

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

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6
7 **Abstract**

8 Both observations and models suggest that large-scale coastal blooms of *Alexandrium fundyense* in the
9 Gulf of Maine are seeded by deep-bottom cyst accumulation zones (“seed beds”) where cysts
10 germinate from the sediment surface or the overlying near-bottom nepheloid layers at water depths
11 exceeding 100 m. The germling cells and their vegetative progeny are assumed to be subject to modest
12 mortality while in complete darkness as they swim to illuminated surface waters. To test the validity of
13 this assumption we investigated in the laboratory cyst viability and the survival of the germling cells
14 and their vegetative progeny during prolonged exposure to darkness at a temperature of 6 °C,
15 simulating the conditions in deep Gulf of Maine waters. We isolated cysts from bottom sediments
16 collected in the Gulf of Maine under low red light and incubated them in 96-well tissue culture-plates
17 in culture medium under a 10:14h light: dark cycle and under complete darkness. Cyst viability was
18 high, with excystment frequency reaching 90% in the illuminated treatment after 30 days and in the
19 dark treatment after 50 days. Average germination rates were 0.062 and 0.038 d⁻¹ for light and dark
20 treatments, respectively. The dark treatment showed an approximately two-week time lag in maximum
21 germination rates when compared to the light treatment. Survival of germlings was considerably lower
22 in the dark treatment. In light treatments, 47% of germinated cysts produced germlings that were able

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1 to survive for 7 days and produce vegetative progeny, i.e. there were live cells in the well along with an
2 empty cyst at least once during the experiment. In the dark treatments 12% of cysts produced germlings
3 that were able to survive. When dark treatments are scaled to take into account non-darkness related
4 mortality, approximately 28% of cysts produced germlings that were able to survive for at least 7 days.
5 Even though cysts are able to germinate in darkness, the lack of illumination considerably reduces
6 survival rate of germling cells. In addition to viability of cysts in surface sediments and the near-bottom
7 nepheloid layer, survivability of germling cells and their vegetative progeny at aphotic depths is an
8 important consideration in assessing the quantitative role of deep-coastal cyst seed beds in bloom
9 formation.

1 **1. Introduction**

2 The life cycle of many dinoflagellates includes a non-motile resting stage (cyst) that remains in bottom
3 sediments or near-bottom nepheloid layers when conditions in the water column are unfavorable for
4 growth (Dale, 1983; Matsuoka and Fukuyo, 2003; Wall, 1971). The switch from cyst to motile cell and
5 vice versa determines the presence of those dinoflagellates in the water column. Numerous studies have
6 shown that germination of dinoflagellate cysts is determined by internal and external factors (Anderson
7 et al., 2005; Rengefors and Anderson, 1998). Among the internal factors, a mandatory dormancy period
8 (maturation) after encystment lasting days to months (Anderson, 1980; Bravo and Anderson, 1994)
9 and, for some species such as *Alexandrium fundyense*, an annual internal biological clock (Anderson
10 and Keafer, 1987; Matrai et al., 2005; Perez et al., 1998) regulates germination. Although oxygen is
11 required for germination (Anderson et al., 1987; Kremp and Anderson, 2000; Rengefors and Anderson,
12 1998), temperature is often viewed as the main environmental determinant for cysts in the surface layer
13 of sediments (Anderson, 1998; Dale, 1983). Light is not necessary for germination in all species but, in
14 some, darkness slows down the process and might reduce the germination frequency and rate
15 (Anderson et al., 2005; Anderson et al., 1987; Bravo and Anderson, 1994; Genovesi et al., 2009).

16
17 Regarding *A. fundyense* bloom dynamics in the Gulf of Maine, germination is the most important factor
18 determining the initial bloom populations, since few overwintering motile cells have been encountered
19 in surface waters in that region (Anderson et al., 2005; Kirn et al., 2005). Although the germination of
20 *A. fundyense* has been thoroughly studied (Anderson et al., 2005) there is a lack of information on the
21 fate of the newly-germinated cells (germlings) and their immediate vegetative progeny. The
22 germination frequency and viability of germlings and their progeny has been estimated for shallow
23 water populations of *A. tamarense* in French coastal waters (Genovesi et al., 2009). The authors found
24 high germination frequencies for natural cysts isolated from field samples (85%) but only 27% of the

1 germlings were observed alive one day after germination. Of the surviving germlings, 76% were able
2 to divide at least once during a 30-day experiment. The length of cold and dark storage of cysts was
3 observed to influence these ratios, with increasing storage time decreasing the cyst quality and thus the
4 ability of the germling and its progeny to survive (Genovesi et al., 2009). The largest cyst seed banks of
5 *A. fundyense* in the northeastern U.S. lie in the Gulf of Maine at depths > 100 m (Anderson et al.,
6 2005). In this environment, the viability of cysts, germlings and their progeny is thus affected by
7 darkness and low temperatures. Darkness has been reported to impair germling survival for other
8 dinoflagellate species (Anderson et al., 1987; Bravo and Anderson, 1994) and it could therefore have an
9 impact on current model parameterizations for *A. fundyense* in the Gulf of Maine.

10

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

19

20 **2. Materials and methods**

21 *2.1. Sample collection and storage*

22 Eight sediment cores were collected using a hydraulically-damped piston corer (Craib, 1965) at a 120
23 m-deep site in the northwestern Gulf of Maine (43° 36' N, 69° 22' W) in October 2009. The site is
24 situated in a high-density cyst accumulation area (seedbed) along the mid-Maine coast. The cores were

1 sectioned, and slices were collected at 0-1 and 1-3 cm depths in the cores. The eight 0-1 and 1-3 cm
2 slices for each core were pooled to yield one homogenized 0-1 cm and one 1-3 cm sample that were
3 stored in completely-filled 50-ml plastic tubes. Based on sediment color, which was black, the sediment
4 was deemed anoxic. The tubes were stored in the cold (4 °C) and in total darkness immediately after
5 filling. The tubes were stored in these conditions until cyst isolation was conducted on 29 March 2010
6 (six months after collection), allowing the cysts to complete their mandatory maturation period
7 (Anderson, 1980; Bravo and Anderson, 1994) and enter the germination window regulated by an
8 internal clock (Anderson and Keafer, 1987; Matrai et al., 2005).

