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Distribution of *Alexandrium fundyense* (Dinophyceae) cysts in Greenland and Iceland, with an emphasis on viability and growth in the Arctic

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ABSTRACT: The bloom-forming dinoflagellate *Alexandrium fundyense* has been extensively studied due its toxin-producing capabilities and consequent impacts on human health and economies. This study investigated the prevalence of resting cysts of *A. fundyense* in western Greenland and Iceland, to assess the historical presence and magnitude of bloom populations in the region, and to characterize environmental conditions during summer, when bloom development may occur. Analysis of sediments collected from these locations showed that *A. fundyense* cysts were present at low to moderate densities in most areas surveyed, with highest densities observed in western Iceland. Additionally, laboratory experiments were conducted on clonal cultures established from isolated cysts or vegetative cells from Greenland, Iceland, and the Chukchi Sea (near Alaska) to examine the effects of photoperiod interval and irradiance levels on growth. Growth rates in response to the experimental treatments varied among isolates, but were generally highest under conditions that included both the shortest photoperiod interval (16 h light:8 h dark) and higher irradiance levels (~146 to 366 µmol photons m^{-2} s^{-1}), followed by growth under an extended photoperiod interval and low irradiance level (~37 µmol photons m^{-2} s^{-1}). Based on field and laboratory data, we hypothesize that blooms in Greenland are primarily derived from advected *A. fundyense* populations, as low bottom temperatures and limited light availability would likely preclude *in situ* bloom development. In contrast, the bays and fjords in Iceland may provide more favorable habitat for germling cell survival and growth and therefore may support indigenous, self-seeding blooms.

KEY WORDS: Arctic · *Alexandrium* · Dinoflagellate · Cysts · Harmful algal bloom

INTRODUCTION

The bloom-forming dinoflagellate genus *Alexandrium* Halim emend Balech (1995) has been extensively studied due to its toxigenicity, particularly those taxa comprising the *tamarense* species complex, which includes *A. acatenella*, *A. catenella, A. excavatum, A. fundyense*, *A. tamarense*, and several closely related species formerly assigned to *Protogonyaulax* Taylor. The human illness caused by the toxins produced by *Alexandrium* is known as paralytic shellfish poisoning (PSP), which is widespread in temperate waters around the world.

One strategy for the success of this dinoflagellate across such a range of habitats is that the life cycle of many species in the genus includes a benthic cyst

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stage. This stage allows cells to enter dormancy during unfavorable temperature or nutrient conditions and survive in sediments during temperature extremes (i.e. overwinter), with seasonal germination inoculating vegetative cells into the water column only when conditions are suitable for growth (Anderson 1998). Population development is thus possible in more locations than would otherwise be the case if year-round persistence in the plankton were the only means for survival. The cyst is also critical in species dispersal, as cells transported to new locations by storms, currents, wildlife, or humans can colonize an area by depositing cysts that germinate in subsequent years.

Due to the widespread and serious impacts of these blooms, the distribution, life cycle, taxonomy, and physiology of *A. tamarense* species complex taxa are relatively well-studied compared to many other globally distributed phytoplankton. Recent morphological, molecular, and mating studies indicate that the strains comprising Group 1 (formerly North American) clade (Lilly et al. 2007) of this complex is composed of a single species: *A. fundyense* (John et al. 2014a,b). There is general agreement that the *tamarense* complex should be split into separate species, but there is some disagreement regarding the name to be used for the Group 1/North American clade, with some arguing that it should be *A. catenella* and not *A. fundyense* (Fraga et al. 2015). Here, we use the name *A. fundyense*, but recognize that this issue is not yet fully resolved.

Although *A. fundyense* is not considered endemic to the Arctic, several recent reports of its cysts, cells, and toxins in Arctic waters suggest that suitable habitat for growth and bloom formation is present in this region. *A. fundyense* has been reported from coastal waters near Barrow, AK (Okolodkov 2005), and recent work by several groups documented *A. fundyense* cysts, vegetative cells, and toxins in the Chukchi and Beaufort Seas (Gu et al. 2013, Natsuike et al. 2013). Notably, the extraordinarily high densities of *A. fundyense* cysts (maximum 10 600 cysts cm^{-3}) observed in surface sediments from the Chukchi Sea are among the highest ever reported for this species (Natsuike et al. 2013).

In waters east of North America and north of Europe, *A. fundyense* has been observed in plankton samples from the Labrador, Greenland, and Norwegian Seas (Scholin 1998, Okolodkov 2005, Baggesen et al. 2012, Tillmann et al. 2016), and in the Northwest Passage in the Canadian archipelago (M. Levasseur pers. comm.). PSP toxins in blue mussels were recently reported for the first time from Iceland, along with record high numbers of toxic *Alexandrium* spp. (>16 000 cells l^{-1}) (Burrell et al. 2013). Additionally, PSP toxins were detected in scallops at levels exceeding regulatory limits for the first time in western Greenland (Baggesen et al. 2012), and *A. fundyense* was isolated and identified from nearby waters. Although it is clear that environmental conditions in at least some areas of the Arctic foster cell growth and bloom development, at this point it is unclear whether and where the establishment of endemic populations (via cyst germination) might be possible, and how climate-driven increases in bottom temperatures might influence the future range and magnitude of blooms in the region.

The aforementioned reports prompted the current investigation, which sought to better characterize the present distribution of this species in western Greenland and Iceland relative to environmental conditions, and to examine growth responses of Arctic isolates compared with those from temperate regions. We examined sediment samples collected in western Greenland and Iceland for *Alexandrium* cyst accumulations (an approach for assessing the presence and magnitude of bloom populations present in previous years) to better understand the prevalence of *Alexandrium* in Arctic waters. Additionally, we characterized the particle size distribution of sediment samples (sediment structure) to assess whether certain areas might favor the accumulation of higher cyst densities.

Data collected on the underwater light field, temperature, and sampling depth were used to infer the potential for germling survival and cell growth. Associated laboratory experiments were carried out with *A. fundyense* cultured isolates established during these surveys to examine their growth responses to the particular light intensities and photoperiod intervals that bloom populations would experience during summer months in the Arctic. Our goals were to (1) provide a preliminary characterization of cyst densities in western Greenland and Iceland, including comparisons between fjord and external coastal habitats, (2) assess environmental factors (temperature, light, and photoperiod interval) that determine viability and growth of germling cells, (3) identify areas that might favor *in situ* bloom initiation, and (4) use these data to generate hypotheses regarding the origins and fate of PSP toxin-producing *Alexandrium* populations in this region. Many dinoflagellate species form cysts, and thus studies like the one presented here provide information about how this adaptive strategy might influence the distributions of many species in a warming climate.

MATERIALS AND METHODS

Field collections

Sediment sampling and data collection were carried out during a research oceanographic cruise aboard the RV 'Maria S. Merian' (27 July to 8 Aug 2012); see also Cembella et al. (2016). This particular cruise leg (field campaign MSM 21/leg 3) included a comparative study of the west coasts and fjords of Greenland and Iceland (Fig. 1). Sediments were collected from a total of 20 stations to characterize the prevalence of *Alexandrium fundyense* cysts in western Greenland and Iceland. Samples were collected from Uummannaq Fjord, the Vaigat, Disko Bay, and 2 stations near Cape Farewell in Greenland, and from Arnarfjörður and Breiðafjörður in Iceland (Fig. 1). Sediments were collected with 2 van Veen grab-samplers, each of which can extract sediments up to 20 cm deep, with a sampling area of 0.04 m^2 (small sampler) or 0.1 m^2 (large sampler). Samples for cyst enumeration, culture establishment, and analysis of sediment characteristics were collected from the sediment surface layer (upper 10 cm) in the grab, and stored in anoxic conditions in the dark at 2°C until further processing (Anderson et al. 1987). Subsequent laboratory analyses were carried out at the Woods Hole Oceanographic Institution.

