Cyst-forming dinoflagellates in a warming climate

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Highlights

- Temperature controls dormancy cycling in dinoflagellate resting cysts
- Dormancy-climate interactions can explain HAB biogeography and phenology
- Temperature seasonality promotes resilience of resting cyst populations to warming
- Cell density dependent encystment triggers can limit bloom intensification
- Longer HABs in response to warming will reflect prolonged cyst bed quiescence

Abstract

Many phytoplankton species, including many harmful algal bloom (HAB) species, survive long periods between blooms through formation of benthic resting stages. Because they are crucial to the persistence of these species and the initiation of new blooms, the physiology of benthic stages must be considered to accurately predict responses to climate warming and associated environmental changes. The benthic stages of dinoflagellates, called resting cysts, germinate in response to the combination of favorable temperature, oxygen-availability, and release from dormancy. The latter is a mechanism that prevents germination even when oxygen and temperature conditions are favorable. Here, evidence of temperature-mediated control of dormancy duration from the dinoflagellates *Alexandrium catenella* and *Pyrodinium bahamense*—two HAB species that cause paralytic shellfish poisoning (PSP)—is reviewed and presented alongside new evidence of complementary, temperature-based control of cyst quiescence (the state in which cysts germinate on exposure to favorable conditions). Interaction of the two temperature-based mechanisms with climate is explored through a simple model parameterized using results from recent experiments with *A. catenella*. Simulations demonstrate the importance of seasonal temperature cycles for the synchronization of cysts' release from dormancy and are consistent with biogeography-based inferences that *A. catenella* is more tolerant of warming in habitats that experience a larger range of seasonal temperature variation (i.e., have higher temperature seasonality). Temperature seasonality is much greater in shallow, long-residence time habitats than in deep, open-water ones. As warming shifts species' ranges, cyst beds may persist longer in more seasonally variable, shallow inshore habitats than in deep offshore ones, promoting HABs that are more localized and commence earlier each year. Recent field investigations of *A. catenella* also point to the importance of new cyst formation as a factor triggering bloom termination through mass sexual induction. In areas where temperature seasonality restricts the flux of new swimming cells (germlings) to narrow temporal windows, warming is unlikely to promote longer and more intense HAB impacts—even when water column conditions would otherwise promote prolonged bloom development. Many species likely have a strong drive to sexually differentiate and produce new cysts once concentrations reach levels that are conducive to new cyst formation. This phenomenon can impose a limit to bloom intensification and suggests an important role for cyst bed quiescence in determining the duration of HAB risk periods.

Key words: microbial life cycles; climate change; resting cyst dormancy

1. Introduction

Many harmful algal bloom species have benthic resting stages in their life histories. Prominent among this group are cyst-forming dinoflagellates like *Alexandrium catenella* and *Pyrodinium bahamense*, two marine species that cause paralytic shellfish poisoning (PSP) through their production of saxitoxins, a potent class of sodium channel-blocking compounds that cause illness and sometimes death to human consumers of contaminated seafood. Therefore, understanding the factors that control bloom timing, intensity, and biogeography of *A. catenella*, *P. bahamense*, and other PSP-causing species has been an important focus for managers and researchers aiming to ensure seafood safety and protect human health (Hallegraeff, 2010).

Numerous works have emphasized the role of cysts in the ecology of *A. catenella* and *P. bahamense*, and studies of these species have contributed greatly to what is known about the role of benthic coupling in phytoplankton ecology (Azanza et al., 2018; Fig. 1). For instance, the locations of benthic ‘cyst beds’—areas where resting cysts accumulate in sediments—often determine where blooms occur (e.g., Anderson and Keafer, 1985; Corrales and Crisostomo, 1996; Azanza et al., 2004; Anderson et al., 2005a, 2014). Bloom timing—both initiation and termination—is also strongly associated with exit from and return to the resting cyst stage of the life cycle through the processes of germination and encystment, respectively (Fig. 1; Wall, 1971; Anderson et al., 2014; Moore et al., 2015a; Brosnahan et al., 2017; Lopez et al., 2019). Cyst beds also serve as reservoirs of genetic diversity, making cyst-formers more resilient to environmental change and enabling them to persist longer in the face of interannual climate variability (e.g., Kremp et al., 2016). While particularly well described in dinoflagellates, benthic life history stages are important for the ecology of other classes of phytoplankton as well, including diatoms (McQuoid and Hobson 1996; Lewis et al., 1999) and cyanobacteria (Livingstone and Reynolds, 1980; Huber, 1984; and Cirés et al., 2017). We focus on *A. catenella* and *P. bahamense* here to highlight recent advances in the understanding of potential climate responses by their resting cysts and to encourage greater consideration of the role of benthic and other non-dividing life cycle stages in predictions about phytoplankton responses to climate change.

The extent to which climate change is affecting HABs has been a major question facing scientists and resource managers for decades (Anderson, 1989; Hallegraeff, 1993, 2010; Wells et al., 2015). Temperature drives the rate of a broad range of microbial processes, including many physiological rates and behaviors that are fundamental to HAB dynamics. A major focus of HAB climate studies has been the effect of warmer temperatures on planktonic, vegetative life stages (e.g., Moore et al., 2008; Wells et al., 2015; Gobler et al., 2017; Seto et al., 2019). Cyst-forming species spend most of their lives in the sediments as resting cysts and only a small fraction of their lives as plankton. Therefore, the factors governing cyst dynamics and survival must be understood and considered to accurately predict these species' responses to warming. In this work, we review the factors known to control cyst germination and explore the implications of newly described temperature-based mechanisms controlling transitions between states of dormancy and quiescence (hereafter referred to as dormancy cycling). We also present recent evidence that intensification of blooms by some cyst-forming species may be limited by an underlying drive to produce new cysts. Finally, we revisit the "window of opportunity" hypothesis (Moore et al., 2008), which predicts earlier and longer lasting blooms as temperatures become increasingly favorable for the growth and division of planktonic vegetative cells.

The window of opportunity hypothesis is built upon a four-decade long record of PSP toxin concentrations in shellfish tissues from *A. catenella* in Puget Sound, WA USA. Examination of PSP records in the mussel *Mytilus edulis* from 1993–2007 found that shellfish harvesting closures occurred earlier in the year (Moore et al., 2009) and are projected to extend an additional 13–30 days into the spring by the end of the 21st century (Moore et al., 2011; Moore et al., 2015b). The hypothesis is based on lengthening periods of conditions that support vegetative cell growth, but other life cycle stages are affected by changing temperature as well (Fig. 1). The consequences of these temperature effects, if not considered, may reduce the accuracy of bloom season projections and limit the generalizability of the window of opportunity hypothesis to other habitats impacted by *A. catenella*, *P. bahamense*, and other cyst-forming species. Consideration of the effect of warming on resting cysts is especially important because they are long-lived and endure nearly the full range of temperatures occurring in many bloom habitats (Fig. 2). Resting cysts also respond to climate in ways that are distinct from planktonic life cycle stages.

Dinoflagellate resting cysts do not grow or divide and may undergo passive mixing within sediments for several decades before they germinate and develop blooms of planktonic vegetative cells (Keafer et al., 1992; Kremp et al., 2000; Feifel et al., 2015). Exit from the resting cyst stage is tightly controlled by both internal and external factors. While buried in sediment, they are prevented from germinating by lack of oxygen (Anderson et al., 1987), a response that ensures germlings only emerge when they have a reasonable chance of returning to the water column. Cyst germination is also inhibited by cold temperatures, preventing germination during wintertime when both light period and water temperature do not support bloom development (Anderson et al., 2005a). Finally, resting cysts cycle between states of quiescence, when they will germinate if exposed to favorable external conditions (e.g., temperature, oxygen; Rengefors and Anderson, 1998; Kremp et al., 2000), and dormancy, when they will not. In temperate systems, this internal mechanism provides an additional barrier to wintertime germination, preventing cysts from responding to occasional spells of unseasonably warm weather. Dormancy also prevents germination late in bloom seasons when germling cells are less likely to successfully form blooms and re-encyst.

There are two distinct types of dormancy in *A. catenella* and *P. bahamense* resting cysts. The first, called mandatory dormancy, occurs immediately after cyst formation and is understood as a maturation period that is required for cysts to germinate (e.g., Anderson and Morel 1979). The second, called secondary dormancy, is the reversible state that underlies dormancy cycling and can recur many times within a single cyst’s lifetime (Fig. 1; Fischer et al., 2018). Prior examinations of *A. catenella* and other dinoflagellates have pointed to an endogenous biological rhythm or “clock” as the mechanism controlling the recurrence of secondary dormancy (Anderson and Keafer, 1987; Rengefors and Anderson, 1998; Matrai et al., 2005). However, more recent work has shown that the duration of secondary dormancy is set by temperature (Fischer et al., 2018; Lopez et al., 2019). A second temperature-based mechanism, shown in *P. bahamense* by Lopez et al. (2019) and parameterized for the first time in this work through experiments with *A. catenella*, controls the duration of quiescence. Together, the two relationships can drive dormancy cycles that are qualitatively similar to past observations of endogenous rhythmicity in the dormancy cycles of *A. catenella* resting cysts. The combination of temperature-based dormancy cycling control and exogenous (temperature- and oxygen-based) triggers for germination of quiescent resting cysts ensures that germination is restricted to times of year and positions within sediments that are favorable for the development of new planktonic blooms.

This work does not aim to make specific predictions regarding changes in the occurrence of blooms of *A. catenella*, *P. bahamense*, or other cyst-forming species in response to global warming. In most cases, the factors driving species' responses to climate change will be far more complex than cells' and cysts' responses to warmer temperatures alone. Climate change is also altering coastal ocean circulation, rainfall, winds, water stratification, incidence of hypoxia/anoxia, and other region- and ecosystem-specific factors, all of which impact HAB ecology in distinct ways. Accurate predictions require investments in persistent, long-term data collection that build upon and complement field-based studies of bloom ecology at a broad range of geographic scales (Ralston and Moore, this issue). However, we do highlight one interesting corollary of warming in many temperate regions, namely increased temperature seasonality, i.e., the difference between summer- and wintertime temperature extremes (Fig. 2). Warming and seasonality have both increased steadily across North America and Eurasia in recent decades (e.g., Santer et al., 2018), and these changes especially impact shallower, inshore habitats where water temperature more closely tracks air temperature. Many species, including both *A. catenella* and *P. bahamense*, also occur across a range of habitats that can differ substantially in the amount of temperature seasonality they experience.

Cyst beds within shallow, long residence time inshore embayments tend to have higher temperature seasonality than those in deep open water areas (Fig. 2). We explore the impact of changes in habitat temperature and temperature seasonality through a simple model that is drawn from experiments with *A. catenella*. Simulations illustrate how cysts' temperature-based dormancy controls may interact with a range of climates. Among the many consequences of dormancy-climate interaction is heightened synchronization of cyst beds with increased temperature seasonality. Under higher temperature seasonality, cyst beds produce greater fluxes of germlings but during narrower temporal windows. A well-studied example that compares favorably with model simulations is the Nauset Marsh (Cape Cod, MA USA), an area that experiences annually recurrent *A. catenella* blooms each spring. In contrast, the model shows lower seasonality produces lesser germling fluxes over longer time spans, desynchronizing populations and promoting the development of successive blooms within a single bloom season. Such may be the case in Puget Sound, an *A. catenella* habitat that experiences far lower temperature seasonality than Nauset and is subject to a much longer annual window of PSP risk.

While the model draws from experiments with *A. catenella*, similar experiments with *P. bahamense* (Lopez et al., 2019) suggests that it may interact with climate in comparable ways. Because this climate-dormancy interaction is only recently discovered in dinoflagellates, knowledge of its effects on the timing and duration of blooms remain to be extended to the broad diversity of dinoflagellates and other meroplanktonic phytoplankton that cause HABs. However, given the global distribution of *A. catenella* and *P. bahamense* (Fig. 3), we suggest that many other cyst-forming species have similar mechanisms for adaptation to climate variability. With the larger goal of encouraging broad consideration of species' life cycles in climate-based predictions, we also present observations related to the production of new cysts. Much like the case of temperature-mediated dormancy cycling, field observations of new cyst formation are limited, but research with *A. catenella* points to a deep-rooted drive to produce new resting cysts during blooms. This encystment drive can impose an upper limit to bloom intensification that is independent of more commonly invoked factors like nutrients and light. The combination of dormancy control and encystment mechanisms may constrain the duration of blooms by cyst-forming species even as climate change promotes conditions that are increasingly favorable for growth and division by the vegetative stage cells of these species.

3. Model species

Alexandrium catenella. *Alexandrium* is one of the most intensively studied HAB genera globally because its species cause most incidents of PSP (Cembella 1998; Anderson et al., 2014). *Alexandrium catenella* is the most widespread of those that produce saxitoxins and was recently the subject of a reclassification involving all species in the "*Alexandrium tamarense* species complex" (John et al., 2014). The final recommendation of the ICN Nomenclature Committee for Algae was that the name *A. catenella* supplant two synonymous names—*A. fundyense* and *A. tamarense* Group I—that had come into common use as a way to differentiate this species from a closely allied sister that was also commonly identified as *A. catenella* but is now known as *A. pacificum* (Prud'homme van Reine, 2017; Litaker et al., 2018).

