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Alexandrium fundyense cyst viability and germling survival in light vs. dark at a constant low temperature

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ABSTRACT

Both observations and models suggest that large-scale coastal blooms of *Alexandrium fundyense* in the Gulf of Maine are seeded by deep-bottom cyst accumulation zones ("seed beds") where cysts germinate from the sediment surface or the overlying near-bottom nepheloid layers at water depths exceeding 100 m. The germling cells and their vegetative progeny are assumed to be subject to mortality while in complete darkness, as they swim to illuminated surface waters. To test the validity of this assumption we conducted laboratory investigations of cyst viability and the survival of the germling cells and their vegetative progeny during prolonged exposure to darkness at a temperature of 6 °C, simulating the conditions in deep Gulf of Maine waters. We isolated cysts from bottom sediments collected in the Gulf of Maine under low red light and incubated them in 96-well tissue culture-plates in culture medium under a 10:14 h light:dark cycle and under complete darkness. Cyst viability was high, with excystment frequency reaching 90% in the illuminated treatment after 30 days and in the dark treatment after 50 days. Average germination rates were 0.062 and 0.038 d^{-1} for light and dark treatments, respectively. The dark treatment showed an approximately 2-week time lag in maximum germination rates compared to the light treatment. Survival of germlings was considerably lower in the dark treatment. In the light treatments, 47% of germinated cysts produced germlings that were able to survive for 7 days and produce vegetative progeny, i.e., there were live cells in the well along with an empty cyst at least once during the experiment. In the dark treatments 12% of the cysts produced germlings that were able to survive for the same length of time. When dark treatments are scaled to take into account non-darkness related mortality, approximately 28% of the cysts produced germlings that were able to survive for at least 7 days. Even though cysts are able to germinate in darkness, the lack of illumination considerably reduces survival rate of germling cells. In addition to viability of cysts in surface sediments and the near-bottom nepheloid layer, survivability of germling cells and their vegetative progeny at aphotic depths is an important consideration in assessing the quantitative role of deep-coastal cyst seed beds in bloom formation.

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1. Introduction

The life cycle of many dinoflagellates includes a non-motile resting stage (cyst) that remains in bottom sediments or near-bottom nepheloid layers when conditions in the water column are unfavorable for growth (Dale, 1983; Matsuoka and Fukuyo, 2003; Wall, 1971; Kirn et al., 2005; Pilskaln et al., this issue). The switch from cyst to motile cell and vice versa determines the presence of those dinoflagellates in the water column. Numerous studies have shown that germination of dinoflagellate cysts is determined by internal and external factors (Anderson et al., 2005; Rengefors and Anderson, 1998). Among the internal factors, a mandatory dormancy period (maturation) after encystment lasting days to months (Anderson, 1980; Bravo and Anderson, 1994) and, for some species such as *Alexandrium fundyense*, an annual internal biological clock (Anderson and Keafer, 1987; Matrai et al., 2005a; Perez et al., 1998) regulates germination. Although oxygen is required for germination (Anderson et al., 1987; Kremp and Anderson, 2000; Rengefors and Anderson, 1998), temperature is often viewed as the main environmental determinant for cysts in the surface layer of sediments (Anderson, 1998; Dale, 1983). Light is not necessary for germination in all species but, in some, darkness slows down the process and might reduce the germination frequency and rate (Anderson et al., 2005, 1987; Bravo and Anderson, 1994; Genovesi et al., 2009).

Regarding *A. fundyense* bloom dynamics in the Gulf of Maine, germination is an important factor determining the initial bloom

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populations, since few overwintering motile cells have been encountered in surface waters in that region (Anderson et al., 2005; Kirn et al., 2005). Although the germination of *A. fundyense* has been thoroughly studied (Anderson et al., 2005) there is a lack of information on the fate of the newly germinated cells (germlings) and their immediate vegetative progeny. The germination frequency and viability of germlings and their progeny has been estimated for shallow water populations of *A. tamarense* in French coastal waters (Genovesi et al., 2009). The authors found high germination frequencies for natural cysts isolated from field samples (85%) but only 27% of the germlings were observed alive 1 day after germination. Of the surviving germlings, 76% were able to divide at least once during a 30-day experiment. The length of cold and dark storage of cysts was observed to influence these ratios, with increasing storage time decreasing the cyst “quality” and thus the ability of the germling and its progeny to survive (Genovesi et al., 2009). The largest cyst seed banks of *A. fundyense* in the northeastern US lie in the Gulf of Maine at depths >100 m (Anderson et al., 2005; Anderson et al., this issue). In this environment, the viability of cysts, germlings, and their progeny is thus affected by darkness and low temperatures. Darkness has been reported to impair germling survival for other dinoflagellate species (Anderson et al., 1987; Bravo and Anderson, 1994) and it could therefore have an impact on current model parameterizations for *A. fundyense* in the Gulf of Maine.

Here, we describe a germination and survival experiment with a light and dark treatment in a constant cold temperature (6 °C) performed with *A. fundyense* cysts collected from the Gulf of Maine. The results show the effect of darkness on cyst germination rates as well as the fate of the newly-germinated cells. Our findings give new insights into factors affecting the bloom-seeding capacity of deep cyst seedbeds. The results can improve estimates of the flux of newly-germinated cells from bottom sediments to the surface layer and thus the output of physical–biological models of *A. fundyense* population dynamics in the Gulf of Maine (Anderson et al., 2005; McGillicuddy et al., 2005; Stock et al., 2005; He et al., 2008; Li et al., 2009).