9 10 2.2. *Sample processing, cyst isolation and experimental setup*

11 All sediment processing was done in a darkened laboratory with the only light source being a soft red
12 light. On 29 March 2010 an aliquot of the cold- and dark-stored sediment from the 1-3 cm layer was
13 taken. To avoid including sediment that might have been oxidized during storage the top layer of
14 sediment from the 50-ml tube was removed and then a sample of arbitrary volume was scooped from
15 the center of the tube, avoiding sediment along the tube walls, into a 50 ml sample tube. The average
16 cyst concentration in the 1-3 cm layer was 2,763 cysts cm⁻³ of wet sediment. Filtered sea water was
17 added to a final volume of 45 ml and the slurry was sonicated with a Branson Sonifier 250 at a constant
18 40W output for 1 minute, and sieved to yield a clean 20-75 µm size fraction (Anderson et al., 2003).
19 The cysts were further concentrated and the sample cleaned of debris with a non-toxic isosmotic
20 density centrifugation method (Schwinghamer et al., 1991). Sucrose and colloidal silica (Nalco) was
21 used to create a solution with two density layers of 1.07 g cm⁻³ and 1.35 g cm⁻³. The mode *A. fundyense*
22 cyst density is 1.2 g cm⁻³ (Anderson et al., 1985), thus most of the cysts will concentrate at the interface
23 of the two liquids after centrifugation. Prior to centrifugation, 5 ml of the processed sediment sample
24 was added to a 50-ml centrifuge tube. Twenty milliliters of the lighter density gradient solution was

1 then carefully delivered under the processed sediment with a Pasteur pipette, after which 20 ml of the
2 denser solution was delivered under the lighter solution. The tube was centrifuged at 2500 RPM for 20
3 min at 4 °C and the clear lighter solution was aspirated and the liquid at the interface of the two
4 solutions was collected with a pipette and sieved through a 20 µm sieve. The contents were collected
5 into a 15-ml sample tube, which was immediately partitioned out into 1-ml Sedgwick-Rafter slides, and
6 cysts were isolated using micropipettes.

7
8 Upon isolation, individual cysts were immediately put into 96-well culture-plates with 300 µl of
9 modified (silica excluded) f/2 medium (Anderson et al., 1994) in each well. It took approximately 45
10 minutes to prepare one plate of isolated cysts. After each plate contained approximately 30 isolated
11 cysts, plates were sealed with electrical tape and put in a zip-lock bag along with a moistened paper
12 towel to reduce evaporative loss from the wells. Plates were then incubated in a 6 °C walk-in incubator.
13 In total, we isolated 10 plates (~300 cysts) of which 5 were designated as dark-treatment plates prior to
14 isolation. The dark-treatment plates were treated similarly to the light treatments, with a few
15 exceptions. Isolations were done in a dark room under low red ambient light and a red filter (Kodak
16 Wratten Red 25) that blocked light < 580 nm, was placed in front of the light source of the microscope
17 (isolations for the light treatment were done under normal laboratory illumination and no light filtering
18 on the microscope). All other light-emitting areas on the microscope were wrapped in red plastic film
19 or covered. The sealed plates were wrapped in foil and put in a dark colored plastic container that was
20 put inside a black plastic bag before incubation at 6 °C. Light treatment plates were incubated at 6 °C
21 with a 14h:10h light:dark cycle with an irradiation of approximately 250 µmol photons m⁻² s⁻¹. Cysts
22 incubated in the dark were only exposed to low levels of red light during sediment processing for
23 approximately 30 min, cyst isolation (approximately 45 min at most) and microscopic examination of

1 the plates. For specific wells there was a brief direct exposure during the examination of that well, and
2 then scattered red light during the examination of the rest of the wells. It took approximately 1 h to
3 examine one dark treatment plate during the germination and viability assessments.

4

5 2.3. Microscopic examination of isolated cysts

6 The well plates were checked immediately after they were sealed, using an inverted Zeiss IM35
7 microscope equipped with a Zeiss 09 filter set (excitation 450-490 nm, emission 515-750 nm) to detect
8 Chlorophyll-*a* (Chl-*a*) autofluorescence. Dark plates were always shielded from multi-spectral light and
9 examined only under low red light in a darkroom. During this initial examination of the plates, the
10 location and number of cysts in each well were recorded. Wells with multiple cysts were eliminated
11 from the experiment. Cysts in the light treatment were checked for onset of Chl-*a* autofluorescence,
12 which is considered an indication of pending germination (Anderson and Keafer, 1985; Anderson et al.,
13 2005) and for germination by detecting empty cysts or swimming *A. fundyense* cells. After cyst
14 germination, we continued to check the plates for swimming cells in order to see how long the
15 germling or the vegetative progeny produced by the germling would survive under our experimental
16 conditions. After cyst germination, the wells were checked by focusing from the bottom to the top of
17 the well several times in order to be able to observe the swimming cells.

18

19 During the first week of the experiment, one light treatment plate was checked on days 1, 2, 3, 4 and 8
20 to detect the onset of cyst Chl-*a* autofluorescence. When cysts started displaying autofluorescence, the
21 rest of the plates were also checked: the light treatment plates for cyst Chl-*a* autofluorescence and
22 germination and the dark treatment plates for germination only, since it was not possible to detect Chl-*a*
23 autofluorescence with red light excitation. During the experiment, light treatment plates were checked
24 on days 1, 2, 3, 4, 8, 9, 11, 12, 15, 18, 22, 29, 36, 44, 50, 57 and 65, and dark treatment plates on days

1 1, 10, 15, 22, 30, 37, 44, 50, 57 and 65.