The overall range of *A. catenella* spans temperate, subarctic, and Arctic waters (Fig. 3). In North America, blooms of *A. catenella* occur along the Pacific Coast from Alaska to California and along the Atlantic Coast from the Gulf of St. Lawrence to Long Island, NY. In South America, blooms occur from central Chile to Tierra del Fuego, and from the northern Argentine Sea to the Magellan Strait. The species also occurs in the Benguela Current region off Namibia and South Africa, in northern regions of east Asia, including Japan, Korea, and the Kamchatka Peninsula of Russia, and in Europe along the northern coasts of the United Kingdom and the west coasts of Norway and Sweden (Lilly et al., 2008). Recent studies have documented *A. catenella* vegetative cells and cysts in the Arctic north of Alaska and Canada (Gu et al., 2013; Natsuike et al., 2013; 2017; Okolodkov, 2005; D. Anderson, unpub. data), Iceland (Burrell et al., 2013), and northwestern Greenland (Baggesen et al., 2012). Along the west Greenland coast, *A. catenella* cysts are present at low concentrations up to 76°N (Richlen et al., 2016; D.M. Anderson, unpub. data).

The timing of blooms varies across this expansive domain and across habitats within single regions. For example, in the western Gulf of Maine, blooms begin in May and last approximately 3 months (Anderson et al., 2014), yet in some shallow inshore embayments within the same region, blooms begin as early as March and typically end 6–8 weeks later (Ralston et al., 2014). Cyst concentrations in these habitats often exceed 10^3 cysts g^{-1} of wet sediment (Anderson et al., 2005a, 2014; Crespo et al., 2011). Cell concentrations regularly exceed 10^5 L^{-1} within inshore blooms (Crespo et al., 2011; Anglès et al., 2014; Ralston et al., 2014), but are typically much lower within offshore populations where peak concentrations are on the order of 10^3 cells L^{-1} (Stock et al., 2005). In contrast, the location, toxicity, and timing of *A. catenella* blooms in Puget Sound exhibits considerable interannual variation within an approximately 5-month long bloom season (Moore et al., 2009) though peak cell concentrations are comparable to those of inshore blooms in the northeast U.S. (Dyhrman et al., 2010). Across its range, *A. catenella* vegetative cells are absent from the water column more often than not, and therefore cyst beds are the most likely source of new blooms rather than revival of remnant vegetative cell populations from the water column.

Pyrodinium bahamense. *Pyrodinium bahamense* is the most common cause of PSP toxicity in tropical and sub-tropical marine waters (Fig. 3). It is a monotypic genus, and Steidinger (2018) recommends distinguishing between its Atlantic and Pacific forms. Blooms occur in many areas of the western Pacific (Furio et al., 2012, Usup et al., 2012), the Persian Gulf, and the Red Sea (Alkawri et al., 2016, Banguera-Hinestroza et al., 2016), as well as in the southeastern U.S. (Phlips et al., 2006, 2011), the Caribbean Sea (Soler-Figueroa and Otero, 2014), Central America (Chow et al., 2010), the Gulf of California (Morquecho et al., 2014), and the Pacific coast of Mexico and southwestern Gulf of Mexico (Morquecho, 2019). Descriptions of *P. bahamense* blooms have been largely restricted to inshore and nearshore coastal areas. Its resting cysts, though, are widespread and abundant relative to other species in both coastal (near where blooms are observed) and deep ocean sediments (Wall, 1967; Limoges et al., 2013; Zonneveld et al., 2013). This distribution may reflect high rates of production and transport of cysts by coastal blooms alone or the occurrence of as yet undetected bloom populations further from shore.

To date, *P. bahamense* ecology has been explored most extensively in the Philippines, where blooms are strongly linked to resting cyst dynamics (i.e. resting cyst abundance, cyst bed locations; Villanoy et al., 1996; Azanza et al., 2004; Siringan et al., 2008; Azanza, 2013). Blooms within Manila Bay and Sorsogon Bay can be especially intense and persist from weeks to months. Water temperatures in the region are favorable for growth throughout much of the year, but the species can be absent from the plankton for long periods. Generally, blooms in the Philippines develop in late summer, a period that marks the start of the southwest monsoon and coincides with more stratified conditions. In other parts of east Asia, blooms occur more sporadically, sometimes with cells present year-round or in multiple peaks within a year (Azanza and Taylor, 2001).

Both vegetative cells and resting cysts have been recorded along the coasts of the U.S. state of Florida, in the Caribbean, and along the coast of Mexico with differences in bloom phenology linked to latitude across the region (Morquecho, 2019). In Florida, high biomass blooms of toxic *P. bahamense* (Atlantic) occur almost every summer in the shallow, estuarine systems of northern Tampa Bay and Indian River Lagoon and more sporadically and at lower abundances in Pine Island Sound, Florida Bay, and other areas of Florida (Phlips et al., 2006, FWC FWRI HAB Monitoring Database). In Tampa Bay, cell concentrations are highest where resting cysts are concentrated ($>10^3$ cysts g^{-1} of wet sediment, Lopez et al., 2017) and water residence times are long (Meyers et al., 2017). Extensive surveys of resting cyst abundance have not been conducted in other areas of Florida, but concentrations of 300–900 cysts g^{-1} of wet sediment are common in Indian River Lagoon sediments and lesser concentrations (~10 cysts g^{-1} of wet sediment) have been recorded in Pine Island Sound (C. Lopez, unpub. data). Tampa Bay and Indian River Lagoon blooms are strongly seasonal—typically beginning in spring, peaking in late summer, and ending during the fall. Peak concentrations (above 10^5 cells L^{-1}) generally persist between two and four months, resulting in ecosystem degradation through shading of seagrass beds and degraded water quality (Lopez et al., 2019, FWC FWRI HAB Monitoring Database). Additionally, in the Indian River Lagoon, extensive shellfish harvesting closures occur each year and harvesting of pufferfish is permanently closed to prevent saxitoxin puffer fish poisoning (SPFP) in humans (Landsberg et al., 2006). In the tropical waters of Puerto Rico, *P. bahamense* is generally present in the water column year-round, and in contrast to Florida, peak concentrations are lower with no clear seasonal signal, although lowest cell concentrations tend to occur more commonly in the dry months (Sastre, 2013, Soler-Figueroa and Otero, 2016). Likewise, *P. bahamense* blooms in bays in Mexico along the southern Gulf of Mexico tend to be present year-round whereas populations in the Gulf of California are more seasonal (Morquecho et al., 2019).

4. Dinoflagellate life cycles

Like most other dinoflagellates, *A. catenella* and *P. bahamense* are haplontic (Fig. 1). Motile, haploid, vegetative cells divide and accumulate in euphotic waters until they are induced to produce gametes that fuse to form swimming diploid cells (planozygotes). Planozygotes then transform into benthic resting cysts, also called hypnozygotes (Anderson and Wall 1978; Pfiester and Anderson, 1987). All resting cysts are highly resistant to temperature and other environmental stressors, but morphology varies among species—*Alexandrium catenella* resting cysts are smooth, elongate, double-walled cells, whereas *P. bahamense* resting cysts are spheroid and covered with distinctive, trumpet-shaped spines (Fig. 4). The mandatory dormancy period of newly formed resting cysts is similar in these species—1–3 months for *A. catenella* (Anderson and Morel, 1979) and 2.5–3.5 months for Pacific populations of *P. bahamense* (Corrales et al., 1995)—despite very different temperature regimes in their respective habitats. Also noteworthy is that mandatory dormancy in *A. catenella* is shorter at warmer temperatures (Anderson, 1980), the opposite relationship from what has been shown for secondary dormancy (Fischer et al., 2018). Germination of a resting cyst produces a planomeiocyte, a short-lived germling stage that reverts back to the mitotically dividing haploid, vegetative stage through a series of meiotic divisions (von Stosch, 1967, 1973).

Resting cysts tend to accumulate in areas that collect fine sediment to form cyst beds (e.g. Anderson et al., 2014; Karlen and Campbell, 2012). There, resting cysts can remain viable for decades, particularly when sediments are anoxic (Keafer et al., 1992; Siringan et al., 2008; Feifel et al., 2015). Within these areas, resting cysts can cycle between states of secondary dormancy and quiescence many times during their lifetimes—a process that may be under control of an endogenous rhythm (Anderson and Keafer, 1987; Matrai et al., 2005) and/or responsive to seasonally varying temperature (Anderson and Keafer, 1987; Rathaille and Raine, 2011; Fischer et al., 2018; Lopez et al., 2019; Moore et al., 2015a). The physiological and molecular underpinnings of dormancy cycles are yet to be described in phytoplankton, but endogenous rhythmicity might preserve germination control in habitats where seasonal signals are absent or muted (e.g., in deep water habitats). Alternatively, temperature-mediated controls may determine rhythm periods (i.e., the time between successive quiescence intervals) by setting the duration of its dormancy and quiescence phases. It is noteworthy that the endogenous rhythm described in Gulf of Maine *A. catenella* is less than one year (~11 months; Anderson and Keafer, 1987; Matrai et al., 2005). Were dormancy cycles solely under the control of this rhythm, resting cysts would enter quiescence increasingly out of phase with optimal growth periods over the course of several years—which would be clearly disadvantageous. Even in the deep cyst beds of the Gulf of Maine (~100 m depths), resting cysts experience seasonal changes in temperature that may override endogenous rhythmicity (Fischer et al., 2018), and in the case of Puget Sound populations, temperature appears to play a more significant role than endogenous rhythmicity (Moore et al., 2015a). In sub-tropical *P. bahamense*, evidence from germination experiments with multiple cohorts of resting cysts have pointed only to temperature-mediated control of secondary dormancy rather than an endogenous mechanism (Lopez et al., 2019).

Both *A. catenella* and *P. bahamense* also produce haploid, pellicle cysts (sometimes called temporary cysts) directly from vegetative cells when exposed to acute stress (e.g., Anderson and Wall 1978; Onda et al., 2014). Pellicle cysts cannot survive long burial periods but can promote recovery and resumption of blooms challenged by ephemeral stressors (e.g., major storms; Azanza, 2013). Increasing frequency of bloom-disruptive events like storms, heatwaves, and cold snaps may favor species that can form pellicle cysts. Indeed, this life history stage may become more prevalent as temperatures warm due to global change. Better understanding of the factors that govern the formation, viability, and germination success of pellicle cysts is therefore needed. However, since dormancy cycling has not been described for pellicle cysts, the discussion presented here is focused on the longer-lived, diploid resting cysts of these two species. All references to 'quiescent cysts' and 'dormant cysts' in this work refer exclusively to resting cysts since it is only the resting cyst life cycle stage that has been shown to experience quiescence and dormancy.

5. Roles of temperature in cyst dormancy and germination

Temperature has been long recognized as an important determinant of cyst dormancy and germination in both freshwater and marine dinoflagellates (Huber and Nipkow, 1922; Binder and Anderson, 1987; Bravo and Anderson, 1994). Rengefors and Anderson (1998) showed how the interaction of endogenous dormancy cycling and the temperature-mediated rate of germination could explain the appearance of the freshwater dinoflagellates *Ceratium hirundinella* and *Peridinium aciculiferum* in the plankton. Germination in these species only proceeds when temperatures fall within a species-specific range; higher and lower temperatures inhibit the germination of quiescent cysts, blocking the introduction of new cells to the water column. Subsequent work by Anderson and Rengefors (2006) extended this concept to six marine species, including *A. catenella*, and found they would not germinate at either low (<5 ˚C) or high (>21 ˚C) temperatures. Later experiments found that *A. catenella* germination rates within the temperature "window" generally increased with temperature and converged asymptotically toward minimum and maximum rates at temperature window boundaries (e.g., Anderson et al., 2005a; Fig. 5). Onset of inhibition at warm temperatures may instead be related to rapid induction of dormancy (discussed below). Similarly, quiescent *P. bahamense* cysts will germinate across the full range of seasonal temperatures experienced in their habitats, but much more slowly in colder conditions (e.g., wintertime, ~17 ˚C in Tampa Bay; Lopez et al., 2016).

Temperature control of germination interacts with anaerobic inhibition to further constrain the flux of plankton into the water column. Oxygen is required for cysts to germinate (Anderson et al., 1987), and the germination rate drastically declines at oxygen concentrations <2 mg L^{-1} (Montani, 1995). As sediments warm, microbial respiration rates increase, reducing oxygen availability in subsurface sediments and constraining fluxes of germling cells. Sediments in productive shallow coastal waters, which represent most cyst beds, are generally characterized by oxygen penetration depths of millimeters (Glud et al., 1994), thus restricting the number of resting cysts that can successfully germinate. Seasonal variations in oxygen penetration are driven by temperature, resulting in the shallowest penetration in summer due to rapid aerobic respiration and fresh detrital inputs. The deepest oxygen penetration occurs in winter due to reduced oxygen demand (Kristensen, 2000), but low wintertime temperatures also inhibit germination (Anderson et al., 1987; Anderson et al., 2005a). Recent investigations using plankton emergence traps in Nauset Marsh suggest that only a small fraction of oxygenated *A. catenella* resting cysts (i.e., those from the uppermost ~1 mm of sediment) germinate in spring, in spite of much deeper wintertime sediment oxygenation (D. M. Anderson, unpub. data). Similar seasonal anoxia also limits germination of *A. catenella* and other dinoflagellates elsewhere on Cape Cod (Keafer et al., 1992; Anderson and Rengefors, 2006). In the case of quiescent cysts that are buried more deeply, germination is frequently inhibited by both temperature (high or low) and anoxia.

