta^{13}C$ analysis. Another goal of this project is to develop a method to study dinoflagellate cysts in a way that uses fewer of the dangerous chemicals required by typical palynological technique. This involved substituting a combination of HCl and sieving to minimize the carbonate and silicate in the samples to remove sample preparations involving HF.

P. bahamense is one of many dinoflagellate species that forms a resistant and protective casing, called a cyst. The germination of cysts from concentrated beds is what drives bloom initiation (Nehring, 1996; Sombrito et al., 2004). Once buried by sediment, a cyst can remain viable for years, but it needs some mechanism to leave the sediment column or it will never germinate (Nehring, 1996; Anderson et al., 2005). In the absence of sediment mixing, only a slow trickle of cysts in the surface sediments will germinate, and many will be permanently buried, but a sediment disturbance can be a powerful bloom-initiating event (Nehring, 1996; Kirn et al., 2005). The study bay is rather unusual in that it contains a standing crop of phytoplankton rather than a resuspension-driven bloom, which improves the accuracy of cysts as a population proxy since there is an uninterrupted stream of cysts being deposited in the undisturbed sediment.

METHODS

Study Core Description

The study core was taken with short-length polycarbonate pipes in shallow water and using a gravity corer in deep water in multiple locations within each bay, both of which are preferable for accurate cyst counting at depth in the sediment column (Anderson et al., 1982). These cores were extruded and sliced open for sedimentary facies characterization, and dinoflagellate cyst samples were preserved in glutaraldehyde. Each sample was taken to represent an average over either a whole sedimentary facies or a 1cm increment.

This study comprises one core sampled at 1cm increments (Fig. 1). The study core is located in Puerto Mosquito in water ~1-1.5m deep. This area is silty, and covered by a bed of seagrass. The core itself was mostly silt and clay near the top with coarse sand sized shell fragments, with larger bivalve shell pieces and whole shells deeper in the core. The samples are composed of carbonate mud and shell fragments, a small amount of quartz sand, biogenic silica (mostly diatoms), and organic carbon. The carbonate fraction becomes more significant deeper in the cores, as most are marked by a shell-dominated storm facies at depth.

Figure 1. Section of core sampled for cysts. Sampled at 1 cm increments until beginning of facies change to storm facies at 14 cm ***(photo by Suzanne O'Connell).***

The study core was chosen based on a variety of factors. Since it was sampled at 1cm intervals, the core is more useful for correlating cyst densities to a particular period of time (rather than a particular facies). This core also has a substantial amount of sediment in the size fraction of silt and below, which is the size fraction that contains cysts. This section lacks ^{210}Pb data, so sedimentation rate estimates come from a core from a geographically similar part of the bay (which is subdivided by facies rather than 1cm increments).

Laboratory Work

Each sample was mixed with water and allowed to settle out for several days to obtain a consistent amount of pore water between samples. Excess liquid was decanted, the sediment was thoroughly mixed to get an even distribution of grain sizes throughout, and a ~1g portion of each sample was removed for analysis. An additional portion was dried and weighed to get the average water content for each sample.

Wet samples were weighed, and each sample was washed through a 105μm sieve and a 20μm sieve. The size fraction between 105μm and 20μm was dissolved overnight in muriatic acid (31.45% HCl), and weighed again.