For cyst enumeration, a homogenized 5 cm^3 sediment sample was removed from each sample, resuspended with filtered seawater, sonicated with a Branson Sonifier 250D at a constant 40 W output for 1 min, and sieved to yield a clean, 20 to 100 µm size fraction (Anderson et al. 2003). Cysts were then concentrated using a single density layer (1.4 g cm^{-3}) of NALCO 1060 colloidal silica (Nalco) (Schwinghamer et al. 1991, Anderson et al. 2003) following procedures described in Vahtera et al. (2014). *A. fundyense* cysts were stained with Primulin (MP Biomedicals) and enumerated in each sample as described in Anderson et al. (2003).

Analysis of sediment structure

Sediment samples were collected at defined stations and frozen at –25°C until analysis at the University of Oldenburg, Germany. For these analyses, the particle size distribution (PSD) from subsamples was determined using a laser scattering particle size analyzer (Horiba LA-950). To remove coarse fragments before measurement, subsamples were sieved through a 2 mm mesh sieve and treated with sodium meta-phosphate ($NaPO_3$, 2% in water) due to presence of aggregates in the sample. The filtrate was examined with the laser particle size analyzer, which has a measurement range of 0.01 to 3000 µm, providing relative composition of 7 granulometric fractions from <2 to 630–2000 µm.

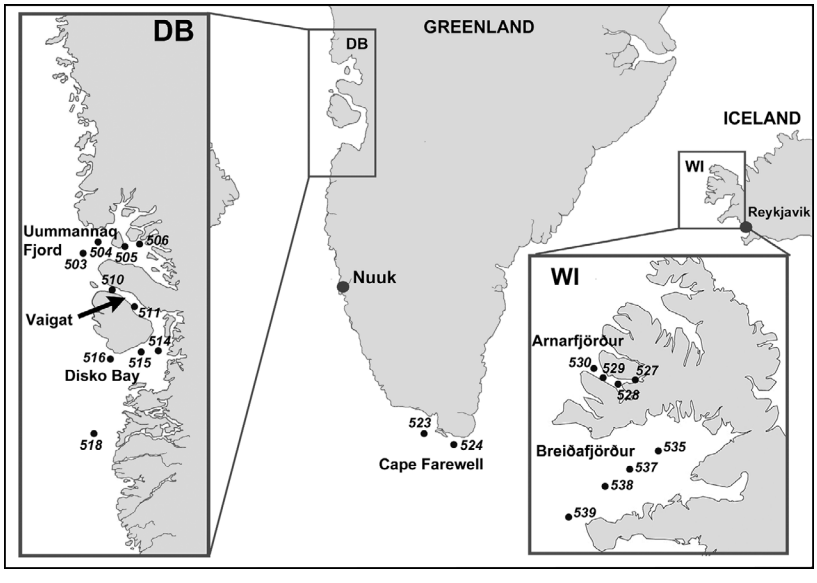

Fig. 1. Sediment sampling locations (stations indicated by numbered dots) in Greenland and Iceland. Intensive sampling was carried out in the Disko Bay region (DB) and western Iceland (WI)

Water column properties and optical measurements

At each sampling location, data on water column properties were collected with a CTD-rosette sampler, and above- and in-water hyperspectral radiometric measurements were collected to investigate the optical properties of water masses (see also Garaba & Zielinski 2013, Holinde & Zielinski 2016). The CTD casts were performed with a Seabird 'sbe911+' CTD probe with sampling rosette at each station as a start-up to determine further key discrete sampling depths (e.g. to locate chlorophyll maxima). Live data acquisition was carried out via CTD-client onboard and data post-processing with Seasoft V2 (Seabird). Salinity and depth were calculated from pressure val-

ues (Fofonoff & Millard 1983), and temperature was corrected to ITS-90 (Preston-Thomas 1990). All CTD data are available from the WDC-Mare database system Pangaea® (doi:10.1594/PANGAEA.819731).

A HyperPro II profiling system (Satlantic) was used to acquire bio-optical data for inherent and apparent optical properties (Holinde & Zielinski 2016). The profiler consisted of one hyperspectral irradiance and one hyperspectral radiance sensor. A second hyperspectral irradiance sensor was mounted at an unshaded elevated position on the research vessel for reference measurements (E_s). On the profiler, the irradiance and radiance sensors measured downwelling (E_d) and upwelling (L_u) light, respectively.

Profiler measurements were conducted at selected stations depending on sea, weather, and daylight conditions. At these stations, 3 casts were typically performed. For each cast, the profiler was lowered until the E_d values were of the same order of magnitude as the background noise level of the sensor. Hyperspectral $E_d(\lambda)$ data were then processed with ProSoft v.7.7.16 (Satlantic) and binned to 0.2 m depth intervals to calculate photosynthetically active radiation (PAR):

$$\mathrm{PAR}(z) = \int_{400}^{700} (\lambda / hc)\, E_d(\lambda).\mathrm{d}\lambda \quad (1)$$

where z is the depth (in m), λ is the wavelength (in nm), h is Planck's constant and c is the speed of light. Additionally, the percentage of PAR reaching depth z relative to surface PAR, PAR(0^+), calculated from $E_s(\lambda)$ was determined according to:

$$\%\mathrm{PAR}(z) = \mathrm{PAR}(z) / \mathrm{PAR}(0^+) \times 100 \quad (2)$$

Based on %PAR(z), the 1% depth of PAR (a common indicator for the depth of the euphotic zone) was derived together with the maximum wavelength present at that depth. The mean values of the available profiles were used for all calculated profiler data.

Irradiance and photoperiod experiments

A subset of plankton and sediment samples was also used to establish *A. fundyense* cultures (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m547p033_supp.pdf), either from single cell isolations from plankton samples (Tillmann et al. 2016) or from germinated cysts. Additionally, isolates were established from cysts in sediments collected from the Chukchi Sea, which were kindly provided by Dr. Haifeng Gu (Third Institute of Oceanography, Xiamen, PR China). Sediment samples were processed as described above, and cysts were isolated via micropipetting and placed in individual wells of 24-well tissue culture plates containing f/2(-Si) growth medium (Guillard & Ryther 1962). Plates were incubated for approximately 1 wk at 10°C under a 14 h light:10 h dark photoperiod cycle. Wells were examined daily for germination and once sufficient motile cells were observed, individual cells were isolated, washed, and placed singly into tubes containing f/2(-Si) medium. Cultures were initially maintained at 10°C, but were subsequently maintained at 15°C due to improved growth at the higher temperature. Species designations of isolates were determined by sequencing the highly variable D1-D2 domains of the large subunit ribosomal RNA gene (LSU rRNA) (Tillmann et al. 2016, D. M. Anderson unpubl. data).