The first evidence for an additional role of cold in releasing resting cysts from dormancy was noted by von Stosch, who found that storage at 3˚C both increased the fraction of germinable cysts and reduced the incubation times required for *Ceratium* (1967), *Gymnodinium*, and *Woloszynskia* (1973) species to germinate. Another study by Montresor and Marino (1996) noted more synchronous germination of *A. pseudogonyaulax* cysts after storage at 7˚C for 40–100 days. More recent studies have confirmed that cold exposure reduces the duration of dormancy in both *A. catenella* (Fischer et al., 2018) and *P. bahamense* (Lopez et al., 2019; Fig. 6). This inverse relationship between temperature and the duration of secondary dormancy is opposite to most other physiological rates (i.e., germination, cell division, and new cyst maturation), which tend to proceed faster at elevated temperatures (at least up to an upper physiological limit).

To date, *A. catenella* is the only species for which the relationship between cold exposure and secondary dormancy passage has been examined quantitatively (Fischer et al., 2018; D.M. Anderson, A.D. Fischer, and M.L. Brosnahan, unpub. data). In a series of experiments with cysts from Nauset Marsh, the duration of dormancy was shown to vary inversely with storage temperature (i.e., colder cysts passed through dormancy more quickly than warmer ones). This relationship between the severity and duration of cold exposure follows a simple chilling-unit model that is commonly used in horticulture, e.g., to describe vernalization in some bulbs (Fischer et al., 2018). *A. catenella* resting cysts exit dormancy by accumulating a set number of chilling units (*CU*), calculated as the integral over time (*t*) of the difference in ambient temperature (*T*) from a chilling threshold temperature (T_c):

$$CU = \sum_{i=t_0}^{t} \begin{cases} (T_c - T_i)\Delta t & \text{if } \; T_c \geq T_i \geq 0 \\ 0 & \text{if } \; otherwise \end{cases}$$

(Eq. 1)

Built into this model are two physiological parameters: the chilling threshold temperature, T_c, which determines the upper limit at which a resting cyst population registers cold exposure, and a chilling requirement, which is the total *CU* needed for transition to quiescence. Nauset *A. catenella* have T_c=15˚C and a chilling requirement of ~800 *CU* (Fig. 7). A subsequent cold storage experiment has confirmed similar dormancy shortening in *A. catenella* from a deep cyst bed in the Gulf of Maine, but further development of the chilling model is needed to determine whether T_c and chilling requirements differ significantly between the Nauset and Gulf of Maine populations (D.M. Anderson, unpub. data). Similar characterizations of other *A. catenella* populations are ongoing and aim to assess if and how their chilling responses can be generalized globally, or instead are region- or population-specific. Comparable experiments with *P. bahamense* suggest that the relationship between its dormancy duration and cold severity is weaker, such that dormancy passage may proceed at a similar rate across a range of chilling temperatures (Lopez et al., 2019, C. Lopez unpub. data). Further exploration of these responses in *P. bahamense* and other species is needed to characterize the nature of chilling requirements across a wider diversity of cyst-forming species.

Like secondary dormancy, the duration of quiescence is also temperature sensitive. The first evidence of this was noted by Anderson and Rengefors (2006) who found that temperatures in excess of 18.5˚C inhibited *A. catenella* germination. Lopez et al. (2019) further showed that quiescent *P. bahamense* cysts returned to dormancy after one month of storage at 30˚C but remained quiescent when stored at 15˚C (Fig. 6). A follow-up study of the relationship between quiescence duration and temperature in *A. catenella* has revealed that quiescent cysts are induced into secondary dormancy more quickly when stored at warmer temperatures and that this relationship follows a heating degree-day (*DD*) formula (Brosnahan et al., in prep). *DD* are calculated as the time integral of temperature above a heating threshold value, T_h:

$$DD(t) = \sum_{i=t_0}^{t} (T_i - T_h)\Delta t \text{ if } T_h > 0$$

(Eq. 2)

The same formulation is commonly applied in agricultural applications to predict the seasonal maturation of plants and insects, and in a prior study, was shown to accurately predict the timing of both PSP toxicity and *A. catenella* bloom peaks in the Nauset Marsh (Ralston et al., 2014).

In the *A. catenella* quiescence experiment (Brosnahan et al., in prep), dormant cysts from a deep cyst bed in the Gulf of Maine were induced into quiescence through cold, anoxic storage at 2 ˚C. Once quiescent (i.e., >90% cysts germinating within 1 week of transfer to favorable conditions), the population was split into three subsamples and warmed at 1 ˚C day^{-1} up to final storage temperatures of 10, 15, and 20 ˚C. The dormancy state of resting cysts in each of the storage temperature treatments was assessed at regular intervals by removing subsamples of approximately 30 from each of the storage treatments and exposing these to oxygen in a 15 ˚C incubator. If the resting cysts germinated within 1 week of exposure to these favorable conditions they remained quiescent. If they did not germinate, they had returned to dormancy. Most resting cysts in the warmest 20 ˚C treatment returned to dormancy within 30 days, while those in the cooler 15 and 10 ˚C treatments returned to dormancy after 40 and 60 days, respectively (Fig. 7). Applying a T_h threshold of 0 ˚C, cysts have an estimated heating requirement of 600 *DD* for induction of secondary dormancy (Fig. 7). The result indicates two additional physiological parameters, T_h and heating *DD* requirement, to describe the rate of quiescence passage.

The effect of temperature on quiescence is opposite to that on secondary dormancy, i.e., quiescence is longer at colder temperatures and shorter at warmer ones. In combination, these heating and chilling relationships point to several simple predictions regarding cyst bed behavior in different climates. First, and perhaps counter-intuitively, dinoflagellate cyst beds are more responsive (i.e., germinate at higher rates) during spells of favorable bloom conditions in colder habitats than in warmer ones. This is because colder temperatures promote cyst quiescence through the cysts' chilling response. Second, the relationships point to an important role for temperature seasonality in determining the synchrony of cyst beds. Cyst populations that experience larger excursions from T_h and T_c thresholds throughout the year— that is, more extreme cold and warmth— will accumulate *CU* and *DD* more quickly during these periods, reducing the differences in the timing of cysts' dormancy and quiescence transitions that arise from small physiological or microhabitat-related differences. Lastly, chilling and degree-day relationships likely underlie (or interact with) endogenous dormancy rhythms observed in cysts from the Gulf of Maine (Anderson and Keafer, 1987; Matrai et al., 2005). The extent to which these mechanisms overlap or reinforce one another remains to be explored and may resolve long-standing conflicts between observations of dormancy cycles in deep water and inshore cyst populations (e.g., Anderson and Keafer, 1987; Moore et al., 2015a; Fischer et al., 2018).

6. Interaction of temperature-mediated controls of secondary dormancy and climate

Chilling- and heating-based controls of secondary dormancy can drive complex responses by cyst beds. This is most easily illustrated through a model, presented here, that combines these relationships using physiological parameters drawn from investigations of *A. catenella*. In the model, passage through secondary dormancy is controlled by chilling accumulation (Eq. 1) with T_c=15 ˚C and a mean chilling requirement of 900 *CU*. Quiescence passage is controlled by the degree-day relationship (Eq. 2) with T_h=0 ˚C and a mean heating requirement of 600 *DD* (Fig. 8). The model is evaluated by considering a large population of cysts with independent, normal variance in their chilling and heating requirements (standard deviation set to 10% of requirement means) reflecting intrinsic and extrinsic differences among resting cysts within a population. Initially, resting cysts are completely synchronized (e.g., 100% quiescent with 0 degree-day accumulation) and are tracked through 100 years of annual temperature fluctuations to assess whether and how dormancy cycles stabilize under regular seasonal forcing. One hundred-year simulations neglect contributions from new resting cysts but were chosen because they produce realistic and stable distributions of secondary dormancy states within model populations. The omission of new resting cysts is an important caveat. Beds that are disproportionately comprised of recently formed resting cysts may behave quite differently, especially if the recently formed cysts exit mandatory dormancy out of resonance with their environment. Little is known about the age structure within cyst beds (e.g., Keafer et al., 1992; Shull et al., 2014), and therefore the model is primarily aimed at exploring the interplay of chilling and heating mechanisms with climate rather than predicting the behavior of resting cyst populations in situ.

Under constant temperature forcing, model cysts' quiescence intervals are shorter in warmer treatments than in colder ones (Fig. 9). Resting cysts at the coldest temperature (2 ˚C) are quiescent 82.1% of the time, whereas those at the warmest temperature (13 ˚C) are quiescent only 9.9% of the time. This type of forcing is similar to storage treatments used in investigations of endogenous rhythmicity in *A. catenella*. Similar to Gulf of Maine resting cysts, initially synchronized model populations exhibit rhythmic phasing of dormancy and quiescence (Fig. 10; Anderson and Keafer, 1987, Matrai et al., 2005). Warmer populations never reach 100% quiescence and return to full dormancy more frequently than colder ones. Notably, the length of the dormancy cycling period varies nonlinearly with temperature. The time between quiescence peaks is shortest for simulated resting cysts at constant 7.5 ˚C and longer for colder and warmer populations (e.g., 2 and 13 ˚C, Fig. 10). Additionally, in all temperature treatments, the cycle period grows longer with model time. Resting cysts at constant 2 ˚C undergo an initial cycle that is 11.5 months long, similar to natural populations from deep water beds in the Gulf of Maine (~11 months; Anderson and Keafer, 1987), but second and third periods are 12.4 and 12.7 months. Oscillations between dormancy and quiescence are also increasingly damped, such that periodicity is hardly evident after year 6. The same damping occurs in other temperature simulations as well, pointing to the importance of temperature seasonality to establish and reinforce dormancy phasing under the heating/chilling model.

In contrast to the constant temperature simulations, model simulations with seasonally varying temperatures drive phasing of dormancy cycles that stabilize over time. Seasonally varying temperatures also prolong quiescence within individual resting cysts. This is illustrated through results from populations forced by four distinct climate regimes (Fig. 11). Regimes 1–3 have the same temperature seasonality (± 3 ˚C) but mean temperatures of 7, 10, and 13 ˚C, respectively. Regimes 1 and 2 are comparable to temperature conditions experienced by cyst beds in the Gulf of Maine (7.4 ± 3.9 ˚C) and Puget Sound (10.3 ± 2.3 ˚C), respectively. Regime 3 is presented as a potential warming scenario for either Regime 1 or 2. Regime 4 has the same mean temperature as the warming scenario Regime 3 but larger seasonality (13 ± 10 ˚C), similar to Nauset Marsh (12.5 ± 11.5 ˚C; Fig. 2). Among these temperature regimes, all but Regime 3 settle into patterns of regular, phased dormancy cycles within five years of initiation (i.e., years 5–100 are highly similar within each regime; Fig. 11). In Regimes 1, 2, and 4, single annual peaks in quiescence are centered during winter but vary in magnitude from 58 to 100% of the total population. While the warming scenario Regime 3 also produces peaks in quiescence, they are irregular and multimodal with maximum quiescence percentages that are lower (38–53%) and less consistent from year to year (Fig. 11).

For simulations with seasonally varying temperatures, temperature-dependent germination of quiescent cysts was also incorporated into the model. Quiescent cysts germinate following a sigmoid function, proceeding at a minimum rate of 1.7% day^{-1} below 5˚C and a maximum rate of ~8.6% day^{-1} above 10 ˚C (Figs. 5 and 8; Anderson et al., 2005a). In Regimes 1, 2, and 4, simulated germling fluxes track the dormancy cycling patterns of populations during the fall and spring but are suppressed during the winter due to cold inhibition of germination. In the coldest simulations (Regimes 1 and 2), distinct peaks in the flux of germlings occur during the fall and spring. In contrast, the warming-scenario Regime 3 effectively releases cysts from cold inhibition such that germination and germling fluxes directly track changes in cyst bed quiescence.

The strongest phasing of quiescence is produced by the highest temperature seasonality (Regime 4; 13 ± 10 ˚C), which is typical of shallow, inshore systems where water temperature more closely tracks air temperature. The cyst population is converted between dormant and quiescent states nearly synchronously, with quiescent intervals spanning from early winter to late spring. This is significantly shorter than regimes with milder seasonality and effectively restricts bloom initiation to spring (as is observed in Nauset Marsh). At the onset of quiescence, model germling flux is initially suppressed by cold winter temperatures and then increases to its peak potential rate as temperatures warm to 10 ˚C (Figs. 5 and 11). Synchronous phasing of quiescence arises from the effects of especially warm and cold periods of the year that rapidly drive resting cysts through quiescence and secondary dormancy, respectively.

Another important effect of temperature seasonality derived from model results is increased duration of quiescence intervals within individual resting cysts. Mild seasonality simulations (Regimes 1–3) produce an inverse relationship between mean temperature and quiescence duration (Fig. 12), just as in the constant temperature model simulation (Fig. 9).

However, for any given mean temperature, as seasonality increases, the duration of quiescence also increases. For example, model resting cysts under constant 13 ˚C forcing are quiescent 9.9% of their lifetimes, whereas resting cysts under Regime 3 (13 ± 3 ˚C) and Regime 4 (13 ± 10 ˚C) are quiescent 11.6% and 24.7% (Figs. 9 and 12). This effect stems from dormancy cycle phasing. Resting cysts in higher temperature seasonality habitats experience greater excursions from threshold T_c and T_h temperatures. This drives more rapid passage from one state (dormancy or quiescence) to the other, and then holds resting cysts in their new state with little progress toward their next transition (i.e., via chilling or heating) until a change in season. Consider a dormant cyst in winter. Severe cold drives its rapid transition to quiescence and then effectively holds it in this state until spring warming because environmental temperatures are near T_h, limiting accumulation of heating *DD*. At the onset of warming in late spring and summer, it will rapidly return to dormancy and remain in this state until the onset of cold in fall and winter.