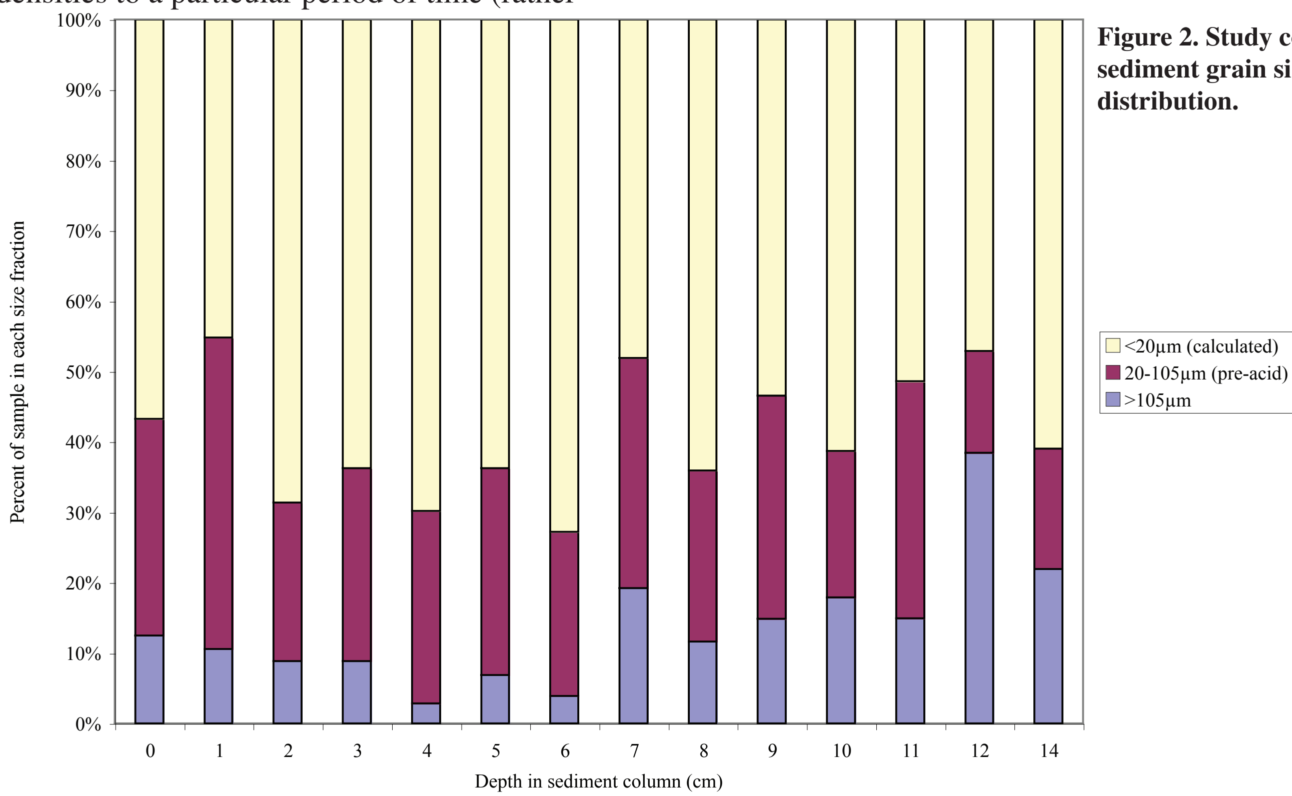


Figure 2. Study core sediment grain size distribution.

The remaining sample was then spread on gridded slides to disperse the sediment for accurate counts, and the cysts were counted on 4 transects on each slide with a light microscope (at 100x and 400x) and multiplied to get a total for each sample (methods modified from Matsuoka and Fukuyo, 2000; Sangiorgi et al., 2006). The age and sedimentation rate of the samples were determined by ^{210}Pb dating (Keck, 2007).

RESULTS

The sample grain size distribution is summarized in Figure 2. There is a decrease in the 20-105μm size fraction and increase in the >105μm size fraction with depth, as the samples approach the depth of the facies change seen in Figure 1. This change is matched by a decrease in pore water content with depth. The amount of carbonate in the samples increases further down in the core, corresponding to a decrease in the organic carbon and silica fraction. Removing the HF preparation means there is no way to remove silica from the samples, so no organic carbon fraction is available.

The calculation of the number of cysts in 1g of dry sediment (Fig. 3) is based on data from the wet sediment counts and the average percent water composition of each sample. The average number of cysts per gram of wet sediment is 4697 ± 1318 (1 standard deviation), and the average number of cysts per gram of dry sediment is 28154 ± 8775 (1 standard deviation). The broad ranges reflect the variability in the cyst counts, which is possibly emphasized by sectioning samples for counts and multiplying to get cyst totals. The $\delta^{13}C$ data for cores from throughout Puerto Mosquito are located in Figure 4.