A series of laboratory experiments were performed to assess the effects of irradiance and photoperiod interval on growth responses of *A. fundyense* under the particular light conditions that bloom populations would experience during summer in the Arctic. These experiments were carried out with 3 isolates each from Greenland, Iceland, and the Chukchi Sea; 3 isolates originating from a temperate location, the Gulf of Maine (GOM), were also examined (Table S1). The Greenland isolate E516 died before the experiments could be completed; it was therefore necessary to use a different isolate (P3H8) in the 24 h light:0 h dark photoperiod treatment (see below). Experiments were performed in an incubator at a constant temperature (12°C); each isolate was grown in triplicate under irradiance levels of 37, 92, 146, 183, 275, and 366 µmol photons m^{-2} s^{-1}, which were established on 4 shelves by combining different light settings on each shelf with nylon window screen (1 or 2 layers) to provide additional shading. The lowest irradiance level was selected based on prior studies of *A. fundyense* from the northwest Atlantic (Etheridge & Roesler 2005), in which growth rates in response to irradiance were lowest under 25 and 50 µmol photons m^{-2} s^{-1} (but at 20 °C, which is higher than the temperature level used in these experiments).

Irradiance received by the cultures was measured with a digital scalar irradiance meter (Model QSP-170, Biospherical Instruments) equipped with a QSL-100 probe. Experiments were replicated under 3 different photoperiod (L:D) intervals: 24:0 h, 20:4 h, and 16:8 h. Preliminary studies confirmed a linear correlation between *in vivo* fluorescence and cell concentrations, and population growth was subsequently monitored by *in vivo* fluorescence measured with a

10-AU fluorometer (Turner Designs) in a 25 mm cuvette. Fluorescence was measured in each tube at the same time (~10:30 h) 3 times per week, and tubes were shaken by hand to distribute the cells uniformly in the medium before measuring fluorescence. Growth data were collected from 3 technical replicates of each isolate.

The intrinsic growth rate was calculated over the exponential phase of growth (as inferred from a semi-log plot of fluorescence versus time; see Guillard 1973, Wood et al. 2005) by the following equation:

$$\mu = \frac{\ln(N_1 / N_0)}{t_1 - t_0} \quad (3)$$

in which μ (d^{-1}) represents the growth rate and N_1 and N_0 represent the fluorescence at times t_1 and t_0, respectively.

Statistical analyses

Statistical analyses to examine the effects of irradiance and photoperiod interval on growth were performed with JMP 11 software (SAS Institute). Datasets were first grouped by region (Greenland, Iceland, Chukchi, GOM) for these analyses. Effects of irradiance on growth were compared among regions but within each photoperiod interval, and growth response data for each photoperiod interval (at all irradiance levels) were also pooled and compared. Growth data were not normally distributed, and it was not possible to achieve normality by transforming the data; non-parametric Welch's ANOVA and Wilcoxon rank sum tests were used instead for these comparisons, with $\alpha = 0.5$.

Additionally, principal components analysis (PCA) was performed with Primer v.6.0 (Primer-E) to examine correlations between cyst abundance, components of the sediment structure and depth, and to evaluate regional clustering among samples.

RESULTS

Temperature profiles and bio-optical parameters

Clear differences in temperature profiles were observed among the sampling locations (Fig. 2). Temperature measured in profiles from Uummannaq Fjord (transect distance 0 to 200 km), the Vaigat (250 to 450 km), and Disko Bay (>450 km) generally ranged from ~0 to 7°C and values throughout much of the water column were <4°C. Maximum temperatures of ~11 to 12°C were only found in surface waters at 2 locations. Cold melt water from the Perlerfiup Sermia glacier at the head of the Perlerfiup Kangerlua (a tributary fjord of the Uummannaq Fjord system), representing the starting point of the transect, was detected throughout section distance (0 to 150 km) between 20 and 200 m depth with temperatures <1°C.

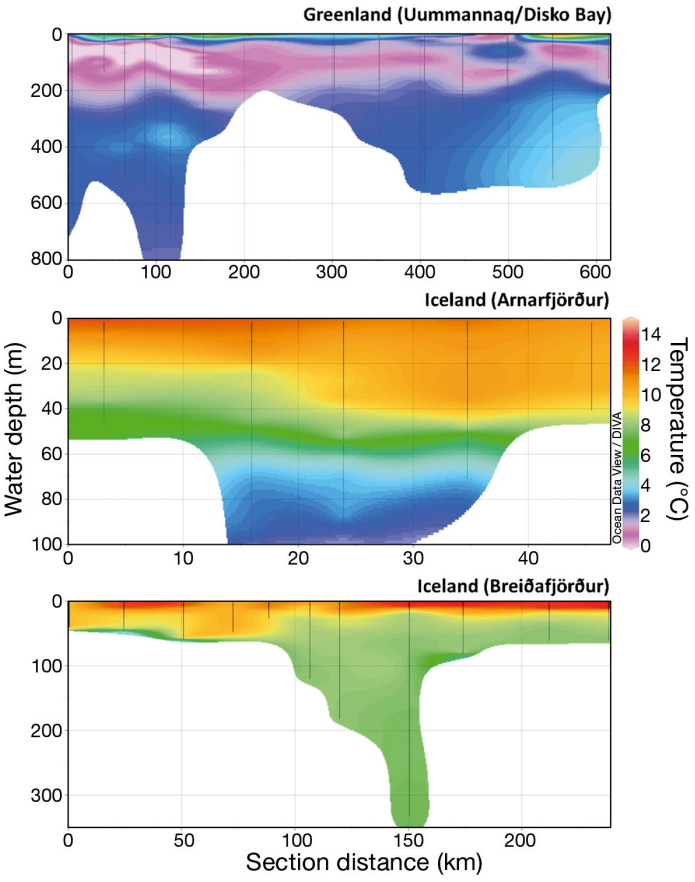

Fig. 2. CTD profiles over the section distance for temperature within selected sections in Greenland (Disko Bay region; top panel) and Iceland (Arnarfjörður and Breiðafjörður; middle and bottom panels, respectively). Stations are identified by dark grey vertical lines. Greenland: starting point was the innermost station in Uummannaq fjord (Perlerfiup Sermia glacier); Vaigat was entered at section distance 250 km; Disko Bay at 450 km. Arnarfjörður and Breiðafjörður: starting points were the innermost stations of the fjords. See Fig. 1 for station locations

Water temperatures in Icelandic fjords were much higher, ranging from 2 to 12°C in Arnarfjörður and 6 to 15°C in Breiðafjörður (Fig. 2). With the exception of the deepest areas of Arnarfjörður (>60 m), water temperatures in this fjord system were generally >8°C throughout much of the water column.