2

3 *2.4. Estimating the germination rate and survival ratio*

4 To quantify germination rates we fitted a simple first-order equation to germination time course data
5 (Anderson et al., 1987). For an initial number of cysts N_0 : $N_t = N_0^{-kt}$, where N_t is the number of cysts
6 remaining at time t and k is the specific germination rate with units of time⁻¹. Germination rates are
7 expressed as averages for the duration of the experiment.

8

9 The survival ratio of germlings and their vegetative progeny was estimated by recording the occurrence
10 of live cells in tissue-culture-plate wells once every week. For each weekly observation after cyst
11 germination the number of wells with live cells was divided by the total number of germinated cysts in
12 each plate to get a survival ratio. Due to the plate checking interval, live cells were not observed in all
13 wells. In cases where an empty cyst but no live cells was observed it was assumed that the germling
14 cell had died after germination and before the checking of the plate. In these cases the germination time
15 was assigned according to the previous checking date. The initial survival time in these cases (first
16 observation of germinated cyst, but no observation of live cell during experiment) was handled by
17 assigning a survival time of one, three and six days. This range of initial survival times is used
18 throughout the calculations made in this study.

19

20 To account for cysts that were inherently of “poor quality” (i.e., could not produce viable germlings
21 even under illuminated conditions) and to account for non-darkness-related mortality, we scaled the
22 dark-survival ratio to the light-survival ratio. Since the checking intervals of our light and dark plates
23 did not match, we used the following approach: (1) the light treatment was used as a control by first
24 fitting a model to the data (Table 1) followed by use of the derived equation to extract the light survival

1 ratios at the specific time intervals when dark survival ratios were estimated; (2) the dark-survival
2 ratios were normalized against these estimated light survival ratios. A model was then fitted to the
3 values of the scaled dark-survival ratios to get estimates of scaled dark-survival ratios for the duration
4 of the experiment, with assumed initial survival times of germling cells of one, three and six days.

5

6 It is pertinent here to make the distinction between the viability of the cysts and of the germlings. The
7 viability of cysts is defined as their ability to germinate, whereas the viability of germlings is defined as
8 their capability to produce living progeny (i.e., undergo meiotic division) or to survive in the dark
9 treatments. Darkness is known to arrest the mitotic cell cycle in G₁ phase and prohibit cell division
10 (Taroncher et al., 1997), and we assume that the lack of light has the same effect on meiotic cell
11 division.

12

13 *2.4. Water column depth-related mortality rate and comparison to model results*

14 A comparison of our results against the existing parameterization in a model for *A. fundyense*
15 population dynamics in the Gulf of Maine described by He et al., (2008) was also made. We calculated
16 a depth-integrated mortality rate from the mortality term used in the model. The model-mortality term
17 (M_m) includes losses due to grazing, encystment, and natural cell death, and is temperature dependent.
18 In these calculations we used monthly averages from a temperature climatology (Lynch et al., 1996) in
19 the area where the cysts were collected. In extrapolation from our experimental data to obtain a
20 survival rate following germination and during ascent to the surface, we assume no growth in darkness.
21 Therefore, the population experiences a net loss of cells after germination before the cells reach the
22 illuminated surface layer where they are able to commence growth. In the calculations based on our
23 experimental results, we used a euphotic zone depth of 30 m, representative of a low-end value for the
24 Gulf of Maine (e.g., Hoepffner and Sathyendranath, 1992). For the calculations based on the mortality

1 parameterization of the model, we compute the temperature-dependent mortality versus depth of
2 germination based on the time required for the germlings to swim upward to the base of the euphotic
3 zone (30 m). For the purposes of modeling germination in areas deeper than the location from which
4 the cores were taken, the profiles were extended assuming uniform temperature from 95 m depth to 150
5 m. This assumption is based on the weak vertical gradients present in the 60-95 m depth interval of the
6 climatological temperature profiles.

7

8 The proportions of cells that survive the transit from the germination depth to the euphotic zone were
9 calculated for 5-m-depth intervals for germination depths ranging from 30 to 150 m using the model
10 parameterization and our observations using equations 1 and 2, respectively.

11

$$12 \quad M_s = 1 - (M_m * t_{aph}) \quad (1)$$

$$13 \quad E_s = y0 + (a / t_{aph}) \quad (2)$$

14

15 M_s is the surviving ratio of cells using the model parameterization and E_s the surviving ratio of cells
16 using the experimental results. M_m is the model mortality term ($M_m = a_m * Q_{10}^{[(T - 10.35) / 10]}$, $a_m = 0.066$
17 and $Q_{10} = 21.75$) and t_{aph} is the time the cells spend in the aphotic zone assuming an average upward
18 swimming speed of 10 m d⁻¹ (Bauerfeind et al., 1986; Eppley et al., 1968; Kamykowski et al., 1992).

19 The aphotic time is defined by $t_{aph} = (G_d - D_{euph}) / S$, where G_d is germination depth in meters, S is
20 swimming speed in m d⁻¹ and D_{euph} is the depth of the euphotic zone.

21

22 Equation 2 is the best-fit inverse first-order polynomial model of the scaled dark-survival data, where
23 $y0$ and a are constants (initial survival time one day: $y0 = 0.125$, $a = 0.881$; initial survival time three
24 days: $y0 = -0.003$, $a = 3.010$; initial survival time six days: $y0 = -0.202$, $a = 6.663$). Since the method of

1 interpolation might affect our results to some degree we tried a suite of different approaches. Inverse-
2 polynomial and exponential-decay functions seem to fit the data best (Table 1). Of these equations, we
3 chose the one with the highest adjusted r^2 and most- significant- parameter estimates. Using any of the
4 other equations that fit the data well will cause differences in the estimated survival ratios during
5 ascent. In Table 1, we present the difference in survival ratio from the chosen equation if any one of the
6 other equations is used to calculate the estimated survival ratio from an arbitrary depth of 100 m.

