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Alexandrium and *Scrippsiella* cyst viability and cytoplasmic fullness in a 60-cm sediment core from Sequim Bay, WA

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ABSTRACT

Many marine protists produce a benthic resting stage during their life history. This non-motile cyst stage can either germinate near the sediment surface to provide the inoculum for subsequent blooms or, be buried by sediment deposits over time and entrained into the sedimentary record. Buried cysts can be resuspended into the water column by mixing events (e.g., storms) or other disturbances (e.g., dredging). It is not clear how long cysts can survive while buried in the sediments and still be capable of germinating given favorable conditions. Here, the germination success of cysts produced by the potentially toxic dinoflagellate genus *Alexandrium* and the non-toxic dinoflagellate genus *Scrippsiella* is reported from a 60-cm sediment core collected in Sequim Bay, WA, in December 2011. Cysts of *Alexandrium* spp. and *Scrippsiella* spp. were isolated from 2-cm sections of the core, placed in individual wells of a 96-well plate with growth medium, imaged, incubated at favorable conditions and monitored for germination. An image analysis program, DinoCyst, was used to quantitatively measure the amount of granular storage products, presumed energy stores, inside the cytoplasm to test the hypothesis that older cysts located deeper in the sediment core will have fewer energy stores available and will be less likely to germinate. An index of the area of the cytoplasm occupied with granular storage products relative to cyst size, termed 'cytoplasmic fullness', and age, based on ^{210}Pb dating of surrounding sediments, was compared with germination success or failure. This research indicates that cysts of *Alexandrium* spp. and *Scrippsiella* spp. can remain viable in sediments for 60 years or longer, show little visual evidence of cytoplasmic deterioration over this timescale (as measured by cytoplasmic fullness), and that germination success is statistically similar for cysts isolated from 0–60 cm deep in the sediment core. These results suggest that a cyst's cytoplasmic fullness is not indicative of viability and that cysts located as deep as 60 cm in the sediments are as likely to germinate as surface cysts given favorable conditions.

1. Introduction

Many planktonic protists produce benthic resting stages in their life histories but little is known about their long-term survival while in the sediments. Resting cells, cysts and spores serve as a seed bank to help a species withstand adverse environmental conditions and repopulate the water column once conditions suitable for vegetative growth arise (Wall, 1971). It has been postulated that resting stages evolved to aid in long-term survival of plankton during the prolonged periods of reduced solar transmission that may have occurred during ice ages and/or after catastrophic meteor strikes (McMinn and Martin, 2013; Ribeiro et al., 2011). A variety of approaches have been used to constrain the lifetime of cysts in the natural environment. Previous studies have estimated cyst degradation and survival rates based on mathematical models (Keafer et al., 1992; Shull et al., 2014), physical examination of a cyst's structural integrity (Head et al., 2006), available energy reserves and respiration rates (Binder and

Abbreviations: FSW, filtered sea water; HAB, harmful algal bloom; PSP, paralytic shellfish poisoning; SD, standard deviation; SPT, sodium polytungstate.

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Anderson, 1990) and long-term viability experiments using both stored and natural sediments (Lewis et al., 1999; Ribeiro et al., 2011).

The potentially toxic genus *Alexandrium* is a cyst-forming dinoflagellate found worldwide in temperate coastal waters (Anderson et al., 2012). Many species within the genus can produce a suite of toxins known to cause paralytic shellfish poisoning (PSP). The presence of PSP-associated toxins in shellfish above regulatory limits for human consumption can cause shellfish harvest closures. These closures result in economic losses to the aquaculture industry and coastal communities dependent upon recreational shellfish tourism as a source of income (Anderson et al., 2000; Hoagland et al., 2002). Because of the human health and economic impacts of PSP, there is broad interest to better understand how long the cyst stage of *Alexandrium* remains viable in the sediments. This is especially true for areas with a history of PSP that experience sediment disturbance events – managers need to know if there is an increased risk of *Alexandrium* blooms due to resuspension of "buried" cysts.

The *Alexandrium* species found in western Washington waters historically has been identified as *Alexandrium catenella* (Whedon & Kofoid) Balech (Nishitani and Chew, 1984). However, other species of *Alexandrium* have been reported in the Puget Sound and along coastal Washington (Horner et al., 1997). Recently, it has been proposed that *A. catenella* is part of the Group I strains within the *A. tamarense* species complex (John et al., 2014a) and that it be referred to as *A. fundyense* (John et al., 2014b). In light of continued taxonomic questions in the *A. tamarense/catenella/fundyense* complex it will be referred to here by its genus. In the U.S. Pacific Northwest, cysts produced by the non-toxic dinoflagellate genus *Scrippsiella* can co-occur in the sediments with *Alexandrium* spp. cysts. Similar to *Alexandrium*, defining a species within the genus *Scrippsiella* is difficult (Montresor et al., 2003; Zinssmeister et al., 2011). Historically, the species of *Scrippsiella* found in the waters of coastal western Washington has been identified as *Scrippsiella trochoidea* (Tomas, 1996) but it will be referred to by its genus here.