Analysis of light availability from radiometric profiles showed that the 1% PAR depth was <46 m at all stations surveyed, ranging from 15.9 m (Stn 535) to

Table 1. Light availability from radiometric profiles. Water depth: bottom depth of the respective station; PAR(0^+): photosynthetically active radiation at the surface; 1% depth of PAR: depth level of PAR relative to PAR(0^+); $\lambda_{max1\%}$: maximum wavelength observed at 1% depth of PAR. All values are the mean of 2 to 3 profiler casts at the specific stations. *indicates stations where *Alexandrium fundyense* cysts were quantified

Location Station	Water depth (m)	1% depth of PAR (m)	PAR (0^+) (µmol photons m^{-2} s^{-1})	$\lambda_{max1\%}$ (nm)
Uummannaq, Greenland				
503*	402.0	38.0	557	496
504*	350.0	35.7	194	496
506*	143.9	32.8	189	496
Disko Bay, Greenland				
514*	259.1	41.3	78	492
515*	543.7	40.3	1291	495
516*	169.5	33.2	354	499
West Greenland coast				
517	112.6	41.5	166	496
521	114.7	36.3	1076	496
522	113.2	45.7	1201	495
Arnarfjörður, Iceland				
527*	54.0	24.6	332	541
528*	106.2	28.5	461	536
529*	103.2	27.8	289	538
530*	81.0	27.1	287	539
531	46.2	33.4	81	498
Breiðafjörður, Iceland				
532	47.0	34.4	131	497
533	53.2	29.7	475	502
534	69.2	20.3	760	565
535*	55.6	15.9	366	567
536	34.2	19.6	88	563
537*	126.6	20.3	676	564
538*	189.9	22.5	966	562

45.7 m (Stn 522) (Fig. 1, Table 1). Maximum wavelength at the 1% level was below 500 nm for all Greenland stations, and 530 ± 30 nm for Iceland stations due to increased presence of colored dissolved organic matter (CDOM) absorbing ultraviolet and blue spectral components (data not shown).

Distribution and abundance of *Alexandrium fundyense* cysts

A. fundyense resting cysts were observed in sediments from all stations surveyed in Greenland and Iceland, with the exception of Stns 523 and 524, located near Cape Farewell, Greenland (Fig. 1). Cysts of several other dinoflagellate taxa (*A. minutum*, *A. ostenfeldii*, *Protoceratium* sp., *Protoperidinium* sp., *Scrippsiella* sp.) were also observed (but not quantified) in many of the samples, including those collected from Cape Farewell. Cyst concentrations in sediments collected from the other Greenland stations ranged from 2 to 37 cysts cm^{-3} (mean ± SD: 9 ± 11 cysts cm^{-3}), with highest concentrations found in Uummannaq Fjord (Fig. 3). Cyst concentrations in Iceland were higher, ranging from 15 to 408 cysts cm^{-3} (109 ± 127 cysts cm^{-3}). In Iceland, highest cyst concentrations were observed at Stn 538 (408 cysts cm^{-3}) in Breiðafjörður, followed by Stn 529 (124 cysts cm^{-3}) and Stn 537 (120 cysts cm^{-3}) in Arnarfjörður and Breiðafjörður, respectively (Fig. 3). The sediment sampling regimes differed substantially between Greenland and Iceland stations with respect to sampling depth. In Greenland, samples were collected from depths ranging from 135 to 550 m, with the majority of samples collected at depths >200 m (Fig. 4). In Iceland, however, sampling depths ranged from 51 to 330 m, and all but one sample were collected from depths <200 m.

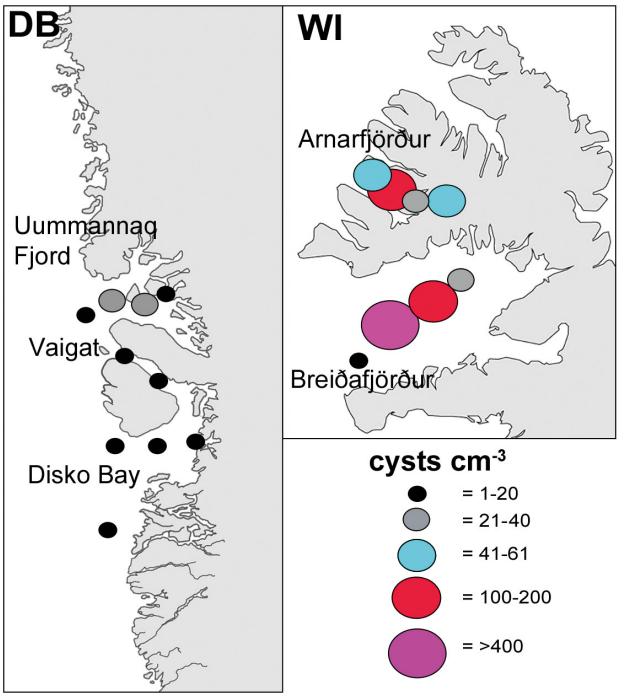

Fig. 3. Abundance and distribution of *Alexandrium fundyense* resting cysts in sediments collected from Greenland (DB: Disko Bay region) and Iceland (WI: West Iceland)

Sediment characterization

Seven granulometric fractions (F) ranging from <2 to 2000 µm were quantified in each of the sediment samples. The most apparent differences among sam-

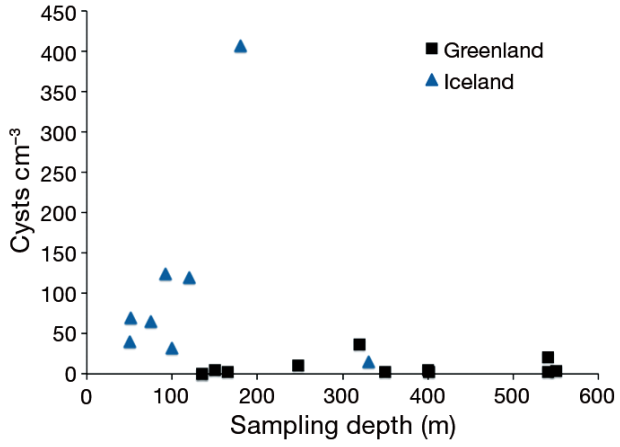

Fig. 4. *Alexandrium fundyense* cyst abundance in sediments collected from Greenland and Iceland versus sampling depth

ples were the higher proportions of fine silt in samples from Greenland compared with those from Iceland (Fig. 5), and the higher proportions of coarser, sandy sediments (*F*63–200, *F*200–630) in samples from Stn 523 and 524, collected near Cape Farewell. With the exception of these 2 stations, finer sedimentary fractions (*F*<63) comprised 50% or more of the particle size fractions of each sample (Fig. 5).

In the PCA based upon data on sediment characteristics, water depth, and cyst abundance, the first 2 principal components accounted for 72.8% of the variance, with the third accounting for an additional 11.8%. The strongest correlations (positive or negative) for the first principal component were with *F*2–6.3 and *F*63–200. For the second principal component, the strongest correlations were with *F*63–200 and cysts cm^{-3}. In the PCA plot, clustering according to region was observed (Fig. 6). The first cluster comprised the samples collected from Iceland and Stn 516 (Disko Bay, Greenland), whereas the second comprised those from the Cape Farewell stations (Stns 523 and 524), and the third comprised the remaining Greenland samples.