7

8 **3. Results**

9 *3.1. Cyst autofluorescence and germination rates*

10 For the first 9 days, cysts incubated in the light displayed no Chl-*a* autofluorescence. The cysts had a
11 small amount of granular cytoplasm and a pronounced yellow accumulation body. The first Chl-*a*
12 autofluorescent cysts were observed on day 10, after which, most of the cysts (up to 94%) showed
13 autofluorescence at some stage of the experiment (Fig. 1). The development of Chl-*a* autofluorescence
14 was generally synchronous, (i.e., all cysts in the light treatments started fluorescing at approximately
15 the same time during the experiment). Germination was also synchronous but differed between
16 treatments, with the dark-incubated cysts showing a one week lag in the germination rate maximum in
17 comparison to the light treatment (Fig. 1). The first germinated cysts were observed on Day 15, both in
18 the light and dark treatments. The average germination rate in the light was 0.062 d^{-1} ($SD \pm 0.051$) and
19 0.038 d^{-1} ($SD \pm 0.029$) in the dark. Both treatments reached similar excystment frequencies (91%) after
20 approximately 50 days (Fig. 1). In the light treatments, on average 97% of cysts that showed Chl-*a*
21 autofluorescence germinated during the experiment. Only one cyst that germinated was not observed to
22 display Chl-*a* autofluorescence.

23

24 *3.2. Germling and progeny survival*

1 In light treatments, 47% of germinated cysts produced germlings that were able to survive and produce
2 vegetative progeny that survived at least 7 days, i.e. a live cell was observed in the well along with an
3 empty cyst at least once during the experiment. In the dark treatments 12% of cysts produced germlings
4 that were able to survive for the same length of time. To account for non-viable germlings (germling
5 mortality not related to lack of illumination), we scaled the ratio of cysts that germinated and produced
6 a live germling cell in the dark by the ratio of cysts that were able to produce live germling cells in the
7 light. When accounting for the non-viable germlings, 24% of cysts in the dark produced germlings that
8 were able to survive for 7 days.

9
10 The cyst progeny experienced marked mortality during the first weeks of the experiment, even in the
11 light treatment. After an initial high mortality the light treatment showed a more slowly-declining
12 survival ratio until Day 42 of the experiment, after which a more rapid decline in surviving cells again
13 took place (Fig. 2). In subsequent calculations and model fitting to the data are cut off at Day 42 due to
14 this enhanced mortality in the light treatments. With the chosen model fit the assumption of a one day
15 initial survival causes the steepest initial slope of the survival curve and thus also the shortest germling
16 half-life (Fig. 3, Table 2). The estimated half-life varies according to the chosen initial survival time,
17 from 2.3 days to 9.5 days for the scaled dark survival results.

18 19 *3.3. Water-column-depth-related mortality rate and comparison to model results*

20 The surviving proportion of cells from hypothetical cyst seedbeds lying at 30-150 m depth
21 experiencing mortality due to darkness was estimated assuming the initial survival times of germling
22 cells to be one, three and six days. The scaled dark survival rates are shown in Figure 3. These survival
23 times are reasonable, taking into account the depth of cyst seedbeds, the depth of the euphotic zone and
24 the average swimming speed of dinoflagellates. These results are compared against the mortality term

1 used in a coupled physical-biological model describing the population dynamics of *A. fundyense* in the
2 Gulf of Maine (Fig. 4). The calculations do not include growth and only describe the mortality of the
3 initial germling inoculum population. Based on our results approximately 25-80% (100% if an initial
4 survival time of 8 days is assumed) of the cells germinating at a depth of 100 m could survive the
5 transit to the euphotic zone, whereas, the model mortality term allows for 75-95% of the cells to reach
6 the surface layer (Fig. 4). However, there are some regimes in which the experimental results suggest
7 mortalities that are less than those predicted by the temperature-dependent model, particularly for
8 longer initial survival times, shallower germination depths, and warmer temperatures. At bottom depths
9 greater than 100 m the survival curves based on the experimental results start to converge as the fitted
10 models approach a crossover point between two and three weeks of aphotic time (Figs. 3 and 4).

11

12 **4. Discussion**

13

14 Information on the fate and survival potential of newly-germinated cells from resting cysts in bottom
15 sediments is critical in the study of population dynamics of cyst-forming HAB species such as *A.*
16 *fundyense*. Here we conducted laboratory experiments to characterize the germination and survival
17 rates of cysts and cells incubated in the light versus the dark. We also compared our measured survival
18 rates to the rates used in the physical –biological model used to simulate *A. fundyense* population
19 dynamics in the Gulf of Maine (McGillicuddy et al., 2005; He et al., 2008). Our results show a marked
20 initial mortality of germling cells in both light and dark treatments, prompting a re-evaluation of how
21 we think about cyst viability. In addition to the potential to germinate, we need to account for the
22 ability of the germling to divide and produce viable vegetative cells. Here we provide direct
23 measurements of survival of germling cells and their vegetative progeny for *A. fundyense* cysts in the
24 deep Gulf of Maine, specifically looking at the effect of darkness on the survival of germling cells. Our

1 aim is to improve existing indirect estimates of cell emergence from coastal sediments.

2

3 *4.1. Chl-a autofluorescence and germination rates*

4 The increase in Chl-*a* fluorescence and germination of cysts were synchronous in the experiment.

5 Synchronous development of Chl-*a* autofluorescence, and also germination, has been previously

6 observed (Anderson and Keafer, 1985; Anderson et al., 2005; Genovesi et al., 2009), even though

7 regional differences exist. In our study 94% of the cysts showed autofluorescence at some point during

8 the experiment (light treatment) and of these, 97% germinated at some point during the experiment. We

9 assume that a similar proportion of dark-treatment cysts showed autofluorescence prior to germination.