2. Materials and methods

2.1. Sample collection and storage

Eight sediment cores were collected using a hydraulically-damped piston corer (Craib, 1965) at a 120 m-deep site in the northwestern Gulf of Maine (43° 36′ N, 69° 22′ W) in October, 2009. The site is situated in a high-density cyst accumulation area (seedbed) along the mid-Maine coast. The cores were sectioned, and slices were collected at 0–1 and 1–3 cm depths in the cores. The eight 0–1 and 1–3 cm slices for each core were pooled to yield one homogenized 0–1 cm and one 1–3 cm sample that were stored in completely-filled 50-mL plastic tubes. Based on sediment color, which was black, the sediment was deemed anoxic. The tubes were stored in the cold (4 °C) and in total darkness immediately after filling. The tubes were stored in these conditions until cyst isolation was conducted on 29 March, 2010 (6 months after collection), allowing the cysts to complete their mandatory maturation period (Anderson, 1980; Bravo and Anderson, 1994) and enter the germination window regulated by an internal clock (Anderson and Keafer, 1987; Matrai et al., 2005a).

2.2. Sample processing, cyst isolation and experimental setup

All sediment processing was done in a darkened laboratory with the only light source being a soft red light. On 29 March, 2010, an aliquot of the cold- and dark-stored sediment from the 1–3 cm layer was taken. To avoid including sediment that might have been oxidized during storage the top layer of sediment from the 50-mL tube was removed and then a sample of arbitrary volume was scooped from the center of the tube, avoiding sediment along the tube walls, into a 50 mL sample tube. The average cyst concentration in the 1–3 cm layer was 2763 cysts cm^{-3} of wet sediment. Filtered sea water was added to a final volume of 45 mL and the slurry was sonicated with a Branson Sonifier 250 at a constant 40 W output for 1 min, and sieved to yield a clean 20–75 μm size fraction (Anderson et al., 2003). The cysts were further concentrated and the sample cleaned of debris with a non-toxic isosmotic density centrifugation method (Schwinghamer et al., 1991). Sucrose and colloidal silica (Nalco) was used to create a solution with two density layers of 1.07 g cm^{-3} and 1.35 g cm^{-3}. The mode *A. fundyense* cyst density is 1.2 g cm^{-3} (Anderson et al., 1985), thus most of the cysts would concentrate at the interface of the two liquids after centrifugation. Prior to centrifugation, 5 mL of the processed sediment sample was added to a 50-mL centrifuge tube. Twenty milliliters of the lighter density gradient solution was then carefully delivered under the processed sediment with a Pasteur pipette, after which 20 mL of the denser solution was delivered under the lighter solution. The tube was centrifuged at 2500 rpm for 20 min at 4 °C and the clear lighter solution was aspirated and the liquid at the interface of the two solutions was collected with a pipette and sieved through a 20 μm sieve. The contents were collected into a 15-mL sample tube which was immediately partitioned out into 1-mL Sedgwick–Rafter slides, and cysts were isolated using micropipettes.

Upon isolation, individual cysts were immediately put into 96-well culture-plates with 300 μL of modified (silica excluded) f/2 medium (Anderson et al., 1994) in each well. It took approximately 45 min to prepare one plate of isolated cysts. After each plate contained approximately 30 isolated cysts, plates were sealed with electrical tape and put in a zip-lock bag along with a moistened paper towel to reduce evaporative loss from the wells. Plates were then incubated in a 6 °C walk-in incubator. In total, we isolated 10 plates (~300 cysts) of which five were designated as dark-treatment plates prior to isolation. The dark-treatment plates were treated similarly to the light treatments, with a few exceptions. Isolations were done in a dark room under low red ambient light and a red filter (Kodak Wratten Red 25) that blocked light <580 nm was placed in front of the light source of the microscope (isolations for the light treatment were done under normal laboratory illumination and no light filtering on the microscope). All other light-emitting areas on the microscope were wrapped in red plastic film or covered. The sealed plates were wrapped in foil and put in a dark colored plastic container that was put inside a black plastic bag before incubation at 6 °C. Light treatment plates were incubated at 6 °C with a 14 h:10 h light:dark cycle with an irradiation of approximately 250 μmol photons m^{-2} s^{-1}. Cysts incubated in the dark were only exposed to low levels of red light during sediment processing for approximately 30 min, cyst isolation (approximately 45 min at most), and microscopic examination of the plates. For specific wells there was a brief direct exposure during the examination of that well, and then scattered red light during the examination of the rest of the wells. It took approximately 1 h to examine one dark treatment plate during the germination and viability assessments.

2.3. Microscopic examination of isolated cysts

The well plates were checked immediately after they were sealed, using an inverted Zeiss IM35 microscope equipped with a Zeiss 09 filter set (excitation 450–490 nm, emission 515–750 nm) to detect chlorophyll-*a* (Chl-*a*) autofluorescence. Dark plates were

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