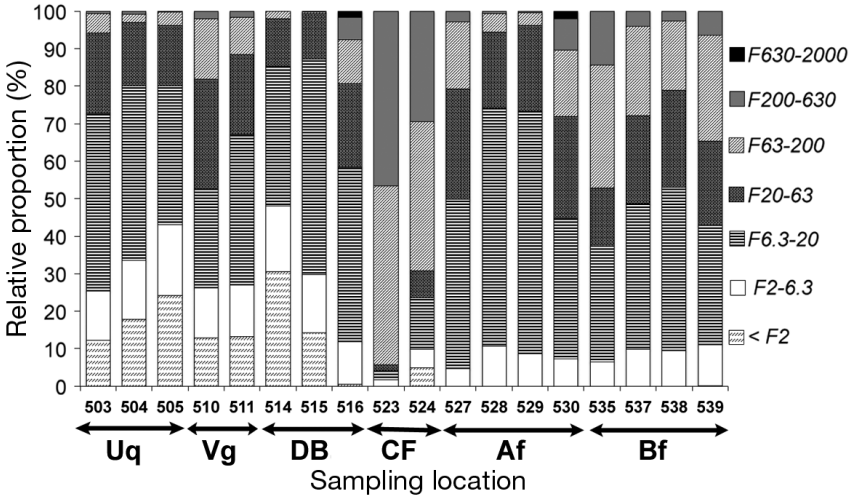

Fig. 5. Proportion of each sediment class in samples collected from Greenland and Iceland. A total of 7 granulometric fractions (µm) were quantified. Uq: Uummannaq; Vg: Vaigat; DB: Disko Bay; CF: Cape Farewell; Af: Arnarfjörður; Bf: Breiðafjörður

Photoperiod interval and irradiance experiments

Patterns of growth responses to the experimental treatments varied widely among isolates but were strain- rather than region-specific. In all cases, both photoperiod and irradiance had significant effects on growth, with the highest growth rate (0.28 d^{-1}) observed in these experiments for Iceland isolate D3 grown under constant light (24 h light:0 h dark) but at the lowest irradiance level (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m547p033_supp.pdf). The next highest growth rates were observed for the same isolate grown under constant light, at the next 2 lowest irradiance levels (~92 and ~147 µmol photons m^{-2} s^{-1}, respectively. The lowest growth rate (0.016 d^{-1}) determined in this study was observed for both P2H7 (Greenland) and F5 (Chukchi Sea) grown under constant light (24 h light:0 h dark) and at the highest irradiance level (~366 µmol photons m^{-2} s^{-1}). The next lowest growth rate was exhibited by isolate E516 (Greenland) under the aforementioned experimental conditions.

Although irradiance level had significant effects on growth, these effects varied according to photoperiod interval and among regions. Under the shortest light period interval (16 h light:8 h dark), growth rates were generally lowest at the lowest irradiance level, and were significantly higher at moderate and higher irradiance levels (Fig. 7). However, under extended light-period treatments, an inverse pattern was observed whereby growth rates were highest under low and moderate light levels; apparent photoinhibition was most pronounced under constant light treatment (24 h light:0 h dark) (Figs. 7 & S1). Comparison of the combined growth response dataset among regions (data pooled from each irradiance level) showed that regardless of irradiance level, growth rates of isolates from the Chukchi Sea

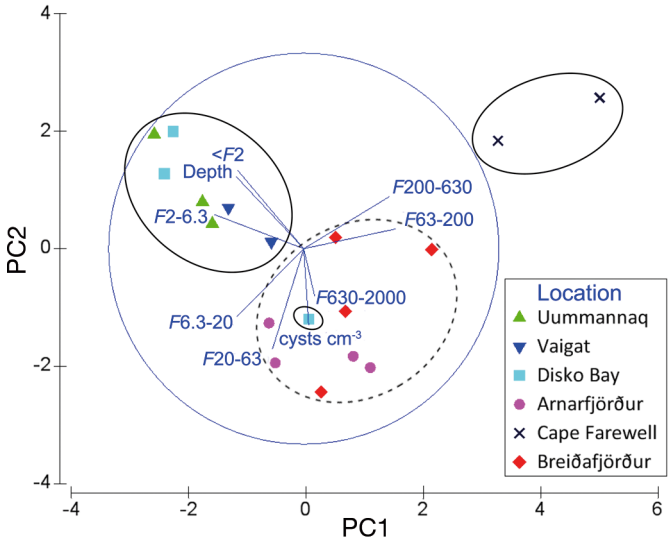

Fig. 6. Principal component analysis (PCA) of *Alexandrium fundyense* cyst abundance, sediment characteristics, and sampling depth of sediments collected from Greenland (Uummannaq, Vaigat, Disko Bay, Cape Farewell) and Iceland (Arnarfjörður, Breiðafjörður). Stations in Greenland and Iceland are delineated by solid and dashed lines, respectively. Symbols denote specific sampling locations

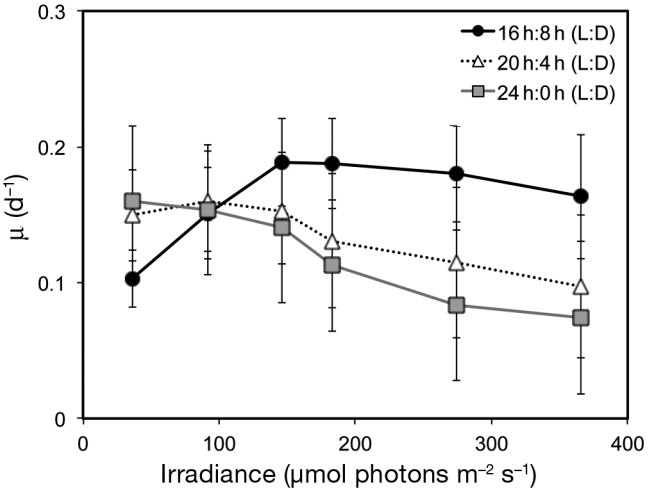

Fig. 7. Growth rates (μ) of *Alexandrium fundyense* isolates from Greenland (n = 3), Iceland (n = 3), the Chukchi Sea (n = 3), and the Gulf of Maine (n = 3) in response to irradiance. Data from 3 different photoperiod intervals (L:D) are shown: 16:8 h, 20:4 h, and 24:0 h

and GOM were significantly higher under the shortest irradiance interval (16 h light:8 h dark) than under an extended interval (20 h light:4 h dark) or constant light regime (24 h light:0 h dark) ($p < 0.05$; Welch's ANOVA, Wilcoxon multiple comparisons). Growth rates of isolates from Greenland grown under the constant light were significantly lower than those grown under the other photoperiod intervals ($p < 0.05$; Welch's ANOVA, Wilcoxon multiple comparisons). In contrast, no statistically significant differences in growth rates were observed among the different photoperiod intervals for the Iceland isolates.

DISCUSSION

To our knowledge, this is the first effort to investigate and compare the abundance and distribution of *Alexandrium fundyense* cysts in bottom sediments of coastal Greenland and Iceland, areas which have been recently impacted by toxic *Alexandrium* blooms. Our surveys of western Greenland and Iceland documented low to moderate *A. fundyense* cyst abundances (~20 to 400 cysts cm^{-3}) throughout these regions; notably, cysts were observed at nearly all stations surveyed, but sediments from Iceland contained substantially more cysts compared to samples from Greenland.

Based on the analysis of field data collected during the cruise and results of physiology experiments examining growth responses of Arctic *Alexandrium* isolates, we hypothesize that light availability and temperature regimes in the water column in fjords and coastal areas of Greenland are largely unfavorable for germling survival during transit from bottom waters to the surface, even during summer. However, many areas in Iceland could support germling survival and vegetative cell growth, which indicates that cyst deposits in Iceland may indeed be functioning for *in situ* bloom initiation. We note that the survival and excystment challenges faced by *Alexandrium* cysts in the deep fjords and cold waters of the Arctic are common to many other cyst-forming dinoflagellate species, and to spore-forming diatoms as well, so there is broad ecological relevance to our findings.