10 If the estimate holds true, 94% of cysts in the dark that would have shown Chl-*a* autofluorescence

11 would have germinated. Our observed germination rates are in line with previous estimates (Anderson

12 et al., 2005). We can conclude that cyst viability in our study was high, and in line with previous

13 studies (e.g., Anderson et al., 2005).

14

15 Anderson et al. (2005) developed an indirect method for estimating the flux of cells germinated from

16 deep coastal sediments. Briefly, the flux was estimated by a combination of laboratory measurements

17 of cyst autofluorescence and germination, and observations of cyst autofluorescence in the field.

18 Sediment slurry containing a natural high concentration of cysts was made up of one part of natural

19 sediment and seven parts of f/2 medium. Aliquots of this slurry were incubated in a range of

20 temperatures, as well as under illumination and in darkness. The number of cysts and the fraction of

21 Chl-*a* autofluorescent cysts were counted at specific time intervals. The difference between the initial

22 cyst number and the cyst number after incubation was used to calculate the percentage of germinated

23 cysts. This approach was implemented in the physical-biological model used for studies of *A.*

24 *fundyense* bloom dynamics in the Gulf of Maine (McGillicuddy et al., 2005; Stock et al., 2005; He et

1 al., 2008). However, this method assumes survival of all germling cells, which is an aspect that has not
2 received much attention in the past. Genovesi et al. (2009) made observations on germling cell viability
3 for shallow water *A. tamarense* populations and concluded that viability was considerably lower than
4 the germination success would have indicated, with approximately 27% of cysts producing germling
5 cells that were able to survive and subsequently divide. In our experiment, 47% of cysts incubated
6 under illuminated conditions produced live germling cells that were able to divide and survive at least
7 seven days. For the dark-incubated cysts, the percentage of germling cells that survived for seven days
8 was 12%. These results may be indicative of the quality of cysts in the specific locale from where the
9 cysts were collected. As such, it would be valuable to conduct studies on the spatial variation of the
10 percentage of cysts able to produce viable germlings.

11

12 Our results show that darkness retards germination timing, as has been previously observed for
13 *Alexandrium* and other dinoflagellate genera (Anderson et al., 1987; Bravo and Anderson, 1994). We
14 observed a similar germination frequency in both light and dark treatments after 50 days, indicating no
15 light requirement for germination. Genovesi et al. (2009) noted that total darkness depressed the
16 cumulative excystment frequency of *A. tamarense* somewhat (62%) compared to treatments that had
17 received light exposures ($100 \mu\text{moles photons m}^{-2} \text{ s}^{-1}$) of 1, 3, 6 and 12 h, before dark incubation
18 (average excystment frequency 80%). However, the cysts in that particular study were harvested from
19 a shallow sea area, which could cause effective selection on the population favoring cysts that have
20 light-inducible excystment.

21

22 4.2. *Survival of germlings and non-darkness related mortality*

23 In our experiment, only a fraction of cysts that germinated seemed to be able to produce viable
24 germlings. This can be due to the fact that we used sediment from the 1 to 3 cm layer from the cores,

1 which would render the cysts slightly older than those at the surface, and also potentially affect their
2 quality (Genovesi et al., 2009). Older cysts of poorer quality, i.e. they have used more of their storage
3 energy during quiescence, would be prone to produce less-viable progeny, especially if germinating in
4 the dark where synthesis of new energy stores is not possible. The cysts would be able to germinate but
5 the germling cells would not be able to divide or the cells would die soon thereafter. The length of cold
6 dark storage has been noted to affect cyst quality, with longer storage times causing a decrease in the
7 ability of cysts to produce viable germlings (Genovesi et al., 2009). Darkness is also known to cause
8 the cell cycle to be arrested in G₁ phase and inhibit mitotic cell division (Taroncher et al., 1997). If
9 darkness affects meiotic cell division in a similar manner, this effectively means that all cells
10 germinating at aphotic depths remain in the germling stage until they reach photic depths, and the cell
11 cycle is able to resume. How this affects cell survival is not known.

12

13 The cells in our experiment were affected by other mortality factors besides the ones caused by
14 darkness directly, as seen from the declining survival ratio in the light treatments. The survival of cells
15 also declined rapidly after day 42 of the experiment for some reason. The most plausible causes that
16 can be evoked for premature cell death are poor cyst quality, producing non-viable germlings, and later
17 on in the experiment, density dependent mortality in the light treatment. However, it could also be other
18 factors related to the incubation conditions. Therefore, we need to account for mortality not caused by
19 darkness in the dark treatments for which the light treatment can function as an effective control.

20

21 The simplest approach is to scale the dark-survival ratio of germlings to the survival ratio of germlings
22 in the light treatment. The scaling was done by first fitting a model to the light treatment data, in order
23 to take into account a range of different initial survival times for the germling cells and to be able to
24 synchronize the light and dark treatment observations. The results from the model fit were used to

1 calculate the scaled dark survival, by dividing the dark-treatment survival ratio by the light-treatment
2 survival ratio. These scaled results were used when we compared our results to model mortality
3 parameterizations. We make the assumption that the same factors (beside darkness) are causing
4 mortality in both the light and dark treatment. It might even be possible that the illumination itself is
5 causing mortality in the light treatments, however, that seems unlikely since the light level we used,
6 $250 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, falls in the interval usually used to culture *A. fundyense*, 200-400 μmol
7 $\text{photons m}^{-2} \text{ s}^{-1}$ (pers. comm. D. Kulis).