The combined response by cyst beds to different climates and climate warming scenarios drawn from this model is complex, but several concepts emerge. Generally, warmer environments promote longer phases of dormancy and shorter phases of quiescence, reducing the potential flux of germling cells from cyst beds for the inoculation of new blooms. However, this effect of warming can be mitigated through increasing temperature seasonality. High temperature seasonality also increases the synchronization of dormancy cycles, promoting larger germling fluxes that are focused over a shorter period of the year. Larger, more synchronous germling fluxes may be advantageous in more seasonal habitats for a number of reasons. Inocula may need to surpass a threshold size for blooms to develop in habitats with high loss rates due to grazing and/or interspecific interactions (e.g., allelopathy; Fistarol et al., 2004). Concentration of germling fluxes over narrower temporal windows may also reduce the depletion of cyst beds and reduce the demand for their renewal through new resting cyst production.

7. Biogeographic implications of interactions between cyst dormancy, warming, and temperature seasonality

Cyst beds of *A. catenella* and other temperate and sub-arctic species are experiencing climate change-associated increases in temperature seasonality but at a scale that is modest relative to differences between shallow inshore and deep coastal habitats. For example, since 1979, there has been <1 ˚C change in tropospheric seasonality (Santer et al., 2018), whereas there is >7.5 °C seasonality difference between inshore and coastal *A. catenella* habitats within the Gulf of Maine region (Figs. 2 and 13). The scale of the seasonality shifts is also modest relative to the rate of climate warming (i.e., changes in annual mean water temperature). Similarly, warming is far more significant than changes in seasonality within equatorial and subtropical habitats where *P. bahamense* occurs. Model results suggest that cyst populations in higher seasonality habitats (i.e., Regime 4) will be more resilient to climate warming than those in habitats with lower seasonality (i.e., Regime 3). In this context, greater resiliency means cyst populations are more likely to persist and inoculate new blooms despite changes in annual mean water temperature. One outcome of warming may be a shift from the relative importance of deep-water (lower seasonality) cyst beds to inshore (higher seasonality) cyst beds for initiation of blooms in many regions, particularly those at the latitudinal limits of their ranges.

The biogeography of *A. catenella* in the northeast U.S., the southern boundary of this species distribution in the northwest Atlantic, is concordant with the prediction that less seasonal, offshore cyst beds will be more sensitive to warming. Expansive cyst beds occur within the Bay of Fundy and along the mid-Maine coast, but to the south, cysts are more abundant within isolated embayments than in deeper waters (Anderson, 1997; Anderson et al., 1994). Georges Bank, an offshore but shallow area, supports substantial blooms of vegetative *A. catenella* cells (McGillicuddy et al., 2014) but does not host a cyst bed of its own (Anderson et al., 2014), instead relying on leakage of vegetative populations from coastal Maine for new bloom initiation. The lack of a cyst bed on the bank itself is likely caused by strong currents that scour fine sediment from its shallowest areas (Harris and Stokesbury, 2010), but even deeper flank areas are characterized by low cyst concentrations (Anderson et al., 2014), suggesting that temperature or other environmental factors are preventing cyst bed formation in these less energetic areas. Despite even higher annual mean temperatures, more southern inshore populations produce localized blooms that are largely self-seeding and persistent, e.g. Nauset Marsh on Cape Cod and areas along the coasts of Connecticut and Long Island, NY (Anderson et al., 1982; Anderson et al., 1994; Crespo et al., 2011; Fig. 13). This distribution is concordant with increasing restriction of *A. catenella* resting cysts to more highly seasonal habitats in warmer areas of its range. Coastal blooms still occasionally extend at least as far south and west as Rhode Island (Anderson et al., 2005b), but offshore cyst beds are restricted to cooler and deeper waters off the coast of Maine and areas to the north.

Mean bottom temperatures within Gulf of Maine have increased >2 ˚C since 2015 (Pershing et al., 2015). With further warming, the mid-Maine coastal cyst bed might wane in its importance. Warming will drive deep cyst bed seasonal temperature cycles from Regime 1-type behavior to Regime 3, releasing cysts from wintertime inhibition of germination and relaxing temperature-based phasing of dormancy cycling. This would further restrict the development of extensive offshore *A. catenella* cyst beds in southern, warmer, low seasonality areas. Range shifts to more northern areas—comparable to what has been observed in many fish species (e.g., Perry et al., 2005; Nye et al., 2009)—are therefore likely to occur first among deep cyst beds in open waters. Over time this may lead to reduced threats from expansive coastal blooms that impact long stretches of the coast (Franks et al., 1992) or cause coastal blooms to rely more heavily on leakage from "upstream", higher latitude cyst beds or localized, inshore populations for their initiation (Anderson et al., 2005a, 2014; McGillicuddy et al., 2005, 2014). Remnant populations at lower latitudes will experience more strongly phased dormancy cycles, tending to concentrate the initiation of new blooms within a shorter period of the year, leading to far more localized PSP risk. At the poleward extreme of its range, warming may instead promote the development of deep cyst beds that have the potential to cause expansive coastal blooms of *A. catenella*, especially as warming enhances cyst germination and vegetative cell growth. The extraordinarily large deposit of *A. catenella* cysts in the Chukchi Sea is noteworthy in this regard as it points to the potential for massive blooms in an area that has no recorded history of PSP (Gu et al., 2013; Natsuike et al., 2013; 2017; Okolodkov, 2005; D. Anderson, unpub. data).

In the case of *P. bahamense*, biogeographic patterns suggest a somewhat different response to climate warming. Near the equator, lower temperature seasonality (Fig. 2) likely drives desynchronization of cyst populations, which may underlie reports that blooms in these lower latitude regions occur more sporadically or are recurrent throughout the year (Usup et al., 2012; Sastre et al., 2013; Morquecho, 2019). In contrast, more seasonal, sub-tropical populations (e.g., Tampa Bay and Indian River Lagoon, FL) peak in summer periods (Phlips et al., 2006), a phenology that likely reflects both heightened germling fluxes in spring and more favorable growth conditions for vegetative cells during late spring and summer (Fig. 2; Lopez et al., 2019). The widespread distribution of *P. bahamense* resting cysts in coastal areas and ocean sediments, which extends beyond the range of known bloom occurrence (Zonneveld et al., 2013), also suggests the potential for the expansion of *P. bahamense* blooms to higher latitudes as warming occurs. But such an expansion may depend on the specifics and plasticity of its temperature-based controls of dormancy and quiescence. Further investigation of these dormancy control mechanisms and how they related to phenology of blooms across warming scenarios is needed to assess how warming may alter sources of *P. bahamense* cells and PSP toxins.

It remains to be shown whether dormancy cycling and cold inhibition do in fact break down with warming in *A. catenella* as illustrated through the Regime 3 simulation. Variability in chilling and heating responses within populations may also enable species to adapt over time. Dinoflagellate cyst beds are phenotypically and genetically diverse, and that diversity can be maintained over decades (Lundholm et al., 2017; Ribeiro et al., 2013). A multigenerational cyst bed provides populations with a reservoir of diverse genotypes that could be resurrected when favorable environmental conditions occur. Kremp et al. (2016) provides experimental evidence that cyst beds do support short-term adaptation of *A. ostenfeldii* to environmental change. The development of relatively small, localized, and self-seeding populations may also promote adaptation to warmer conditions (Anderson et al., 1994). In other cases, warming may cause established cyst beds to erode as germination delivers more germlings to the water column during periods that are unfavorable for bloom development, and, thus cyst bed replenishment.

It is also true that additional temperature effects not considered in the model may be more decisive in driving changes in the range of *A. catenella*, *P. bahamense*, and other species. Dormancy is just one of many factors that determine germling fluxes in natural systems. Other factors that control the supply of resting cysts to surficial sediments and the water column are not considered here but are critical for release of resting cysts from anaerobic inhibition, which, likewise, is not considered in the model. Similarly, the model ignores enhanced heat stress that may contribute to higher mortality (Haellegraeff et al., 1997). It also neglects the potential for resting cysts to exploit temperature gradients in the water column, e.g., deep populations may reach surface waters as quiescent cysts through winter resuspension and mixing, then germinate at elevated rates within warmer euphotic waters (Kirn et al., 2005; Pilskaln et al., 2014). Many, if not all, of the factors controlling germling fluxes will be impacted by climate warming and their responses will have interacting effects that sometimes enhance and other times negate one another. Those temperature-related impacts that directly affect the physiology of resting cysts are of paramount interest here because critical thresholds may delimit the conditions under which germination (and initiation of new blooms) is possible or effectively regulated.

8. Importance of new resting cyst formation and limits to bloom intensification

Production of new resting cysts by blooms is important for renewal of cyst beds and initiation of future blooms. Given this importance to bloom ecology, descriptions of new resting cyst formation in situ have been widely sought after for decades, yet few exist because they present a formidable observational challenge. Like all other plankton, HAB cell distributions are spatially patchy and dynamic. Gametes and planozygotes—the planktonic sexual stage precursors to new cysts—are short-lived and therefore relatively rare compared to vegetative cells in most bloom populations (Fig. 1; Badylak and Phillips, 2009; Brosnahan et al., 2017). Most descriptions of new cyst formation therefore come from laboratory observations.

In culture, vegetative cells of *A. catenella* (and many other photosynthetic dinoflagellates) can be induced to form cysts through nutrient limitation (e.g., Anderson and Lindquist, 1985). *Pyrodinium bahamense* is perhaps an exception since conditions promoting encystment of cultures of this species are yet to be described (Blackburn and Oshima 1989; Usup et al., 2012). That nutrient limitation can drive encystment is consistent with the paradigm that evolutionary pressure pushes species to favor self-replication and forego sexual recombination for as long as a population's environment will allow. Through combination of sexual recombination and encystment, dinoflagellates and many other protists are able to defer gene repair and recombination from periods that support vegetative cell division (e.g., Margulis et al., 1985). In the field, however, many reports fail to link new resting cyst production to nutrient limitation (e.g., Anderson et al., 1983; Anglès et al., 2012; McGillicuddy et al., 2014; Brosnahan et al., 2015, 2017), suggesting that other stimuli may commonly drive sexual induction and new resting cyst production by blooms (e.g., Bravo and Figueroa, 2014).

Blooms of *A. catenella* have been shown to produce large pulses of new cysts shortly after their peaks. Different sampling methods used across studies make comparisons of peak cell concentrations preceding encystment challenging, but work from the Nauset Marsh has shown remarkable consistency across years and at three distinct kettle holes, each of which hosts its own localized bloom (Anderson et al., 1983; Ralston et al., 2014). More recent observations from Nauset Marsh using an in situ phytoplankton imaging sensor called Imaging FlowCytobot (IFCB) has revealed that blooms undergo mass gametogenesis once thin layer concentrations exceed 10^6 cells L^{-1} (Brosnahan et al., 2015, 2017; Fig. 14). Gamete fusion and planozygote formation proceed within hours of mass gametogenic events and are associated with localization of *A. catenella* near the surface producing highly ephemeral red water discoloration (Ralston et al., 2015; Brosnahan et al., 2017). Wholesale conversion of a coastal *A. catenella* bloom to sexual stages, coinciding with red water and cell concentrations in excess of 10^6 cells L^{-1}, was also observed in a population that spanned much of the coast of western Maine and New Hampshire (McGillicuddy et al., 2014). In both of these works, concerted sexual transformation led to rapid and complete bloom termination, suggesting that intensification of *A. catenella* blooms is limited by an overwhelming drive to form new resting cysts once cell concentrations surpass the 10^6 cell L^{-1} threshold. Similarly, Uchida (2001) has reported cell concentration and cell contact thresholds for sexual induction of the dinoflagellates *Scrippsiella trochoidea* and *Gyrodinium instriatum*. In the case of *P. bahamense*, resting cyst production by field populations remains to be characterized, but Florida monitoring data reveals a comparable limit to *P. bahamense* bloom intensification (~10^6 cells L^{-1}, Fig. 15). Unlike with *A. catenella*, however, high *P. bahamense* concentrations often persist for weeks or months. Thus, while *P. bahamense* planktonic populations can be composed of mixtures of vegetative and sexual stage cells (e.g., Azanza et al., 2004; Azanza, 2013; C. Lopez and S. Shankar unpub. data), plateaus in bloom intensification, do not immediately precede rapid decline of blooms.

In Nauset Marsh, mass gametogenesis of *A. catenella* blooms typically occurs when growth rates (determined through IFCB analysis) are fastest, temperatures are favorable for growth, and when ambient concentrations of phosphate and nitrogen salts are relatively high (Ralston et al., 2014; Brosnahan et al., 2015, 2017 and unpublished). Blooms also do not typically resurge within a bloom season once sexual transformation has occurred, likely due to the lack of inocula from cyst beds (Fig. 11; Regime 4). This limit to bloom intensification and duration through sexual transformation in a population with highly synchronized dormancy cycling of cysts adds nuance to the window of opportunity hypothesis that predicts prolonged blooms with expanded windows of conditions supporting bloom development. At the very least, these works show that the seasonal window within which blooms might occur is much narrower than would otherwise be predicted by only considering conditions that support vegetative growth.