Cyst distribution

The accumulation rate and total abundance of cysts at a particular location reflects the net balance between deposition versus advective and germination losses, and is thus affected by bathymetric and hydrographic characteristics and processes that determine bloom and/or cyst retention, as well as external and biological controls of cyst germination and bloom initiation. *Alexandrium* spp. cyst densities ranging from 100s to 1000s of cysts cm^{-3} of sur-

face sediments have been reported from areas around the world impacted by annual *Alexandrium* blooms and PSP, including the GOM in Canada and the USA, Puget Sound (USA) in the northeast Pacific, several coastal regions of Japan, and the western Mediterranean (Thau Lagoon, France). In the northwestern Atlantic, densities as high as 2000 and 6700 cysts cm^{-3} were reported from the Bay of Fundy and GOM, respectively (Anderson et al. 2014), and abundances >12 000 cysts cm^{-3} were observed in the Puget Sound region, in an area known to be a hotspot for PSP toxins in shellfish (Horner et al. 2011). Notably, extraordinarily high *A. fundyense* cyst densities (>10 000 cysts cm^{-3}) in the Chukchi Sea were recently reported by Natsuike et al. (2013). However, in contrast with the aforementioned regions in which high cyst densities were generally associated with massive seasonal blooms, cyst concentrations in the Chukchi Sea sediments may well reflect the deposition of cysts year after year during a series of smaller blooms over time (rather than cyst deposition following a major bloom) with little or no germination losses, leading to the high abundances observed. As an example of the magnitude of cyst deposition following a major bloom, McGillicuddy et al. (2014) documented a red-water *A. fundyense* bloom in the GOM (cell densities in excess of 3×10^6 cells l^{-1}) that deposited only 10% as many cysts as observed in Chukchi Sea sediments (Natsuike et al. 2013).

With the exception of the 2 stations near Cape Farewell, *A. fundyense* cysts were found in all Greenland samples collected during the cruise. Cyst accumulations in Greenland sediments were generally low, and the maximum abundance of 37 cysts cm^{-3} was observed in Uummannaq Fjord (Fig. 3). In contrast, much higher cyst abundances were observed in sediments from Iceland, ranging from 15 to 408 cysts cm^{-3}. Although cyst accumulations in Greenland and Iceland were low to moderate compared with areas impacted by large-scale, annual *Alexandrium* blooms (e.g. Anderson et al. 2014), the concentrations we found are well within the range reported from areas with seasonal *Alexandrium* blooms and recurrent PSP toxin accumulation in shellfish. One example of relatively low cyst abundance levels leading to *Alexandrium* blooms comes from the Nauset estuary in Massachusetts, USA, where cyst densities of 150 to 418 cysts cm^{-3} have been associated with blooms and recurrent PSP-related shellfish harvesting closures in several of the embayments within that system (Crespo et al. 2011). Likewise, in a lagoon-wide survey of the Thau Lagoon in France, which is impacted annually by PSP toxin contamination in shellfish, the mean density of *Alexandrium* cysts was relatively low (<20 cysts g^{-1} dry sediment [DS]), with the highest density (~440 cysts g^{-1} DS) recorded at one location within the system where dense blooms were previously observed (Genovesi et al. 2013). Using the relationship between cyst abundance normalized to sediment dry weight versus sediment volume determined for *A. fundyense* in the GOM (Anderson et al. 2014), these Thau Lagoon values equate to between 34 and 185 cysts cm^{-3}. The GOM relationship may not be entirely appropriate for Thau Lagoon because of differences in sediment consistency and granularity, but is considered suitable for a rough approximation of cyst cm^{-3} levels.

Sediment structure

Although cyst distributions are frequently heterogeneous and site-specific, prior investigations seeking to better define the physical dynamics underlying the occurrence of cyst seedbeds have identified several important characteristics common to many important cyst accumulation zones. First and perhaps most importantly, higher cyst densities have been reported from protected or enclosed areas such as fjords, embayments, and harbors (Anderson 1997, Godhe & McQuoid 2003, Crespo et al. 2011), which serve to entrain blooms and promote local cyst deposition. Cyst abundance is also positively correlated with the proportion of finer grains and levels of total organic carbon (TOC) in sediments (Horner et al. 2011, Genovesi et al. 2013, Anderson et al. 2014). Finally, higher abundances have been linked with higher summer surface water temperatures, which serves to stimulate dinoflagellate growth and promote the vertical stratification of the water column (Godhe & McQuoid 2003), leading to both a higher potential inoculum and reduced advective loss of cells within the system.

Dale (1976) first proposed that cysts tend to behave as fine silt particles in sediment dynamics, and as such, increase in abundance as the proportional abundance of finer sediment increases (often at depth). This hypothesis is supported by reports of higher *Alexandrium* spp. cyst accumulations in finer sediments or mud compared with sandy areas (Nehring 1994, Gayoso 2001, Yamaguchi et al. 2002, Anderson et al. 2005), and by subsequent investigations of the correlation between cyst densities and sediment characteristics. In surveys of Puget Sound,

Horner et al. (2011) observed a positive correlation between cyst abundance and the percentage of clay and silt in sediments during small scale surveys in Quartermaster Harbor, located in the south basin of Puget Sound. This pattern was not observed, however, in the large-scale, sound-wide surveys, potentially due to variable and site-specific physical forcing conditions within the system (Moore et al. 2008, Horner et al. 2011).

Genovesi et al. (2013) documented a significant correlation between *Alexandrium* cyst densities and the *F*20–50 sediment fraction in the Thau Lagoon, along the French Mediterranean coast, reporting that the cysts in this ecosystem effectively behaved like 20 to 50 µm particles (the approximate size range of *Alexandrium* cysts). With the exception of the 2 stations sampled near Cape Farewell, sediments in Greenland and Iceland were characterized by a high proportion of finer grained sediment fractions (<63 µm) (Fig. 5), and would thus favor cyst accumulations in these areas. The most apparent difference among locations was the higher proportion of fine silt (<2 µm) in the majority of samples collected from Greenland (Uummannaq and Disko Bay) compared with those from Iceland. These very fine particles, also referred to as glacial flour, are transported to the estuary with the melt water (Lund-Hansen et al. 2010). A second apparent difference was the higher proportion of coarser, sandy sediments (>63 µm) in samples from Stns 523 and 524. Both stations are located near Cape Farewell on the southern coast of Greenland, and are typically exposed to open sea conditions with higher turbulent energy, thus preventing the accumulation of finer sediments. Notably, these were the only samples in which *Alexandrium* cysts were absent. Regional differences were also evident in the PCA of data on the sediment structure, cyst densities, and sampling depth (Fig. 6). This analysis identified at least 3 major clusters, grouped according to geographical region. One exception was Stn 516 (Disko Bay), which clustered with stations sampled from Iceland in the PCA plot (Fig. 6), and was characterized by a lower proportion of sediment fractions <6.3 µm and a higher proportion of fractions >63 µm compared to the other samples from Uummannaq, Disko Bay, and the Vaigat (Fig. 5). This analysis also linked depth with the finest sediment fractions (<6.3 µm), whereas cyst densities were linked with the intermediate (*F*6.3–63) and coarsest size fraction (*F*630–2000), the latter of which was only found in samples collected from Stns 516 and St 530 (Arnarfjörður).