DISCUSSION

Sediment Analysis

The changes in sediment sample characteristics can be attributed to the transition to a shell-filled facies past 14cm. The facies change explains the increase in carbonate, the larger grain size, and the decrease in pore water space, since the shells are made of carbonate, are larger than the fragments found in the sample section, and do not have significant amounts of pore

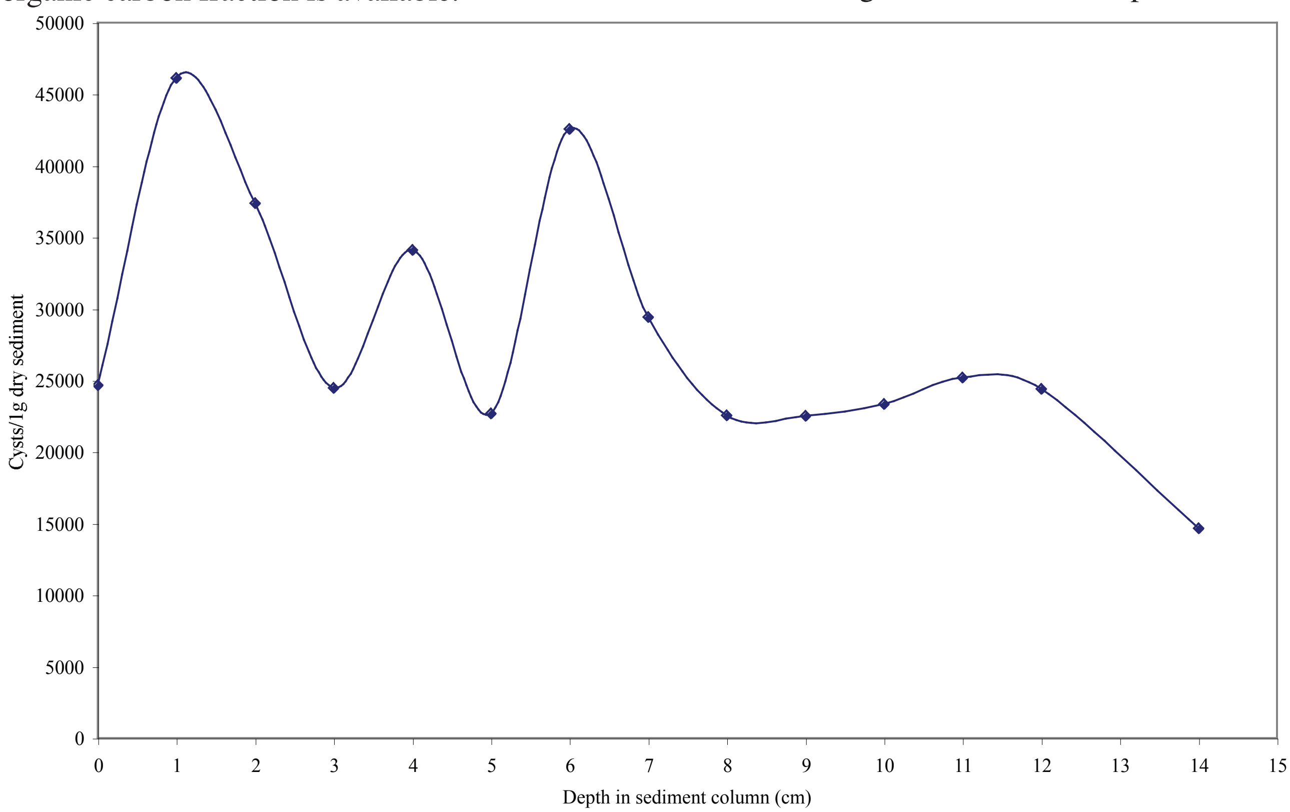
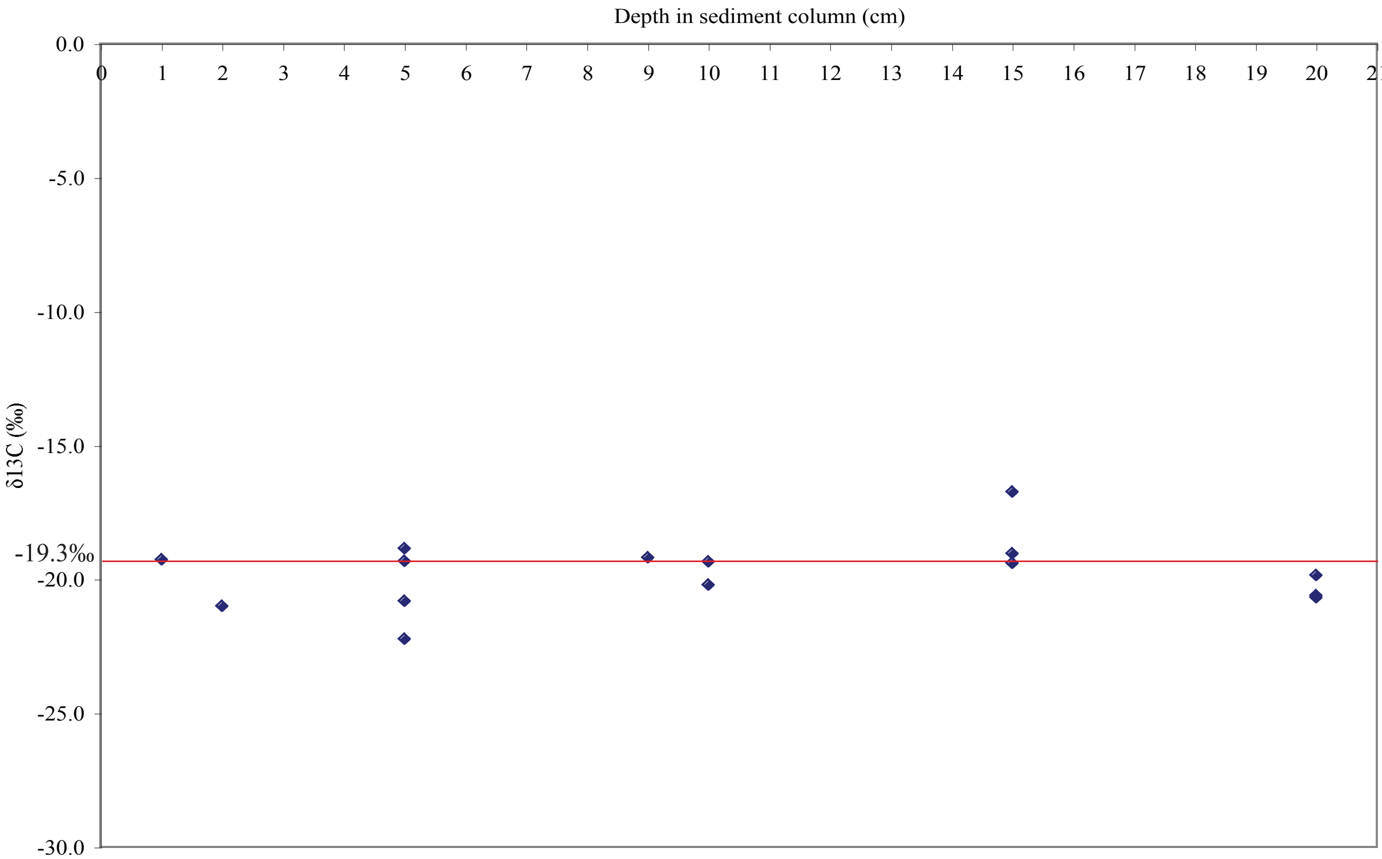