8
9 Because of the lack of frequent observations, the assumption of initial survival time of germlings of
10 one, three and six days cause a marked variation in the estimated half-lives of the germlings/cells. The
11 difference in half-life between the one- and six -day initial survival time assumption scenarios for the
12 scaled dark treatment is 7.2 days. However, a previous study has shown that germlings of poor quality
13 do not survive for long (Genovesi et al., 2009). Only 27% of cysts in the study produced germlings that
14 lived longer than 1 day. Thus, rather short initial survival times seem a more plausible assumption than
15 longer ones. These results apply for experiments with more frequent observations of germling survival
16 in the dark for coastal *A. fundyense* populations.

17

18 *4.3. Survival during ascent: model versus data comparisons*

19 In order to estimate the effects of darkness on the survival of germlings and their progeny in natural
20 waters, we calculated the survival of germinated cells moving upward through a deep water column.
21 We also compared these results to parameterizations from an existing coupled physical-biological
22 model used to simulate the population dynamics of *A. fundyense* in the Gulf of Maine (Fig. 4). The
23 model assumes that 100% of the cysts in the top 1 cm of surface sediments can germinate, with the
24 survival of the germling cells and their progeny determined by a temperature-dependent mortality term

1 (He et al., 2008). This mortality term allows 75-95% of the population that derive from germinated
2 cysts to reach the illuminated surface layer at 30 m depth, in a 100 m water column overlying bottom
3 sediments where the germination occurs.

4

5 Dinoflagellate cells have been reported to reach swimming speeds of 5 to 15 m d⁻¹ (Bauerfeind et al.,
6 1986; Eppley et al., 1968; Kamykowski et al., 1992). Without any effect from hydrodynamic transport,
7 a cell germinating on the bottom at 100 m depth and swimming at an average speed of 10 m d⁻¹ would
8 require approximately 10 days to reach the surface, and seven days to reach the euphotic zone where
9 photosynthesis and cell division can commence, assuming a euphotic layer depth of approximately 30
10 m for the Gulf of Maine (Hoepffner and Sathyendranath, 1992). The spatial distribution of cysts and
11 cyst germination appear to be an important part of bloom dynamics, as indicated by coupled physical-
12 biological models (McGillicuddy et al., 2005; Stock et al., 2005; He et al., 2008; Li et al., 2009;
13 Anderson et al., this issue). Most of the cysts lie at depths below 100 m depth, either in bottom
14 sediments (Anderson et al., 2005; Anderson et al., this issue), or in a near-bottom benthic nepheloid
15 layer (Pilskaln et al., this issue). Therefore, it is important to be able to evaluate how large of a
16 proportion of cysts actually produce living progeny that survive for a long enough period to be able to
17 further divide and contribute to population growth in illuminated water layers, assuming that the cells
18 do not grow in the dark (pers. comm. D. Kulis; Vahtera et al., unpublished data; Taroncher et al., 1997).

19

20 Our study showed that germling survival is lower in the dark, which is the condition that prevails in
21 deep waters of different parts of the Gulf of Maine. In darkness, the half-life of germling cells is on
22 average approximately 30-35% shorter than in the light. Depending on the assumption of initial
23 survival time (1, 3 or 6 d) for germling cells, 27-35%, 50-75% or 90-100% of cells would survive a
24 transit to 30 m depth from bottom depths ranging from 90 to 70 m, respectively. This percentage of

1 surviving cells is the subject of additional mortality due to grazing and other factors, which are not
2 considered in this study. The chosen model fit to our data obviously affects the results of this
3 comparison. The curves for the three different initial survival-time estimates start converging when
4 approaching 17 days (Fig. 3). After this, the development of survival becomes the opposite for the one
5 and six day initial survival time estimates. In our calculations the longest time span is 12 days. The
6 curves do not converge, however, they are approaching each other, which can be seen as a decreasing
7 difference in survival ratio when germination depth increases (Figs. 3 and 4). Different model fits will
8 behave slightly differently and give somewhat different results, e.g. a single, one-parameter exponential
9 decay model will yield a survival ratio that is 0.16 units smaller than what the currently- used model
10 yields. What is important though is the fact that not all cysts are able to produce germlings that can
11 survive the ascent to the illuminated surface layer and that most of the mortality takes place during the
12 first several days after germination. This indicates that caution should be exercised when evaluating the
13 quantitative impact of cyst seed banks on bloom formation based solely on cyst concentrations in the
14 surface sediments. Survival ratios are affected by cyst quality, so both bottom depth and the average
15 age of cysts in the seed bank will affect the ability of the cysts to seed blooms effectively.

16
17 Our data are obviously compromised by the fact that the experiment was carried out in the laboratory.
18 Further, the plate-checking interval presents an important caveat to the interpretation of the data.
19 Survival of cells during the first ten days of our experiment is the most important time interval with
20 regard to the model comparisons. During this interval we have only one observation. By changing the
21 length of time of initial survival to one, three and six days we can markedly affect the results. If 100%
22 of the cells would have survived for six days, 100% of germinated cells would make it up to the
23 euphotic zone from a bottom depth of approximately 85 m. Germling viability and initial survival time
24 of germling cells needs to be further investigated in order to make our estimates more accurate.

1 However, the relative differences in initial survival between light and dark treatments (47% vs. 12% of
2 cysts producing germlings that were able to divide) should prompt a more accurate description of
3 germling emergence, survival and vertical movement of these cells in models. An effort should be
4 made to more accurately estimate the proportion of germlings and their progeny in relation to
5 germinated cysts that reach the illuminated layers of the water column.