Water temperature, depth, and bio-optical parameters

Cyst germination, and subsequent germling cell survival and growth, are highly dependent on light availability and temperature (Anderson 1980, Rengefors & Anderson 1998, Kremp & Anderson 2000, Vahtera et al. 2014); thus, the striking differences in these water column characteristics observed among sampling sites suggest that bloom development might only occur at certain locations within the study area. Temperatures throughout much of the water column in Greenland were generally <4°C, with the maximum temperature of ~12°C only detected in surface waters at 2 locations (Fig. 2). Previous temperature measurements from Disko Bay during the summer months (June to August) ranged from ~4 to 7°C (Madsen et al. 2001, Heide-Jørgensen et al. 2007), thus the frequency and extent of the warmer surface waters we documented (>10°C) is yet unknown. In contrast, water column temperatures measured in Iceland were much higher, ranging from 2 to 12°C in Arnarfjörður and 6 to 15°C in Breiðafjörður; with the exception of the deepest areas of Arnarfjörður (>60 m), water temperatures were generally >8°C. Notably, Burrell et al. (2013) observed high cell concentrations (>10 000 cells l^{-1}) of *Alexandrium* spp. (which they tentatively designated as *A. tamarense* along with small numbers of *A. ostenfeldii*) in Breiðafjörður and in Eyjafjordur (northern Iceland) in 2009. Low to moderate *Alexandrium* spp. cell concentrations were also observed in Breiðafjörður and Eyjafjordur between 2005 and 2008 (Gudfinnsson et al. 2010, Burrell et al. 2013), indicating that blooms may be recurrent at these locations.

Differences in bio-optical properties affecting light availability and quality over depth are important determinants of photosynthetically driven growth potential among *Alexandrium* populations at various locations. Based on the results of our laboratory experiments, variation in day length expected in the study region would be most likely to promote growth in August, during which the highest seawater temperatures would also be expected. Day length during the summer months (July to August) in western Greenland (Disko Bay) and Iceland ranges from >20 h during much of July, to between ~15 or 20 h in August. In our laboratory experiments, highest growth rates were measured at irradiance levels of ~150 µmol photons m^{-2} s^{-1} or greater under the 16 h photoperiod interval. However, comparatively high growth rates were also observed at low light levels under extended light-period treatments, indicating

that longer photoperiod intervals may also be suitable for growth.

The 1% depth of PAR derived from our field data was interpreted to indicate the lowest depth of sufficient light for positive *Alexandrium* cell growth in the study region. In general, 1% PAR was surface bound to the upper water column, and was restricted to the top 50 and 35 m for Greenland and Iceland, respectively (Table 1). Considering water depth, we inferred that the distance to be covered by vertically motile germling cells from bottom water to reach sufficient light for positive growth ranges from <70 m for most Iceland fjord stations to 500 m for Stn 515, the deepest Greenland station in this dataset. Assuming an average swimming speed of 10 m d^{-1} (Eppley et al. 1968, Bauerfeind et al. 1986, Kamykowski et al. 1992), cells germinating at depths of 70 m would require approximately 7 d to reach the surface, whereas cells germinating at 500 m would require 50 d. These transit times may be shorter, however, during upwelling conditions which could rapidly transport cells to surface waters. The ability of germling cells to survive vertical transit to the euphotic zone in these areas will determine the potential for bloom initiation at these locations (see next section).

Cyst viability and germling cell survival

Whether or not *A. fundyense* cysts in the Arctic and sub-Arctic (Greenland, Iceland, Chukchi Sea) are able to germinate, and the corresponding vegetative cells are able to transit to the euphotic zone under the specific temperature and light conditions present (i.e. very cold, deep, and dark waters) to initiate *in situ* blooms remains unknown. If not, the observed cyst deposits could represent end points or terminal deposits, with the bloom populations that ultimately produce those cysts originating from sub-Arctic systems in the south through transport by coastal currents. Alternatively, cyst deposits from nearby shallow areas could serve as an initiation site for local blooms that lead to deposits in the deeper fjord sections.

To our knowledge, the potential for cyst germination and cell growth of *A. fundyense* (or any cyst-forming dinoflagellate) from Arctic and sub-Arctic regions have not been studied. At high latitudes, cyst behavior and germling survival and growth at low temperatures and under an extended light-period interval in summer are of fundamental importance to bloom development and life cycle completion. Low temperatures can maintain cyst quiescence for extended periods (months, years, even decades) after cyst deposition, and where germination is possible, will also regulate the rate of excystment. Following excystment, the germling cell must survive the transit to surface waters, which is influenced by distance travelled in the dark (depth), availability of temperature and light, and cellular energy reserves (Vahtera et al. 2014). For the *A. fundyense* strains tested thus far from temperate waters, cyst germination either did not occur or proceeded at extremely low rates at temperatures between 0 and 4°C (Anderson & Morel 1979, Anderson 1980). Based on the CTD measurements collected during our surveys, bottom temperatures in Greenland are expected to be in this range or lower during much of the potential *Alexandrium* bloom season (Fig. 2). Anderson et al. (2005) showed that at 2°C, *A. fundyense* cysts from the GOM required up to 2 mo of incubation to reach 50% germination, whereas at 8°C, this only took 1 to 2 wk. At the low rates expected for cold Arctic waters, the bloom inoculum from excystment would be very gradual and slow, and might therefore introduce cells into the water too late in the season for successful bloom formation and new cyst deposition.

Following excystment, temperature and light are both important limiting factors that determine germling survival and vegetative cell growth. For many *A. fundyense* strains, including the few isolates examined from Greenland, Iceland, and the Chukchi Sea, a temperature range for survival and growth of 2 to 24°C has been observed, with rates that are <25% of maxima at 6°C or less (Watras et al. 1982, Anderson & Rengefors 2006, D. M. Anderson unpubl. data). Although they were collected from Arctic and sub-Arctic locations, the isolates we examined did not appear to be physiologically adapted for growth and survival in the extremely cold bottom water temperatures in the Arctic. Instead, their growth responses to temperature were similar to those of temperate isolates, with the maximum growth rate for all isolates found between 16 and 18°C. These data will be published separately, along with a detailed analysis of the toxin contents of the isolates (Tillmann et al. 2016, D. M. Anderson unpubl. data). The CTD temperature profiles collected during the cruise indicated that summer water temperatures in the Uummannaq/Disko Bay region only ranged from 4 to 8°C, well below the temperature range for optimal growth, and bottom temperatures were much lower (Fig. 2).

Furthermore, the depth from which sediments were collected suggests that the survival of germinated cysts would be low at many of the locations surveyed in Greenland. Laboratory experiments examining the effects of dark treatment on cyst ger-

mination and survival estimated that <50% of germinated cells would survive a 70 m transit from bottom sediments to the surface, and only 20% could survive a 200 m ascent in the dark (Vahtera et al. 2014). In Iceland, the estimated distances germlings would have to travel from germination depth to reach the 1% PAR depth ranged from 13 to 167 m; however, these estimated distances are substantially greater in Greenland, where this travel distance ranged from 68 to 503 m. Using the equations derived by Vahtera et al. (2014) describing the depth-related mortality rate, and assuming an initial survival time of 1 d, the proportions of cells estimated to survive the transit from germination depth to 1% PAR ranged from 14 to 26% in Greenland, and 18 to 82% in Iceland.