The cyst stage of dinoflagellates has an important role in seeding annual blooms. Once a newly-formed cyst has completed a mandatory dormancy period and is matured, it can remain in a quiescent stage on the seafloor and may be able to germinate under favorable environmental conditions (Anderson, 1980; Bravo and Anderson, 1994). Depending on the species and environmental conditions, dormancy requirements have been estimated to range from <15 to 180 days for *Alexandrium* spp. (Anderson, 1980; Genovesi-Giunti et al., 2006) and 25 days to 6 months for *Scrippsiella* spp. (Binder and Anderson, 1987; Kremp and Anderson, 2000). Upon germination, a motile vegetative cell is released from the cyst, and in the case of some *Alexandrium* spp., can form potentially toxic blooms. However, some mature dinoflagellate cysts do not germinate and instead become buried by sediment deposits and entrained into the sedimentary record. If *Alexandrium* spp. and *Scrippsiella* spp. cysts are buried below the depth that oxygen can penetrate, the cysts cannot germinate (Anderson et al., 1987; Kremp and Anderson, 2000). Presumably over some length of time, the dinoflagellate cysts in the anoxic sediment deteriorate to the point that germination becomes impossible even if reintroduced into surface sediments and favorable conditions.

Understanding the long-term viability of *Alexandrium* spp. and *Scrippsiella* spp. cysts in subsurface sediments will help to elucidate the potential for older cysts that are disturbed and mixed to the sediment surface to germinate and initiate blooms (e.g., Butman et al., 2014). Estimates of how long *Alexandrium* spp. cysts remain viable while buried in the sediments vary considerably. Keafer et al. (1992) suggest a half-life of 2–10 years for *A. tamarense* cysts buried in a shallow salt pond in Massachusetts. However, Miyazono et al. (2012) germinated an estimated 100-year old *A. tamarense* cyst from Funka Bay, Hokkaido, Japan. *Scrippsiella* spp. cysts have been identified in sediments from Koljö Fjord, Sweden that were deposited 90 years ago and cysts estimated to be 40 years old were able to germinate (Lundholm et al., 2011). In the U.S. Pacific Northwest, *A. catenella* cysts have been identified 1 m deep in sediments estimated to have been deposited 120 years ago (Feifel et al., 2012), but it is not known if these cysts were able to germinate.

Most photosynthetic protist resting stages are thought to lose their ability to photosynthesize and survive in the sediments by reducing respiration rates and relying on internal energy stores in the cytoplasm for survival. The internal contents within a dinoflagellate cyst cytoplasm have been referred to as "storage compounds" (Binder and Anderson, 1990), "intracellular storage products" (Bravo and Figueroa, 2014; Genovesi et al., 2009), and "granular cytoplasm" (Vahtera et al., 2014). There has been scant research to confirm that the amount and content of material inside the cytoplasm represents the amount of energy available in the cyst to germinate and produce a dividing cell. Binder and Anderson (1990) found that cysts produced by *S. trochoidea* displayed a 60-fold reduction in cyst metabolism compared to its vegetative stage and that newly-formed cysts were primarily composed of lipids and carbohydrates. Light microscopy work has shown that the energy storage products found in the cytoplasm of dinoflagellate cysts tend to be granular in appearance but there can be quite a bit of inter and intra-species variation (Bravo and Figueroa, 2014). Here, the golden-brown, granulated intracellular contents in the cytoplasm of cysts produced by *Alexandrium* and *Scrippsiella* will be referred to as "granular storage products". The amount of granular storage products and the presence of an accumulation body in the cytoplasm are commonly cited by researchers in cyst isolation work as a potential indicator of cyst health and viability (e.g., Lundholm et al., 2011; Moore et al., 2015).

The cytoplasm of *Alexandrium* spp. and *Scrippsiella* spp. cysts can range from almost entirely full of granular storage products to almost empty with only a few, colorless vacuoles (Genovesi et al., 2009; Miyazono et al., 2012). Genovesi et al. (2009) suggest that the internal cellular contents of laboratory-produced *A. tamarense* cysts systematically evolve from a newly-formed resting cyst with a cytoplasm full of golden-brown granular storage products to a final-stage cyst that exhibits transformation of colored granular storage products to colorless vacuoles in the cytoplasm as the cysts age over one year. In contrast, Binet and Stauber (2006) found that the viability of laboratory-produced *A. catenella* cysts could not be predicted based upon cyst size, natural fluorescence, or the internal cell structure found in the cytoplasm. They did not find a correlation between a cyst's physical appearance and its ability to germinate. These studies examined laboratory-produced cysts; there have not been any studies that examine the amount of granular storage products in the cytoplasm of *Alexandrium* and *Scrippsiella* cysts produced in the environment (termed 'naturally-produced' cysts) in relation to cyst viability or age.

In this study, the long-term viability of dinoflagellate cysts produced by *Alexandrium* spp. and *Scrippsiella* spp. in a 60-cm sediment core was assessed. The following hypotheses were tested: (1) cysts buried deeper in sediments and hence, older relative to surface cysts, will have fewer granular storage products inside the cytoplasm as energy stores are respired over time; (2) older cysts will be less likely to germinate compared to the younger cysts isolated from the sediment surface; and (3) the amount of granular storage products in the cytoplasm can be used