Two warmer than normal years in Nauset Marsh from which suitable monitoring data are available (i.e., 2012 and 2016) offer a chance to evaluate the window of opportunity hypothesis in this system. During these warm years, rapid in situ vegetative growth led to bloom development approximately one month earlier than in other, more typical (and cooler) years. Detection of the early 2012 bloom led to an emergency shellfish harvesting closure of the Nauset system prior to the start of sampling by the Massachusetts state shellfish monitoring program in that year (Ralston et al., 2014). Spring warming proceeded nearly monotonically through the bloom period from early March through mid-April when the bloom in Salt Pond surpassed 10^6 cells L^{-1} and then underwent a rapid and total decline driven by encystment as the water temperature reached 15 ˚C. From start to finish the bloom persisted for only about five weeks, slightly shorter than typical, even though favorable conditions for vegetative cell growth persisted well into May (Brosnahan et al., 2015). The bloom in 2016 proceeded similarly through mid-April when it too surpassed 10^6 cells L^{-1}, triggering a mass conversion to sexual stage cells and rapid bloom decline (Fig. 14). Unlike 2012, however, a series of cold spells during the bloom's development kept water temperatures below 10 ˚C for most of April, extending germling production by prolonging cyst quiescence. Continued germination likely led to the renewal or second phase of the 2016 bloom in early May, and a second sexual induction-linked bloom peak and decline in mid-May (Brosnahan et al., 2017; Fischer, 2017). These results are instructive in that they emphasize the importance of cyst bed quiescence for the window of opportunity hypothesis. Warmer than normal temperatures in 2012 and 2016 were projected to expand the window of opportunity for *A. catenella* in Nauset Marsh leading to earlier and longer lasting blooms. Blooms occurred one month earlier than normal during both years, but the bloom duration was extended only in 2016 because cool spring conditions prolonged cyst quiescence. In 2012, the bloom duration was the same as other years but was simply shifted earlier in the year. In regions like Puget Sound where conditions promote longer and less synchronized fluxes of germlings from cyst beds, blooms may go through several cycles of development, new cyst production, and revival, prolonging the risk of PSP until conditions no longer support vegetative cell growth. In areas like Nauset that experience greater temperature seasonality and more synchronized fluxes of germlings from cyst beds, fewer cycles are possible because warmer temperatures in late spring and summer tend to drive cyst beds back into dormancy. It is worth noting that more intense spring and summer warming may also drive greater anoxia in sediments, causing anaerobic inhibition of germination and further reducing the flux of germlings that might otherwise sustain and renew blooms. The kettle holes within the Nauset

Marsh commonly experience anoxia during summer periods and blooms often end just as anoxia sets in within the deepest areas of the system. Most resting cysts, however, are present in shallower areas that remain oxygenated for several weeks after blooms terminate (Crespo et al., 2011; Brosnahan et al., 2017).

Collectively, these observations support the notion that the life cycle of a cyst-forming species is more oriented toward the production of resting cysts rather than favoring continued production of vegetative daughter cells. Blooms of some of these species will undergo mass gametogenesis once they reach concentrations that are conducive to gamete pairing and fusion, limiting bloom intensification. In many cases, this will reflect an imperative that cells return to their resting cyst stage to survive periods between favorable bloom conditions. The implications of this encystment trigger are significant in the context of global warming impacts on blooms. Instead of vegetative populations continuing to grow as long as favorable temperatures persist, cell density thresholds may be reached that terminate blooms “prematurely” unless they are renewed through fluxes of new germlings. It remains to be shown whether characteristic peak concentration and encystment-driven termination observed in Nauset and the Gulf of Maine can be generalized to Puget Sound and other areas within *A. catenella*’s extensive geographic range, or if similar mechanisms for sexual induction hold for *P. bahamense* and other species, but the observations from northeastern U.S. *A. catenella* blooms expand the window of opportunity hypothesis to also consider the effects of temperature and temperature history on the flux of germlings from cyst beds. Especially for populations whose termination can be driven by mass encystment, the potential for blooms to exploit more favorable conditions for vegetative growth may depend on conditions also promoting continued germination of cysts.

9. Future directions

Recent intensive study of *A. catenella* blooms in Nauset Marsh demonstrates the value of rigorous, quantitative field investigations that can test and validate inferences and predictions born from analysis of long-term data sets and laboratory-based studies of HAB organisms. While temperature is undoubtedly a major determinant of HAB physiology, other factors that drive dynamics in natural blooms remain to be elucidated. As one example, the factors driving enhanced growth by Nauset *A. catenella* in situ in comparison to laboratory cultures remain to be described (Brosnahan et al., 2015). While division rates in situ retain a strong temperature dependence, growth is also restricted to a far narrower range of temperatures than has been shown for growth by cultures (5–15 ˚C vs. 5–26 ˚C; Fig. 16). Similarly, coastal blooms in the Gulf of Maine are restricted to waters between 5 and 15 ˚C (Townsend et al., 2005), but blooms in Northport Harbor, NY, a more southern inshore system, occur between 10 and 20 ˚C (Anglès et al., 2012). It remains to be shown whether *A. catenella* can bloom in even warmer waters in nature and if so, how cells behave in terms of peak bloom intensity, production of new resting cysts, and resilience to stress and changing interspecific interactions.

Like *A. catenella*, the relationship between the growth of *P. bahamense* and temperature is well described by an asymmetric bell-shaped curve with low growth at low temperatures increasing to a maximum and then falling rapidly at high temperatures (Usup et al., 1994). Some differences are apparent however between Pacific and Atlantic isolates, the latter being more tolerant of higher temperatures (Omura et al., 1994). Unlike *A. catenella*, *P. bahamense* blooms occur more commonly at temperatures near or exceeding those that support optimal growth of laboratory cultures (i.e., >28–30 ˚C; Usup et al., 2012; Fig. 16) and can persist in these environments for weeks to months (FWC FWRI HAB Monitoring Database). It may be that *P. bahamense* is adapted to bloom nearer to, and even above, its upper temperature limit to growth in culture. Evidence from experiments with Florida isolates suggests cells can maintain slow cellular division for extended periods under conditions that induce cell stress (S. Shankar, unpub. data). *Pyrodinium* bloom dynamics may also be driven to a larger extent by cycles of temporary cyst formation and excystment (Azanza et al., 2013), which is a topic that requires further exploration.

Deployment of in situ biosensors like the IFCB at bloom hot spots will better characterize in situ division rates and the role of different life cycle transformations in determining bloom dynamics of *A. catenella*, *P. bahamense*, and many other species across a wide diversity of habitats. With expanded use of these tools, more comprehensive understanding of the factors that limit bloom intensity and duration will be developed. Continuous recording and real-time sharing of phytoplankton diversity and abundance at bloom hot spots also has obvious value for managers and stakeholders who must protect public health and natural resources from both established and emerging HAB species affecting their regions (e.g., Campbell et al., 2010). Records produced through these activities characterize HAB responses to interannual climate variability and anomalous weather events. Because these events often mimic climate change scenarios (see Trainer et al, this issue), their analysis can provide further insights into the response of blooms to warming and other climate-related environmental changes (e.g., Moore et al., 2010; Anderson 2014; Anglès et al., 2015; Wells et al., 2017).

Because the distribution and abundance of resting cysts is a strong predictor of bloom locations in subsequent bloom seasons, understanding the evolution of cyst beds in response to warming and climate variability will be invaluable for managing HAB impacts like PSP (Anderson et al., 2014). Cyst beds reflect both the location of new cyst production and hydrodynamic factors—tides, seasonal weather patterns, etc.—that scour and redistribute cysts and other fine sediment particles in coastal systems (Butman et al., 2014; Aretxabaleta et al., 2014). These factors can produce consistent patterns of cyst distribution within both inshore and coastal cyst bed habitats (Anderson et al., 2014; Crespo et al., 2009), which, once known, can be leveraged for design of efficient monitoring schemes (Solow et al., 2014). Expanded use of molecular methods like quantitative PCR in benthic monitoring programs will also improve detection of emergent species and toxins of concern (e.g., Erdner et al., 2010; Murray et al., 2019). The combination of benthic monitoring with increased use of in situ monitoring tools like the IFCB will improve understanding of HAB responses to warming and preparation of appropriate management responses.

New efforts to understand relationships between changing temperatures and HAB species must also develop new observational, experimental, and analytical approaches. The characterization of temperature-based controls of cysts' dormancy cycles remains in its early stages. New approaches are needed to assess the prevalence of these mechanisms across the diversity of cysts and other benthic stages formed by dinoflagellates and other classes of phytoplankton. Similarly, evaluation of the plasticity of chilling- and heating-type responses within and between populations will require adoption of new experimental and analytical approaches. It is noteworthy that the initial descriptions of chilling-mediated dormancy passage in *A. catenella* and *P. bahamense* were based on studies of cyst beds that were naturally synchronized by relatively high temperature seasonality (Fischer et al., 2018; Lopez et al., 2019). Strong phasing of dormancy cycles in these populations helped to reveal chilling- and heating-mediated physiologies through simple experiments that applied constant temperature storage conditions. More recent experiments have demonstrated alternating temperature schemes that synchronize populations by mimicking habitats with high temperature seasonality (results presented here and D.M. Anderson, A.D. Fischer, and M.L. Brosnahan, unpub. data). Further exploration of these more dynamic storage schemes may be required to determine differences between populations. Additionally, progress may be made through experimentation with cyst dormancy in species that readily produce viable cysts in culture. Lab-based investigations of cultured cysts are likely better suited to investigations of the molecular underpinnings of these responses and can better leverage new genetic tools (e.g., Chan et al., 2019).

10. Summary

Life cycle dynamics introduce complexity in efforts to predict the response of cyst-forming dinoflagellates to climate change. These complications arise from heating and chilling requirements for secondary dormancy and quiescence of resting cysts that are only now becoming recognized in two dinoflagellate species (*A. catenella* and *P. bahamense*) that span nearly all latitudes. The model presented here for one of these species (*A. catenella*) is a first step towards incorporating this type of physiological process into projections of bloom response to climate change. Preliminary indications from model simulations are that warming will promote longer phases of dormancy and shorter phases of quiescence, leading to shorter windows for bloom initiation and renewal through cyst germination. This, in turn, may mean that species with a density-dependent trigger for encystment that would otherwise take advantage of an expanded window of bloom development, will instead bloom and decline earlier. Another inference is that resting cyst populations will be more resilient to warming in areas that experience greater temperature seasonality. This may alter the geographic distribution of HAB impacts, with more localized populations persisting in estuaries and embayments at the latitudinal extremes of a species' geographic range and deeper cyst beds in these areas gradually diminishing. Enhanced warming may also lead to greater dependence upon pellicle cyst formation as a life-cycle based adaptation to environmental change. All of these issues highlight the need for expanded consideration of life cycles in climate change assessments.

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Figures

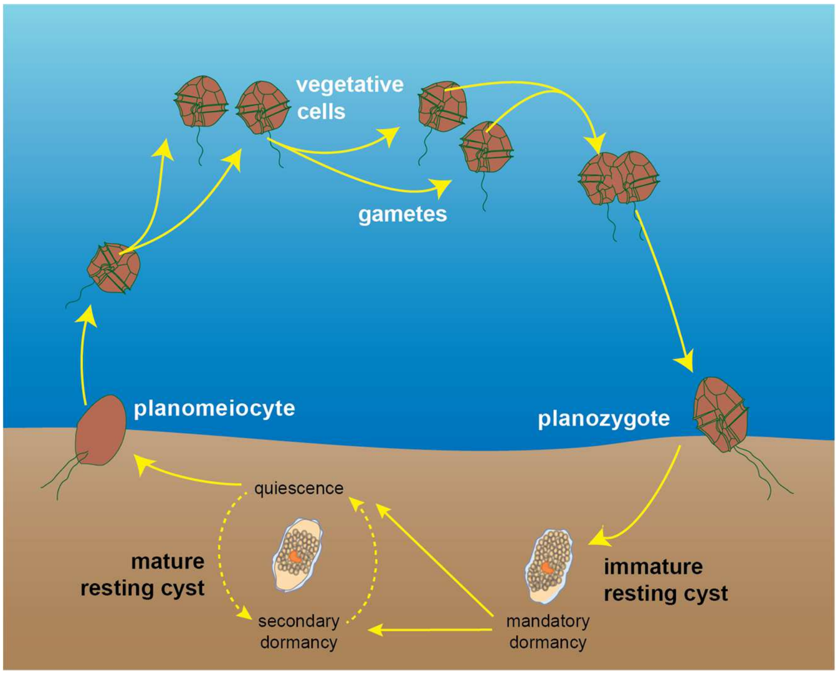

Figure 1. Dinoflagellate life cycle and cyst dormancy cycling. Most dinoflagellates divide and form blooms as haploid vegetative cells. Under certain conditions, these vegetative cells will form short-lived gametes that fuse in pairs to form a swimming diploid stage called a planozygote. Planozygotes may then transform into benthic resting cysts. Resting cysts must pass through mandatory dormancy before they can become quiescent and germinate in response to favorable oxygen and temperature conditions. They may also be induced into secondary dormancy and undergo many cycles of dormancy and quiescence before germinating to produce a diploid germling stage called a planomeiocyte. Planomeiocyte germling cells return to the vegetative stage through a series of meiotic divisions.