Figure 3. Cyst counts per gram of dry sediment with depth in study core.

space. Therefore, these changes in sediment characteristics are not caused by changes in cyst population.

Figure 4. Stable carbon isotope ratios in top 20 cm of cores throughout Puerto Mosquito. Red line shows sediment average isotopic composition of phytoplankton. Deviations at 2 cm, 5 cm, and 15cm correlate with drops in cyst counts.



The sediment size distribution can be used as a check to see what size fraction dinoflagellate cysts sort with hydrologically. The correlations between cyst counts and the size fraction examined in this study can be found in Figure 5. There is no correlation between cysts and the <20μm size fraction, a strong positive correlation between cysts and the 20-105μm size fraction, and a negative correlation between cysts and the >105μm size fraction. Cysts are usually ~45μm in diameter, so even though they are less dense than inorganic sediment they sort with the silt size fraction rather than the lighter <20μm fraction (Williams and Bujak, 1985). There is no correlation with the smallest grain size fraction (rather than a negative correlation) because clay-sized particles can be deposited in the same areas as silt due to flocculation, meaning the rates of deposition of the two smaller size fraction are unrelated to each other. The more sediment that is deposited in the largest size fraction, the more likely it is that smaller sediment particles will remain suspended in the water column.

Even though cysts may be formed across a variety of depositional regimes, they are hydrodynamically unstable where larger grain sizes are deposited, so they will stay suspended in the water column. Therefore, even though a bloom can spread to a shallow sandy area given enough light, new blooms are unlikely to initiate from that location, since the cysts would have been carried elsewhere.

Cysts

Using cysts is the primary way to get information about past populations of dinoflagellates, and *P. bahamense* is a particularly good species to use for this type of analysis. Some dinoflagellates form cysts only when they are stressed by environmental conditions, so the correlation between dinoflagellates in the water column and cysts being deposited is low (Godhe et al., 2001; Peperzak, 2006). However, since *P. bahamense* form resting cysts as a part of their life cycle, there is a continuous stream of cysts deposited into the sediment, so cyst densities can be used as proxies for population size (Anderson and Wall, 1978).

are known for being particularly resistant to oxidation, despite having an organic wall (Zonneveld et al., 2001). This excellent preservation potential means that population counts will not be significantly affected by the age of the cysts or small-scale sediment conditions given the age of sediment in the study cores, so compensation for decay with depth is unnecessary. The cyst preservation potential is confirmed by the data from this study.