6

7 **Acknowledgements**

8 E. Vahtera was funded by the Academy of Finland (grant #130934) and B. Gomez-Crespo was
9 supported by a Xunta de Galicia Ángeles Alvariño fellowship. Additional funding support was also
10 provided by the National Oceanic Atmospheric Administration ECOHAB program through grants
11 NA06NOS4780245 and NA09NOS4780193 and from National Science Foundation grants OCE-
12 0430724 and OCE-0911031 and National Institute of Environmental Health Sciences grant 1P50-
13 ES01274201 through the Woods Hole Center for Oceans and Human Health. We are also grateful for
14 technical assistance from Z. Bonin, B. Keafer, K. Smith, and D. Kulis. R. He, and Y. Li are
15 acknowledged for their valuable comments on the manuscript. This is ECOHAB contribution number
16 734.

17

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1 **Tables**

2

3 Table 1. The types of models evaluated for scaling of dark survival data, adjusted r^2 values and
 4 statistical significance of all parameter estimates. The difference in calculated survival ratio between
 5 the chosen first order inverse polynomial model and the other evaluated models, when assuming
 6 germination at 100 m depth.

7

Type of function		Adjusted r^2	Significant par. estimates	Difference from chosen function
Inverse polynomial	¹ $f = y0 + (a/x)$	0.9889	Yes	
	$f = y0 + (a/x) + (b/x^2)$	0.9906	No	-0.04
Exponential decay	$f = a*exp^{(-b*x)}$	0.8404	Yes	-0.16
	$f = y0 + a*exp^{(-b*x)}$	0.9724	Yes	-0.08

8 ¹ function chosen to calculate the scaled survival ratio

9

10 Table 2. The calculated half-life of germlings/cells in the experiment calculated based on the first
 11 degree inverse polynomial model fitted to the data for the raw light and dark treatment data and the
 12 scaled dark treatment data. Initial survival time refers to the survival time of the germling cell before
 13 the first observation was made.

14

Initial survival time (d)	Half-life of germlings/cells (d)		
	Light treatment	Dark treatment	Scaled dark treatment
1	3.6	2.0	2.3
3	8.1	5.3	6.0
6	11.7	8.6	9.5

15

16

17 **Figures**

18

19 Figure 1. The average cumulative percentage of cysts showing Chl-*a* autofluorescence (light treatment
 20 only) and the average cumulative percentage of cysts that germinated during the experiment. Error bars
 21 show standard deviation.

22

23 Figure 2. Survival ratios of cells in light and dark treatments during the experiment. The symbols
 24 overlay each other at the first point in time.

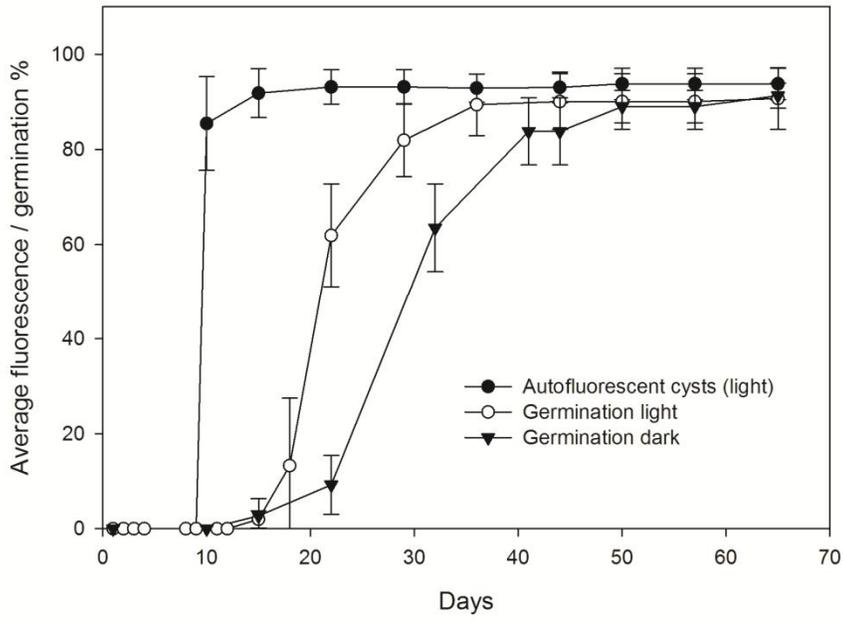
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26 Figure 3. The calculated scaled dark survival ratio. The scaled survival ratio is calculated based on
 27 models (table 1) fitted to data shown in figure 2 assuming initial survival times of 1, 3 and 6 days (see
 28 text for details).

29

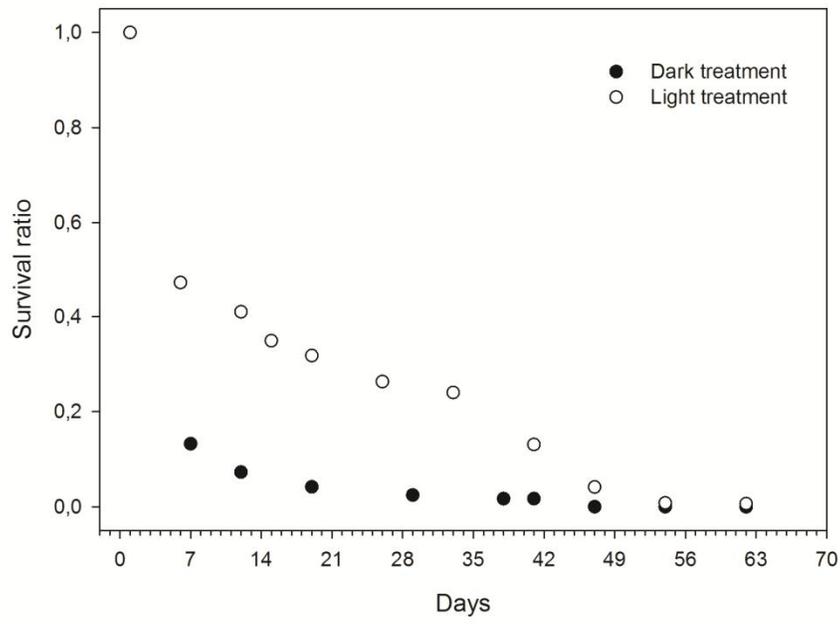
30 Figure 4. Temperature climatology used for model calculations of the survival of cells migrating
 31 upward from a specific depth at an average swimming speed of 10 m d⁻¹ (upper panel); and comparison
 32 of the survival ratio of cells germinating at different water column depths between our experimental
 33 scaled dark treatment values (denoted experiment 1-8d) and the model parameterization (lower panel)