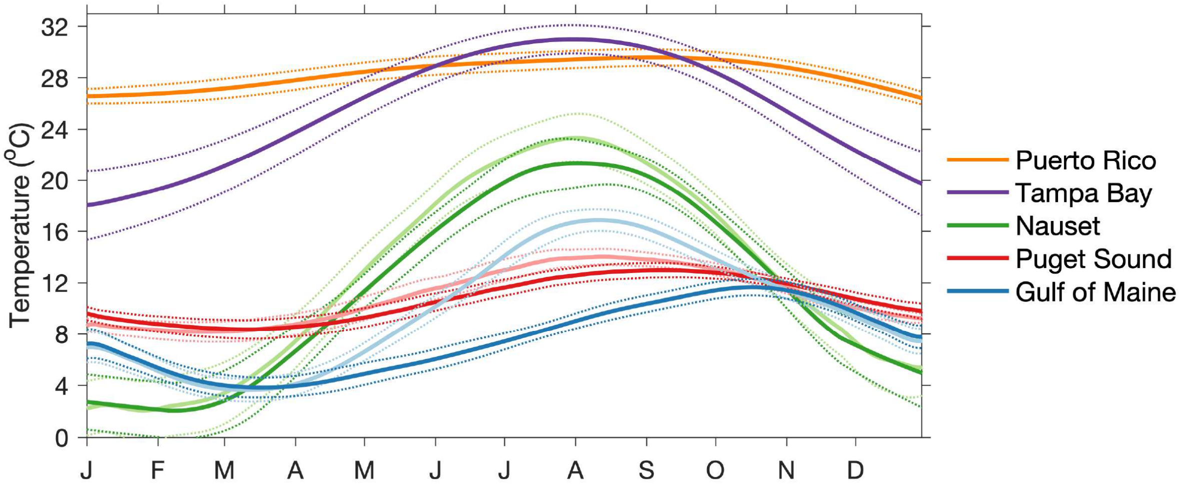

Figure 2. Seasonal water temperatures of Puerto Rico, Tampa Bay, Nauset, Puget Sound, and the Gulf of Maine. Surface water temperatures are shown in light colors and bottom water temperatures are shown in darker colors, except for the shallow Tampa Bay and Puerto Rico which show temperature only from a single depth. Solid lines are mean temperatures and dashed lines are standard deviations. Puerto Rico data are from Caleta Parguera at Magueyes Island (sensor depth ~0.1m, 2010-2015; NOAA buoy 9759110l; www.tidesandcurrens.noaa.gov), Tampa Bay data are from Port of St. Petersburg, FL (sensor depth ~4 m, average Tampa Bay depth 3.6m; 2009-2018; NOAA buoy 8726520; www.tidesandcurrents.noaa.gov), Nauset data are from Salt Pond (surface and ~5 m depth; 2013–2017), Puget Sound data are from the Seattle Aquarium (surface and ~10 m depth; 2009–2018; http://green2.kingcounty.gov/marine-buoy/), and Gulf of Maine data are from NERACOOS E01 buoy (1 and 50 m depths; 2009–2018; http://www.neracoos.org).

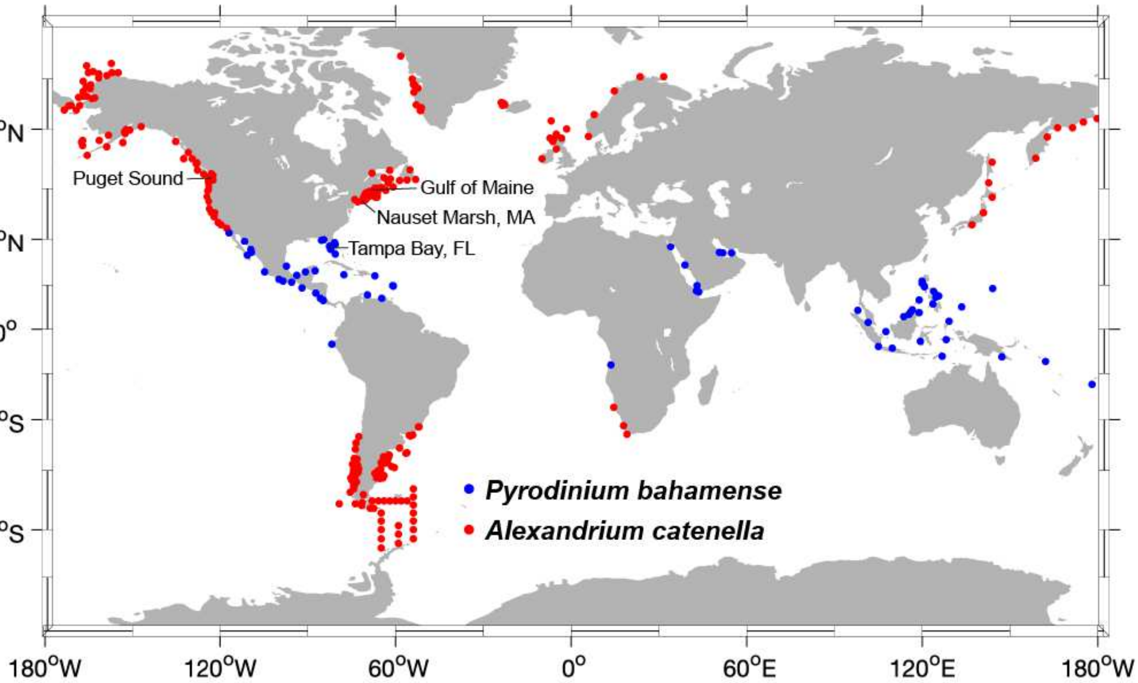

Figure 3. Global distribution of *A. catenella* and *P. bahamense* blooms. Bloom locations are taken from reports in the Ocean Biogeographic Information System (obis.org) and observations compiled by the authors and colleagues.

Figure 4. Examples of *A. catenella* (left) and *P. bahamense* resting cysts (right).

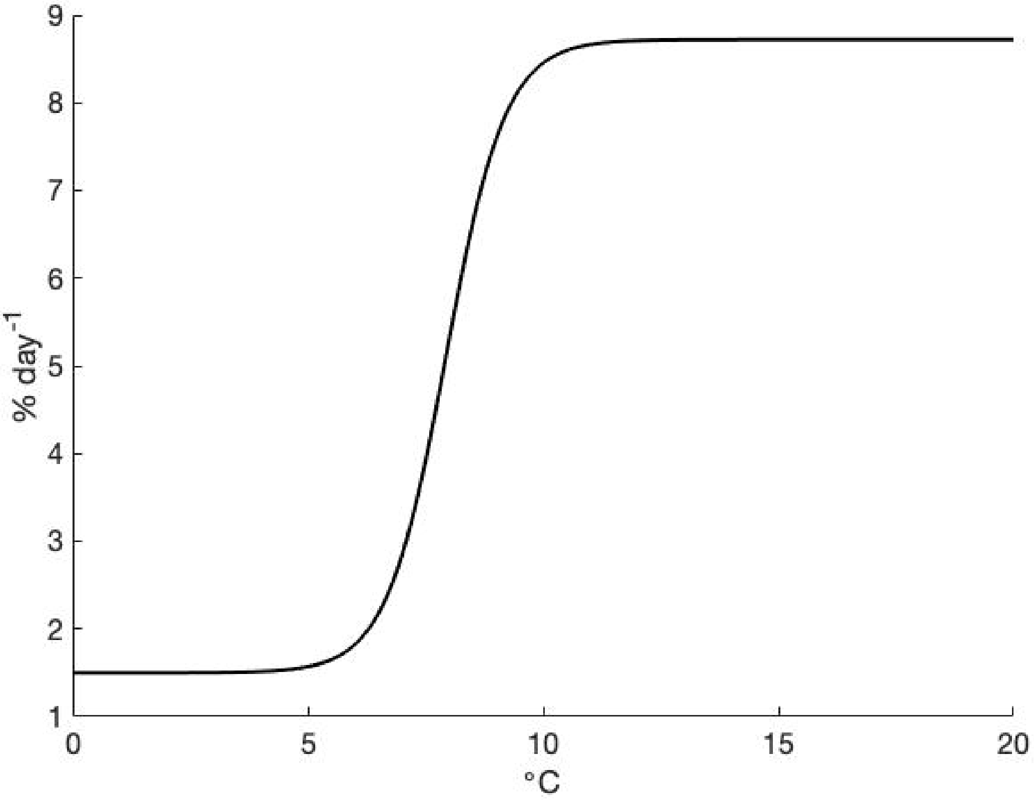

Figure 5. Relationship between temperature and the germination rate of quiescent cysts under aerobic conditions and light exposure. Parameters describing this relationship are taken from description of cysts from a deep-water seedbed within the eastern Gulf of Maine (Anderson et al., 2005a).

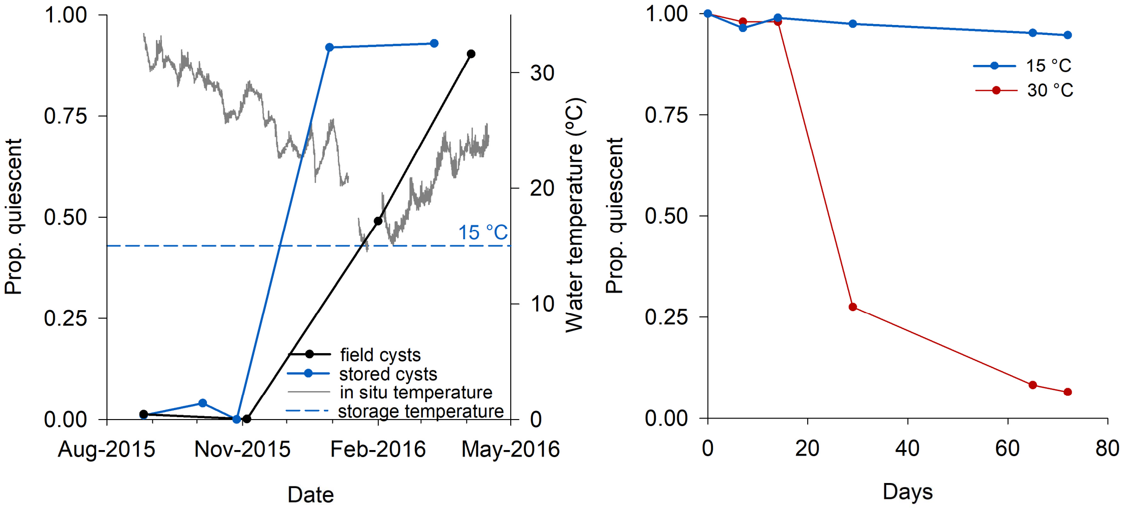

Figure 6. *Left:* Proportion of *P. bahamense* cysts quiescent in situ August 2015-April 2016 (field cysts, black line) compared to those collected in late August 2015, then stored at 15 ˚C (stored cysts, blue line). *Right:* Induction of dormancy through warm (30 ˚C) storage of quiescent *P. bahamense* cysts (from Lopez et al., 2019).

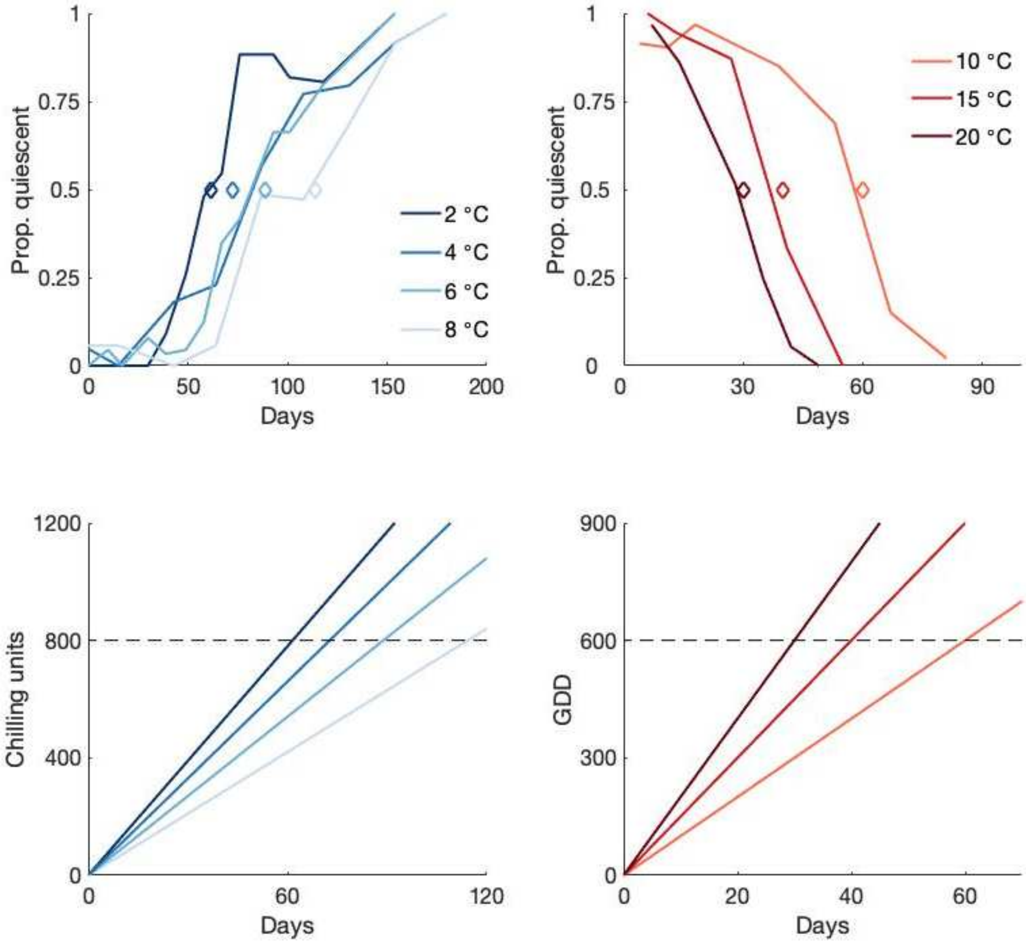

Figure 7. Temperature regulation of dormancy and quiescence passage in *A. catenella* from the northeast U.S. Temperatures in bottom axes are colored as indicated in top row legends. *Top left*: Passage through dormancy is fastest at 2 ˚C and slowest at 8 ˚C for cysts from Nauset Marsh (Fischer et al., 2018). Open diamonds indicate median transitions to quiescence predicted by the chilling model (Eq. 1) given T_c=15 ˚C and a chilling requirement of 800 *CU*. *Bottom left*: Accumulation of chilling during exposure to constant temperatures under a simple chilling model (Eq. 1). Dashed line indicates the 800 *CU* chilling requirement of Nauset cysts. *Top right*: Passage through quiescence by *A. catenella* cysts from the Gulf of Maine after dormancy passage through storage at 2 ˚C (Brosnahan et al., in prep). Open diamonds indicate median transitions to dormancy predicted by the degree-day model (Eq. 2) given T_h=0 ˚C and a heating requirement of 600 *DD*. *Bottom right*. Accumulation of heating under the degree-day model. Dashed line indicates the 600 *DD* heating requirement of Gulf of Maine cysts.