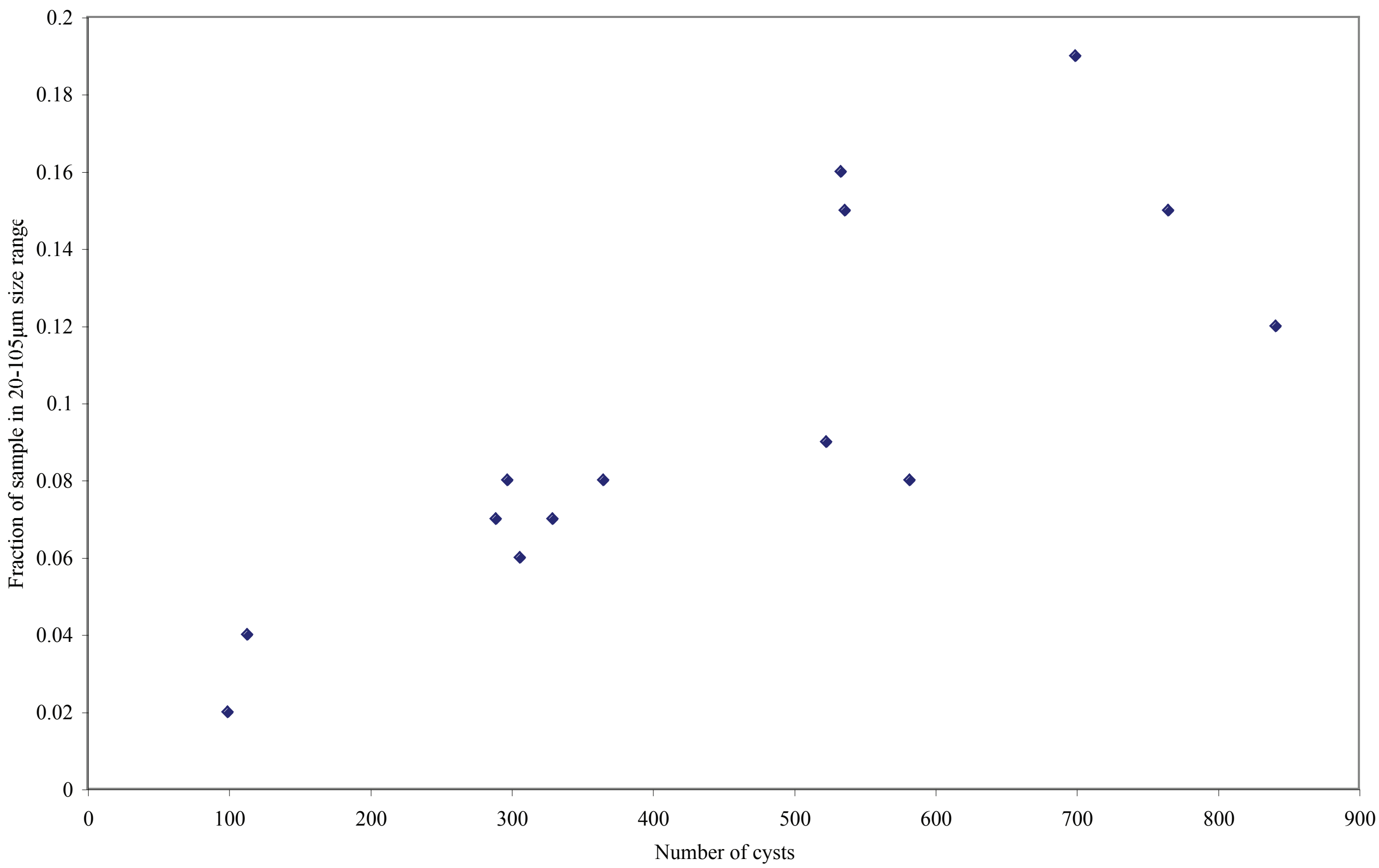


Figure 5. Number of cysts shows positive correlation with 20-105 µm grain size.

Different sediments are affected by decay processes to a different extent, often based on oxygen content. If the cysts are prone to aerobic degradation, the population signal in the sediment record can quickly be obscured by varying decay rates (Versteegh and Zonneveld, 2002). However, the cysts of this species

There is a gradual decrease in the number of cysts in the sediment with depth (Fig. 12), but there is no exponential drop that would be expected if the cysts were heavily affected by decay.

Carbon Isotope Data

The average isotopic composition of sediments in Puerto Mosquito indicates mixed carbon inputs. The primary sources of this organic matter include the mangroves surrounding the bay, macrophytes growing on the bottom of the bay (mostly seagrasses), and phytoplankton. Assuming inputs from mangroves and macrophytes stay the same over time, any variation in $\delta^{13}C$ should come from changes in the dinoflagellate population. Carbon isotope data thus serve as a check to verify the cyst population data.

The cyst data show good correlation with stable carbon isotope values from other cores in the study bay. The $\delta^{13}C$ values for the top 20cm of other cores in Puerto Mosquito (with incubated cores and a mangrove peat core removed) are shown in Figure 18. A $\delta^{13}C$ value of -19.3‰ shows a phytoplankton-dominated carbon source (Gu et al., 2006). This data is confirmed for the study bay by the $\delta^{13}C$ value of -19.3‰ for the top 1cm of sediment from a core taken in the middle of the bay (Keck, 2007). Since terrestrial mangrove organic carbon does not extend beyond the fresh and brackish parts of the bay, and this core location is too deep for light to penetrate for seagrass beds, this value should reflect the phytoplankton carbon input for the sediments (Fry and Sherr, 1984). Therefore, deviations from this value indicate a decreased influence of phytoplankton as a carbon source, which should correspond to a drop in the cyst counts.

There are three deviations in the $\delta^{13}C$ value from the typical sediment value of around -19.3‰. These variations indicate that fewer cysts and phytoplankton were being deposited in parts of the bay, since a carbon source with a more positive (seagrass) or negative (mangrove) $\delta^{13}C$ signature than phytoplankton contributed relatively more to the sediment organic carbon at the time the sediment was deposited. The isotope deviations correlate with decreases in the cyst counts, so the carbon isotope data confirms the accuracy of the cyst counts in the sediment and variations in the rate of deposition of phytoplankton.

Puerto Mosquito is likely at a constant carrying capacity for phytoplankton. Therefore, variations in cyst numbers in the sediment mainly reflect hydrological variation, rather than drastic changes in the size of the bloom, which is corroborated by the close correlation between cyst counts and grain size fractions of the sediment samples. Changing water flow conditions altered the distribution of sediment in the particular grain size that carries cysts, which changed the relative cyst density and thus the $\delta^{13}C$ signature of that particular sediment layer. This demonstrates the need for careful grain size control in any cyst-based dinoflagellate population studies.

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