1 used by He et al. (2008), (denoted March-July) see text for details. Calculations are done with a mean
2 euphotic zone depth of 30 m representing the natural variation found in the Gulf of Maine.
3



1
2 Figure 1.
3

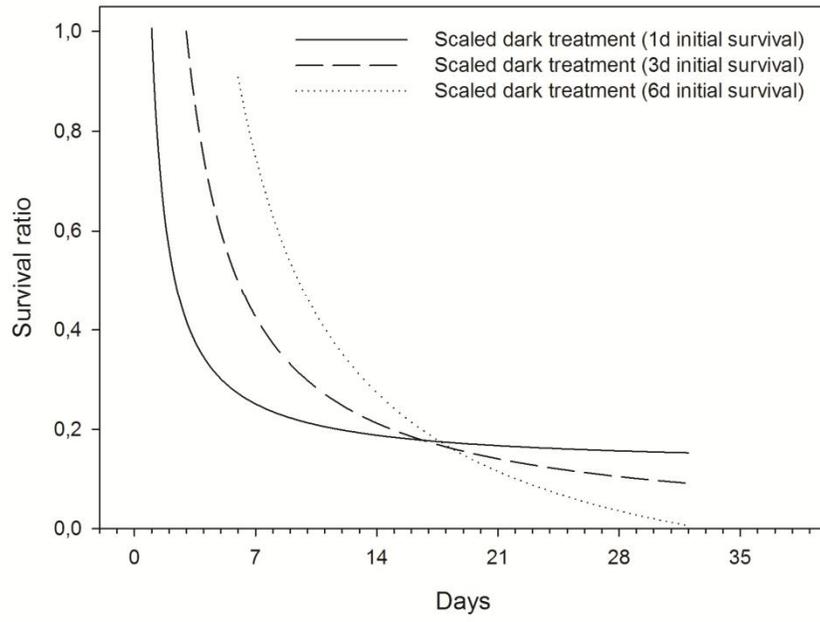
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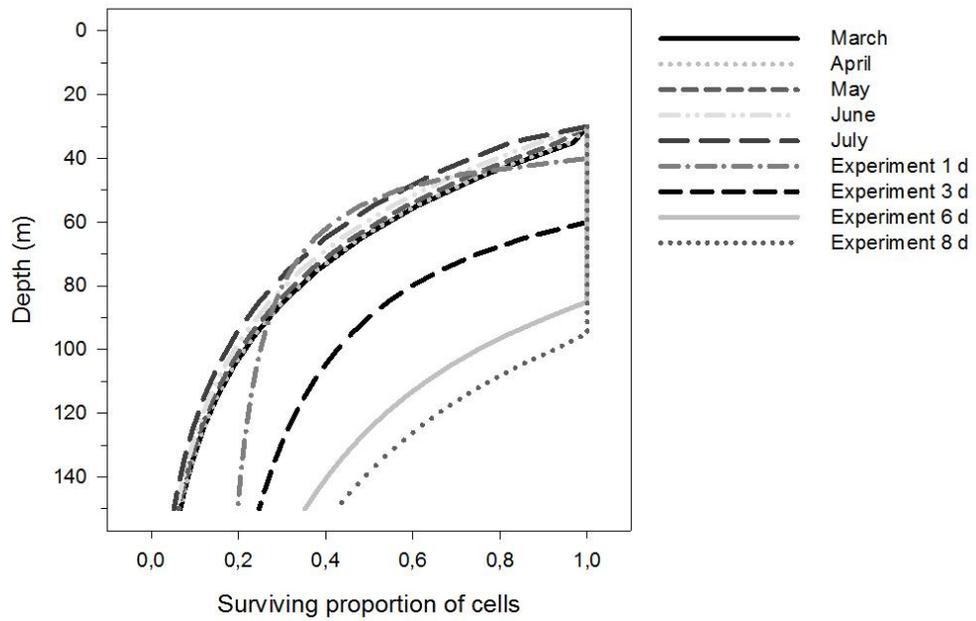
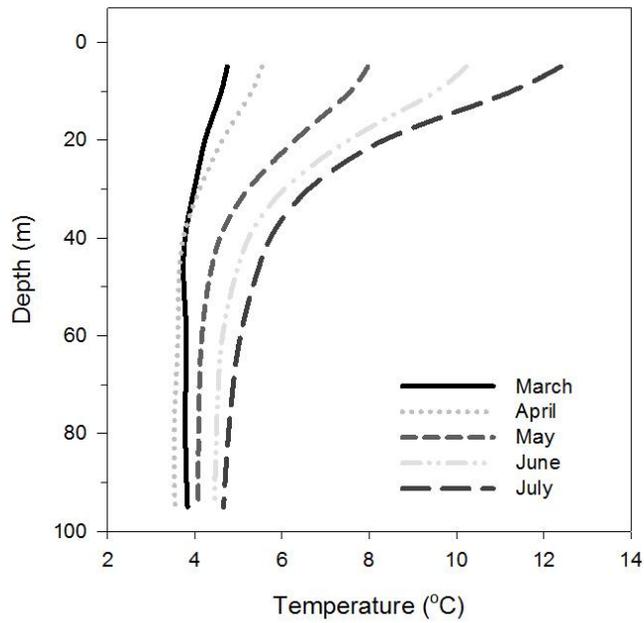
3 Figure 2.

4



1

2 Figure 3.



1

2 Figure 4.