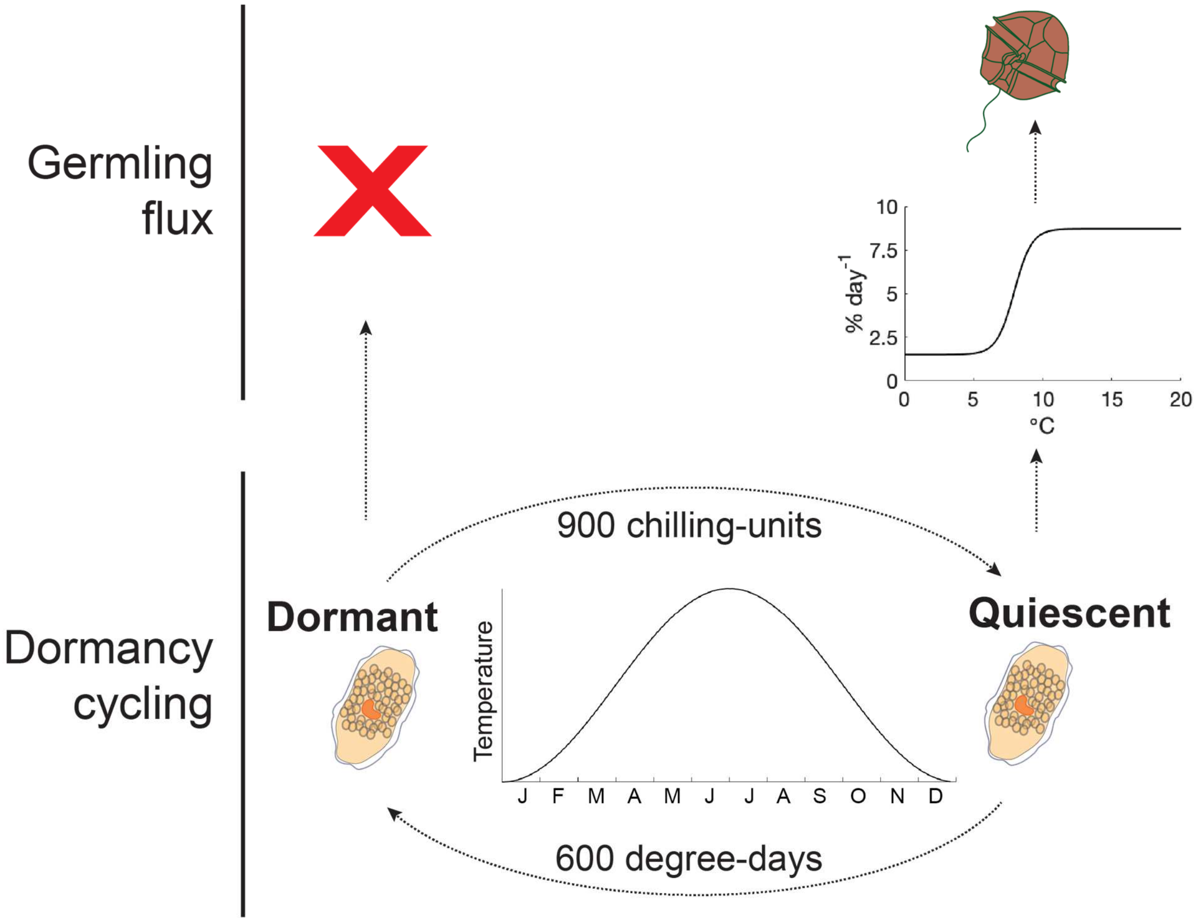

Figure 8. Schematic diagram of temperature controls considered in the heating and chilling based model of dormancy cycling and germling flux. Populations of *Alexandrium*-like cysts with mean chilling requirement 900 *CU* and heating requirement 600 *DD* are forced by seasonally oscillating temperatures. Dormancy cycles of model populations reflect phasing of individual cysts' dormancy and quiescence periods. Germling fluxes from model populations are calculated as the product of the quiescent fraction of the population and a temperature dependent rate of germination (Anderson et al., 2005a; Fig. 5).

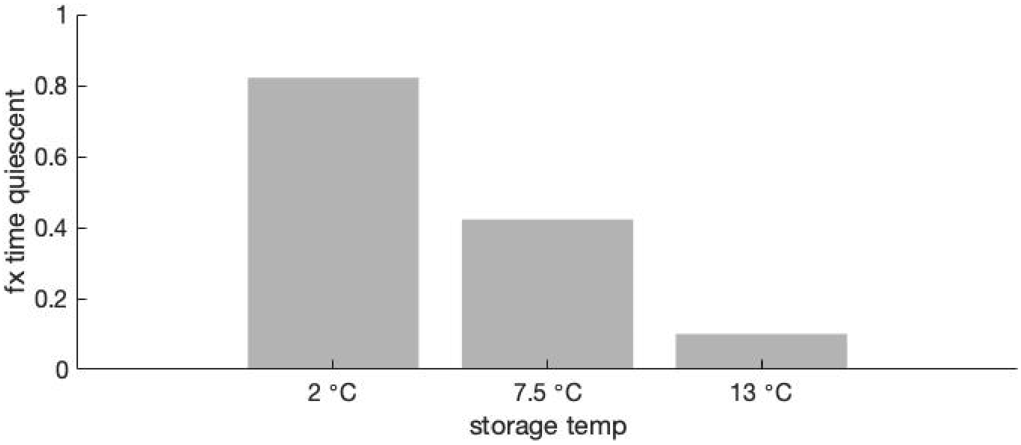

Figure 9. Mean proportion of time that *A. catenella* cysts are quiescent during constant temperature storage under a simple chilling-heating model of dormancy cycling.

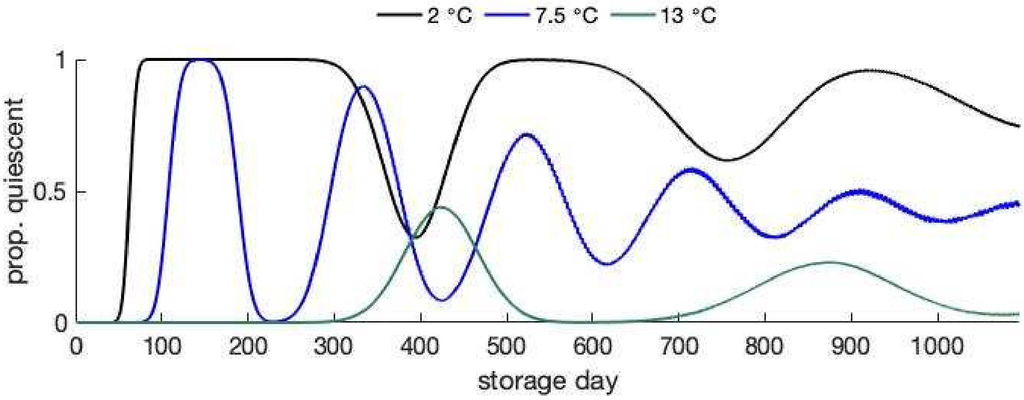

Figure 10. Dormancy cycling during constant temperature storage in a simulated cyst population controlled by the chilling and heating mechanisms described in *A. catenella*. At the coldest storage temperature (2 ˚C), initial cycles of quiescence and dormancy occur with an approximate period of 11.5 months. At the warmest (13 ˚C), cycle periods are ~14.5 months long.

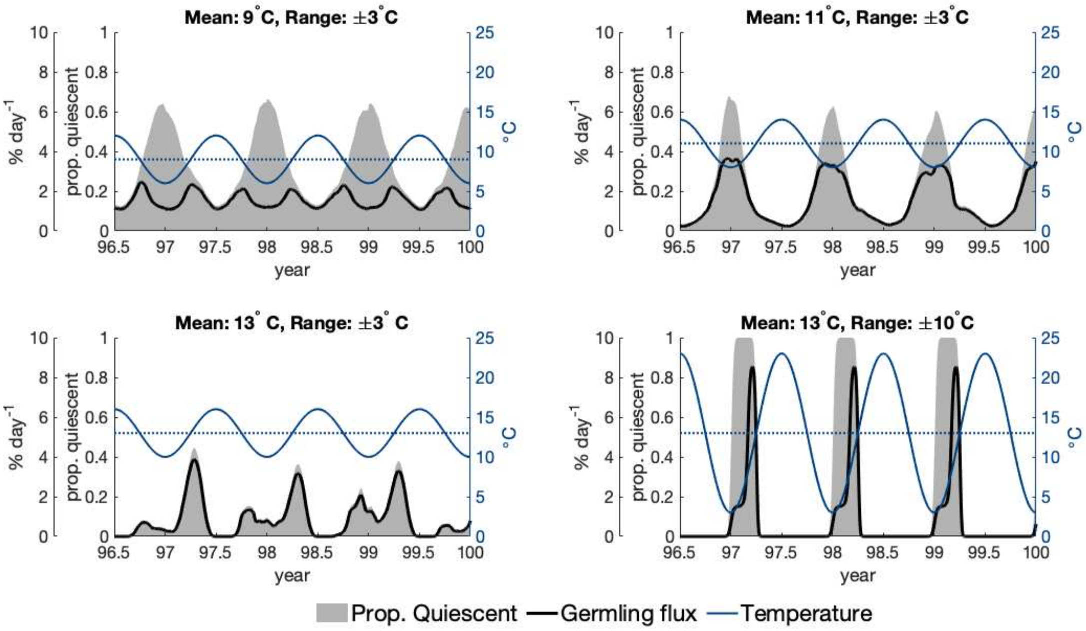

Figure 11. Numerical simulation of dormancy cycling and germling fluxes through the *Alexandrium*-derived chilling- and heating-based model. *Upper left:* Regime 1 (7 ± 3 ˚C), an analog of temperature seasonality experienced within Gulf of Maine cyst beds, produces stable dormancy cycling and spring and fall peaks in germling fluxes. *Upper right:* Regime 2 (10 ± 3 ˚C), an approximate analog of temperature seasonality experienced within Puget Sound cyst beds. Like Regime 1, Regime 2 produces stable dormancy cycling and spring and fall peaks in germling fluxes but lower overall cyst bed quiescence and germling fluxes. *Lower left:* Regime 3 (13 ± 3 ˚C), a warming scenario with mean temperature 6 and 3 ˚C warmer than Regimes 1 and 2, respectively. Dormancy cycles are not consistent year to year and quiescent cysts do not experience wintertime inhibition of germination. *Lower right:* Regime 4 (13 ± 10 ˚C) is an approximate analog of temperature seasonality experienced within Nauset Marsh cyst beds. Dormancy cycles are essentially synchronized and germling fluxes are restricted to spring warming periods.

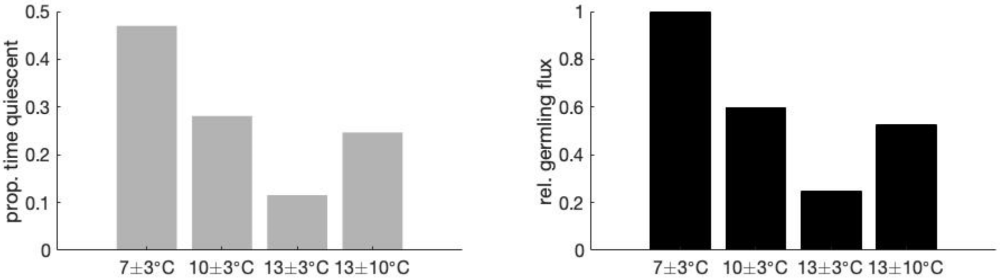

Figure 12. *Left:* Mean proportion of time that cysts are quiescent when forced by temperature regimes described in Figure 11. *Right:* Relative fluxes of germlings under the temperature regimes described in Figure 11 under an assumption that only dormancy cycling and temperature control germination (no anaerobic inhibition). Germling fluxes were calculated as the product of the quiescent fraction of the cyst population and the temperature dependent germination rate (Figs. 5 and 8), assuming constant replenishment of cysts in surficial sediments. Relative germling flux is calculated via comparison to Regime 1 (7 ± 3 ˚C), which produced germlings at the highest mean rate over the last 10 years of the 100 year simulations explored in the model.

