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Quantitative Response of *Alexandrium catenella* Cyst Dormancy to Cold Exposure

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Many dinoflagellate cysts experience dormancy, a reversible state that prevents germination during unfavorable periods. Several of these species also cause harmful algal blooms (HABs), so a quantitative understanding of dormancy cycling is desired for better prediction and mitigation of bloom impacts. This study examines the effect of cold exposure on the duration of dormancy in *Alexandrium catenella,* a HAB dinoflagellate that causes paralytic shellfish poisoning (PSP). Mature, dormant cysts from Nauset Marsh (Cape Cod, MA USA) were stored at low but above freezing temperatures for up to six months. Dormancy status was then determined at regular intervals using a germination assay. Dormancy timing was variable among temperatures and was shorter in colder treatments, but the differences collapse when temperature and duration of storage are scaled by chilling-units (*CU*), a common horticultural predictor of plant and insect development in response to weather. Cysts within Nauset meet a well-defined chilling requirement by late January, after which they are poised to germinate with the onset of favorable conditions in spring. Cysts thus modulate their dormancy cycles in response to their temperature history, enhancing the potential for new blooms and improving this species' adaptability to both unseasonable weather and new habitats/climate regimes.

Key words: Resting cyst; dinoflagellate; dormancy; chilling; germination; endogenous clock.

Introduction

The phenology of phytoplankton blooms at higher latitudes is seasonal, a direct consequence of the large changes in temperature and photoperiod that occur over the course of a year and directly affect species' growth. In response, many phytoplankton have evolved specific behavioral and life-cycle strategies for surviving seasonally-induced stress and exploiting periods that are more favorable for growth and reproduction (Ji et al. 2010). An especially common strategy among dinoflagellates is the formation of resting cysts. Dinoflagellate cysts can survive extended periods of unfavorable environmental conditions, and eventually germinate to enter the reproductive, planktonic phase of their life cycle when conditions improve (Dale 1983; Wall 1971). New resting cysts are formed as blooms decline and remain in bottom sediments until some germinate, providing an inoculum for subsequent blooms (e.g. Brosnahan et al. 2017).

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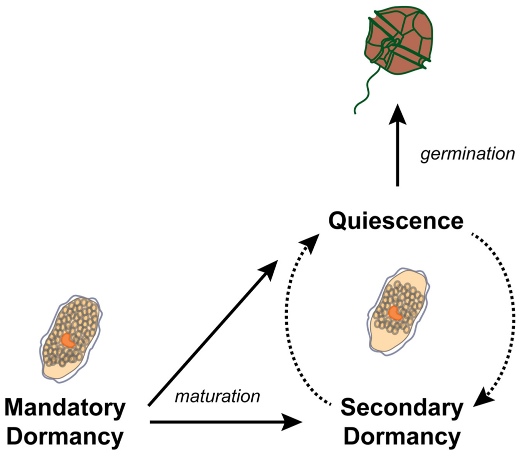

Figure 1. Schematic of dormancy–quiescence transitions in *Alexandrium catenella* cysts. Mandatory dormancy begins at cyst formation and inhibits germination for several weeks to months until cysts have matured (indicated by the loss of starch granules within the cyst on the right). At this point, mature cysts either become quiescent – a state in which they will germinate if environmental conditions are favorable – or may be induced into secondary dormancy by either internal and/or external factors, e.g., via endogenous rhythmicity, external temperature conditioning, or other as yet undiscovered mechanisms.

In many species, cyst germination is inhibited immediately after a bloom and during certain periods of the year because of dormancy (Anderson et al. 2003). Dormancy is an internal state that impedes germination under otherwise adequate environmental conditions and is directly comparable to the dormancy described in the seeds of higher plants (e.g. Bewley et al. 2013). On exit from dormancy, cysts are termed quiescent, a state in which they will germinate if environmental conditions are favorable (Pfiester and Anderson 1987). Among the factors that are essential for germination is temperature, which must be within an optimal range (e.g. Anderson and Morel 1979; Bravo and Anderson 1994; Huber and Nipkow 1923), and oxygen, which must be present (Anderson and Keafer 1987; Kremp and Anderson 2000; Rengefors and Anderson 1998). Two types of dormancy have been described (Fig. 1). The first, termed mandatory dormancy, begins at cyst formation and can last from several weeks to months until maturation is completed (e.g. Anderson 1980; Perez et al. 1998; Pfiester 1977). The second type (termed secondary dormancy) only occurs in mature cysts and can recur multiple times during a cyst's lifetime (Anderson and Keafer 1987; Matrai et al. 2005).

Many dinoflagellates are harmful algal bloom (HAB) species and therefore a quantitative understanding of the internal and environmental factors controlling cyst dormancy is needed to improve bloom prediction and mitigation of impacts. One example is *Alexandrium catenella* (= *A. fundyense*[1]), a species that causes paralytic shellfish poisoning (PSP) in temperate and subarctic coastal areas worldwide (Anderson et al. 2012). Mature *A. catenella* cysts from deep "seedbed" areas of the Gulf of Maine (GOM) cycle between dormancy and quiescence under control of an endogenous circannual clock (Anderson and Keafer 1987; Matrai et al. 2005). This rhythmicity ensures that dormancy is broken prior to the onset of favorable bloom conditions within the euphotic zone, even with dampened seasonal signals at depth. In contrast, dormancy cycling in shallow water populations seems to be determined by cysts' external environment and so has been frequently likened to the secondary dormancy of terrestrial plant seeds (e.g. Anderson and Keafer 1987; Matrai et al. 2005; Rathaille and Raine 2011). In seeds, secondary dormancy is induced by unfavorable environmental conditions and many different species annually cycle between secondary dormancy and quiescence (e.g. Batlla and Benech-Arnold 2010). Here, we examine an *A. catenella* cyst population from a shallow temperate estuary. In previous studies of dinoflagellate cysts, secondary dormancy and endogenous rhythmicity have been juxtaposed as opposing mechanisms of dormancy control, yet our results suggest that a common feature may underlie both behaviors – a chilling response detailed herein.

Chilling has previously been shown to shorten dormancy and improve the viability of dinoflagellate cysts (Montresor and Marino 1996; von Stosch 1967, 1973), suggesting that cysts may have a chilling requirement much like the seeds of many terrestrial plants (e.g. Vegis 1964). The goal of this study was to determine if *Alexandrium* cysts have such a chilling requirement for exit from dormancy, and if so, to determine how it regulates germination in the context of environmental temperature fluctuations. Here, we present the results of a series of experiments that applied different cold storage temperatures to naturally-formed *A. catenella* cysts.

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[1] *Alexandrium catenella* and *A. fundyense* are conspecific, and "*catenella*" was determined to have nomenclatural priority (Prud'homme van Reine 2017).