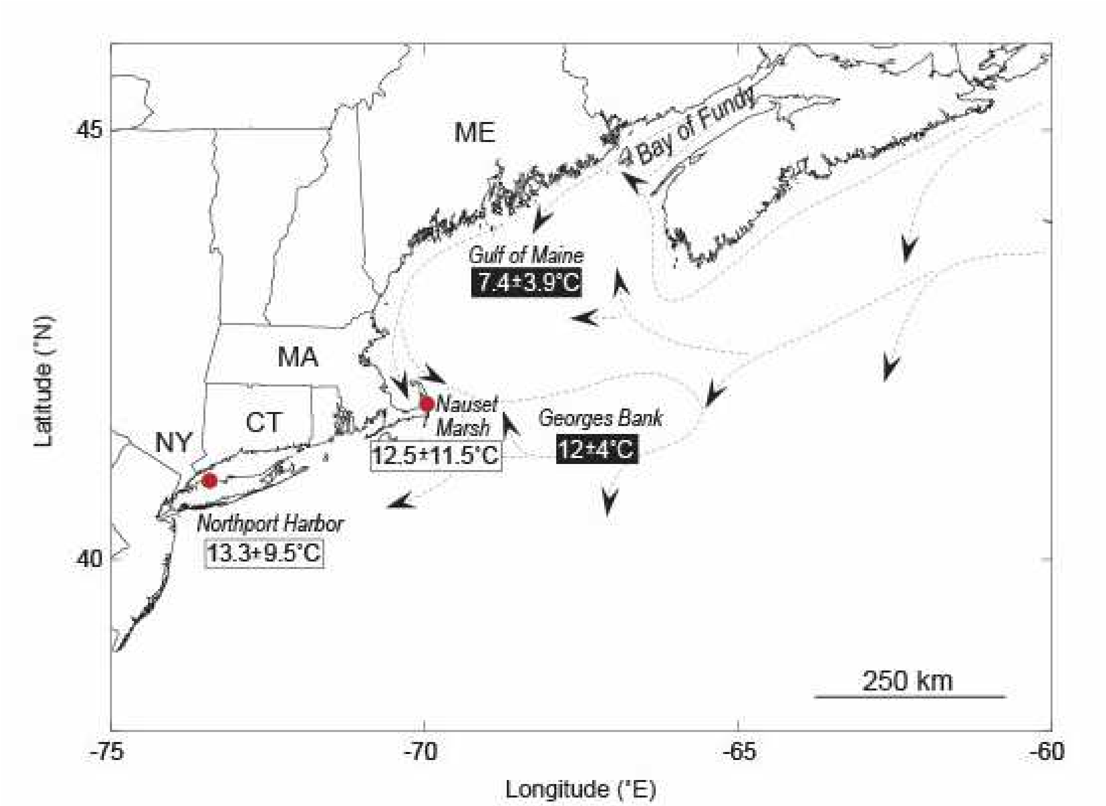

Figure 13. *Top (A):* Location of Gulf of Maine (northeast U.S.) and Puget Sound regions impacted by *A. catenella* blooms. *Bottom (B):* Northeast U.S. map with mean and range of temperatures of cyst bed habitats. Offshore habitats (e.g., Gulf of Maine and Georges Bank; black highlight) experience low temperature seasonality and inshore habitats (e.g., Nauset Marsh and Northport Harbor; white highlight) experience high temperature seasonality. Extensive cyst beds along mid-coast Maine and within the Bay of Fundy inoculate large coastal blooms within the region annually. Georges Bank also experiences large blooms but does not support a cyst bed. Nauset Marsh (Cape Cod, MA) and Northport Harbor (Long Island, NY) experience annual localized blooms and both support cyst beds despite higher annual mean temperatures than Georges Bank.

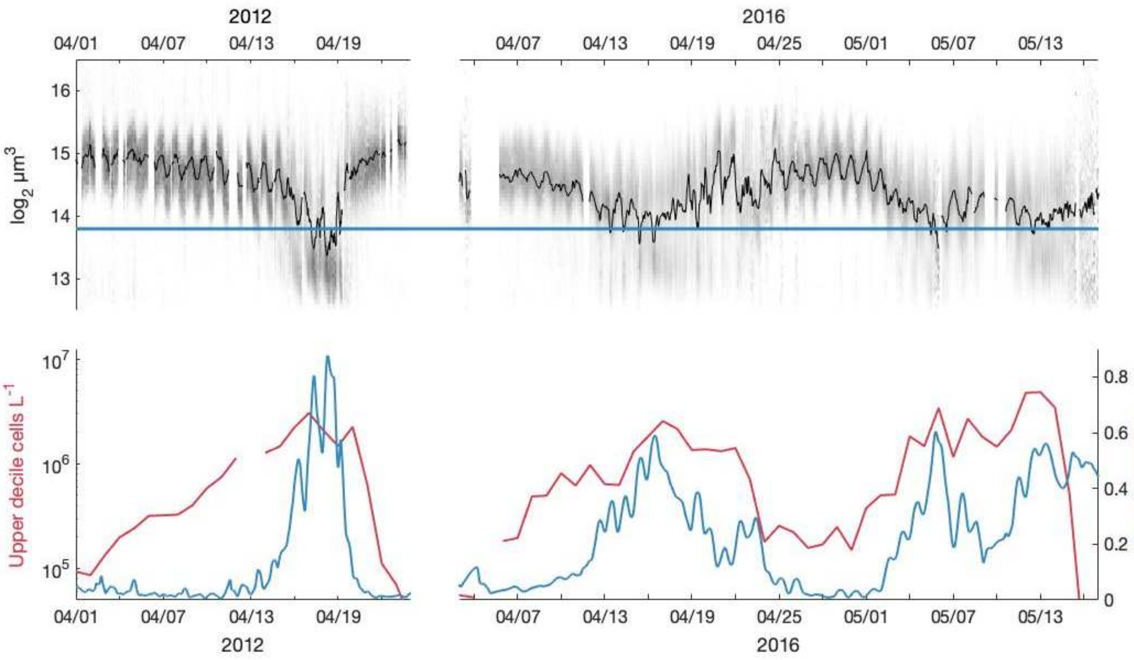

Figure 14. IFCB time series of *A. catenella* bloom development, sexual induction in Nauset Marsh during the 2012 and 2016 spring bloom seasons. In both years, blooms subsided when temperatures and nitrogen and phosphorus concentrations remained at levels normally expected to support further growth of vegetative cells. *Top:* Distribution of cell biovolume through time estimates from IFCB images. Cells having biovolume less than $2^{13.8}$ µm^3 (blue line) are gametes. *Bottom:* The daily upper decile cell concentration observed (red, left y-axis; a measure of concentration within vertically migrating thin layers) and the proportion of cells in the gamete size class (blue, right y-axis). Gametogenesis is induced once maximum cell concentrations exceed 10^6 cell L^{-1}, limiting the intensification of blooms. New cyst formation can drive rapid declines in bloom intensity (e.g., late April 2012 and 2016, late May 2016; Brosnahan et al., 2017). Revival of blooms as observed in 2016 may be stimulated by continued cyst germination and the production of new germling cells.

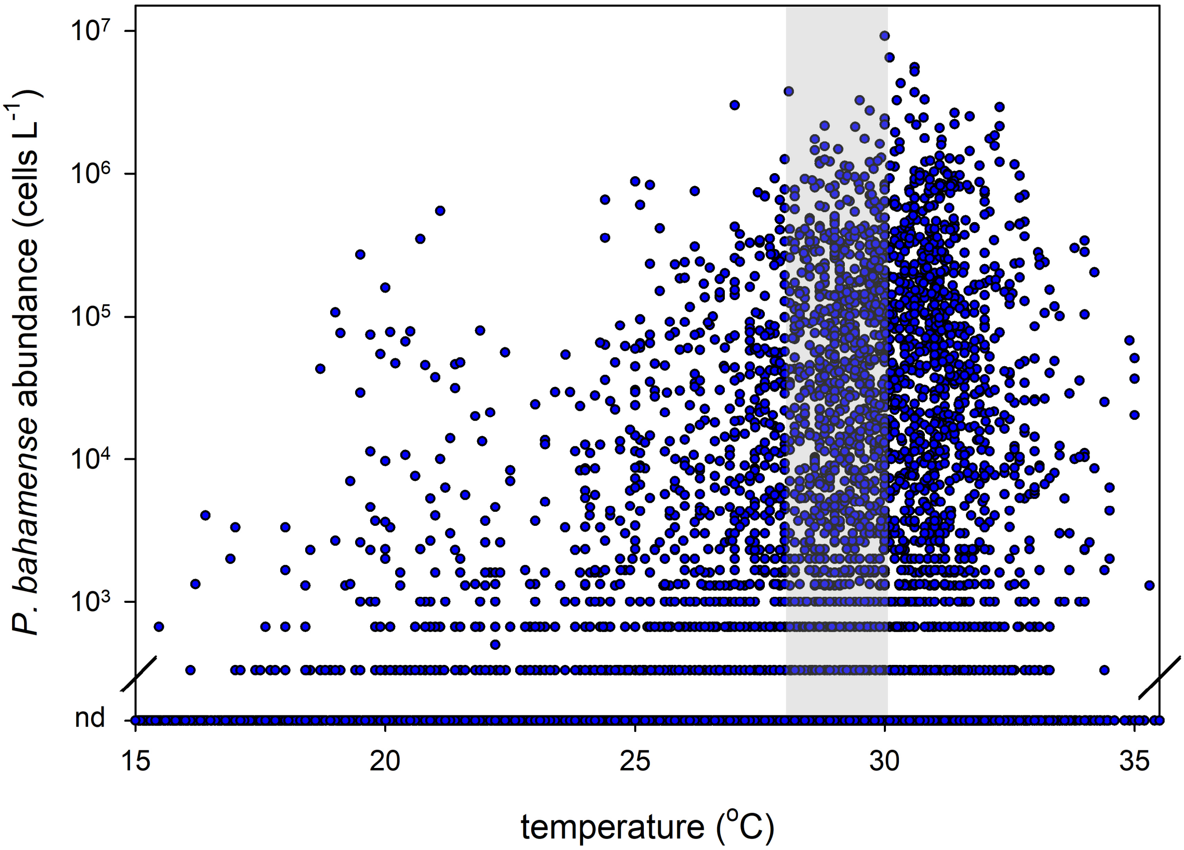

Figure 15. Abundance of *P. bahamense* in Florida waters from 1965-2019 versus water temperature (FWC FWRI HAB Monitoring Database). Detection limit is 333 cells L^{-1}; samples where cells were not detected are represented by nd on the y-axis. The gray shaded area represents optimal growth temperatures from culture experiments (Usup et al 1994, Omura et al., 1994).

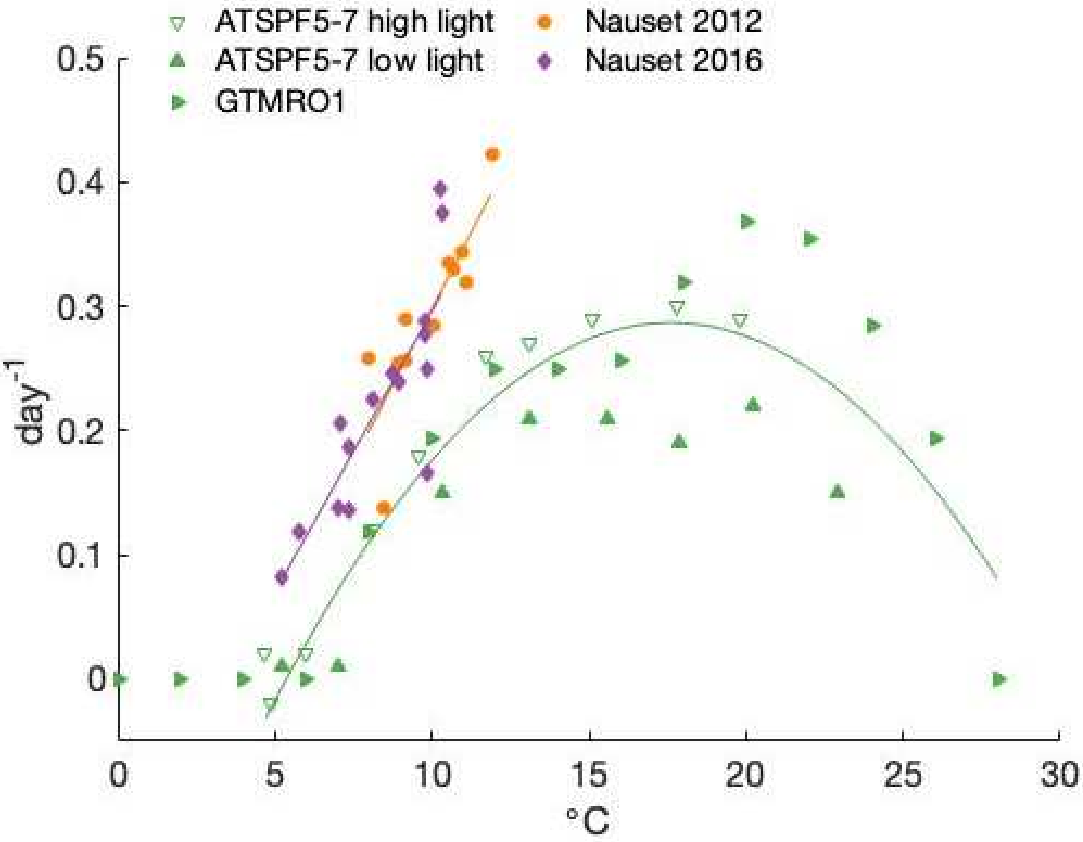

Figure 16. Growth rates from two *A. catenella* cultures – ATSPF5-7 and GTMR01 – isolated from Nauset Marsh and from in situ observation of Nauset blooms in 2012 and 2016. Growth rates estimated from in situ observation are estimated conservatively but are still ~2-fold higher than rates from cultures (e.g., Brosnahan et al., 2015).