Based on these field and experimental data, it is likely that *Alexandrium* cells and associated toxins in shellfish from Greenland are primarily derived from advected *Alexandrium* populations. However, there may also be certain shallow, nearshore areas not explored in this study that could provide favorable habitat for cyst germination and germling survival. Conditions in the bay and fjords we surveyed in western Iceland are suitable for germling survival and vegetative cell growth, and therefore may support indigenous, self-seeding blooms. The potential for *Alexandrium* bloom initiation in Greenland and other Arctic areas may be enhanced in the future, as Arctic Ocean bottom temperatures are projected to increase at a rate of 1 to 5°C per 100 yr, with a higher rate in nearshore regions (Biastoch et al. 2011). This will clearly have an impact on the germination and survival rate of *Alexandrium*, but also will affect the distribution and bloom timing of many other meroplanktonic phytoplankton species.

CONCLUSIONS

Our field investigation documented low to moderate densities of *Alexandrium* cysts in most areas surveyed in Greenland and Iceland, with highest densities observed in western Iceland. We know that *A. fundyense* strains disperse readily and are highly adaptable to new regions due to their ability to form cysts, overwinter, and germinate to initiate blooms. Based on data collected on temperature and light availability (as influenced by water depth), we hypothesize that blooms in Greenland are primarily derived from advected *Alexandrium* populations, as extremely low bottom temperatures and travel distance from germination depth to the euphotic zone would preclude *in situ* bloom initiation at most of the locations we surveyed. Alternatively, cyst deposits from nearby shallow areas could serve as an initiation of local blooms that lead to deposits in the deeper fjord sections. We further hypothesize that, in contrast with the situation in Greenland, the bays and fjords in Iceland provide favorable habitat for germling cell survival and growth, and therefore may support indigenous, self-seeding blooms.

The potential for *Alexandrium* blooms in Greenland and other Arctic areas may change, as projected increases in water temperatures could expand habitat suitable for *Alexandrium* germling survival and cell growth, particularly at nearshore locations. The human health and ecosystem impacts of this potential expansion will be significant, as marine bioresources are extremely important to the economies of both Greenland and Iceland. Additional studies that examine the physiology of *Alexandrium* cysts and cells from the Arctic are needed, particularly with regard to the potential for cyst germination under ambient conditions in the region. These data will help to further characterize processes that determine the distribution of endemic versus introduced populations of *Alexandrium* and other toxin-producing phytoplankton in the Arctic, and will be useful for understanding the potential for dispersal in the region under warmer conditions.

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LITERATURE CITED

Anderson DM (1980) Effects of temperature conditioning on development and germination of *Gonyaulax tamarensis* (Dinophyceae) hypnozygotes. J Phycol 16:166–172

Anderson DM (1997) Bloom dynamics of toxic *Alexandrium*

species in the northeastern US. Limnol Oceanogr 42: 1009–1022

Anderson DM (1998) Physiology and bloom dynamics of toxic Alexandrium species, with emphasis on life cycle transitions. In: Anderson DM, Cembella A, Hallegraeff G (eds) The physiological ecology of harmful algal blooms. Springer-Verlag, Heidelberg, p 29–48

Anderson DM, Morel FMM (1979) The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. Estuar Coast Mar Sci 8:279–293

Anderson DM, Rengefors K (2006) Community assembly and seasonal succession of marine dinoflagellates in a temperate estuary: the importance of life cycle events. Limnol Oceanogr 51:860–873

Anderson DM, Taylor CD, Armbrust EV (1987) The effects of darkness and anaerobiosis on dinoflagellate cyst germination. Limnol Oceanogr 32:340–351

Anderson DM, Fukuyo Y, Matsuoka K (2003) Cyst methodologies. In: Hallegraeff GM, Anderson DM, Cembella AD (eds) Monographs on oceanographic methodology, Vol 11: manual on harmful marine microalgae. UNESCO, Paris, p 165–190

Anderson DM, Stock CA, Keafer BA, Bronzino Nelson A and others (2005) *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep-Sea Res II 52:2522–2542

Anderson DM, Keafer BA, Kleindinst JL, McGillicuddy DJ Jr and others (2014) *Alexandrium fundyense* cysts in the Gulf of Maine: long-term time series of abundance and distribution, and linkages to past and future blooms. Deep-Sea Res II 103:6–26

Baggesen C, Moestrup Ø, Daugbjerg N, Krock B, Cembella AD, Madsen S (2012) Molecular phylogeny and toxin profiles of *Alexandrium tamarense* (Lebour) Balech (Dinophyceae) from the west coast of Greenland. Harmful Algae 19:108–116

Bauerfeind E, Elbrächter M, Steiner R, Throndsen J (1986) Application of Laser Doppler Spectroscopy (LDS) in determining swimming velocities of motile phytoplankton. Mar Biol 93:323–327

Biastoch A, Treude T, Rüpke LH, Riebesell U and others (2011) Rising Arctic Ocean temperatures cause gas hydrate destabilization and ocean acidification. Geophys Res Lett 38:L08602, doi:10.1029/2011GL047222

Burrell S, Gunnarsson T, Gunnarsson K, Clarke D, Turner AD (2013) First detection of paralytic shellfish poisoning (PSP) toxins in Icelandic mussels (*Mytilus edulis*): links to causative phytoplankton species. Food Contr 31:295–301

Cembella A, Zielinski O, Anderson D, Graeve M and others (2016) ARCHEMHAB: interactions and feedback mechanisms between hydrography, geo-chemical signatures and microbial ecology, with a focus on HAB species diversity, biogeography and dynamics. Cruise Report MSM21/3, DFG-Senatskommission für Ozeanographie, Bremen

Crespo BG, Keafer BA, Ralston DK, Lind H, Farber D, Anderson DM (2011) Dynamics of *Alexandrium fundyense* blooms and shellfish toxicity in the Nauset Marsh System of Cape Cod (Massachusetts, USA). Harmful Algae 12: 26–38

Dale B (1976) Cyst formation, sedimentation, and preservation: factors affecting dinoflagellate assemblages in recent sediments from Trondheimsfjord, Norway. Rev Palaeobot Palynol 22:39–60

Eppley RW, Holm-Harisen O, Strickland JDH (1968) Some observations on the vertical migration of dinoflagellates. J Phycol 4:333–340

Etheridge SM, Roesler CS (2005) Effects of temperature, irradiance, and salinity on photosynthesis, growth rates, total toxicity, and toxin composition for *Alexandrium fundyense* isolates from the Gulf of Maine and Bay of Fundy. Deep-Sea Res II 52:2491–2500

Fofonoff NP, Millard RC Jr (1983) Algorithms for computation of fundamental properties of seawater. UNESCO Tech Pap Mar Sci 44:1–53

Fraga S, Sampedro N, Larsen J, Moestrup Ø, Calado AJ (2015) Arguments against the proposal 2302 by John & al. to reject the name *Gonyaulax catenella* (*Alexandrium catenella*). Taxon 64:634–635

Garaba SP, Zielinski O (2013) Comparison of remote sensing reflectance from above-water and in-water measurements west of Greenland, Labrador Sea, Denmark Strait, and west of Iceland. Opt Express 21:15938–15950

Gayoso AM (2001) Observations on *Alexandrium tamarense* (Lebour) Balech and other dinoflagellate populations in Golfo Nuevo, Patagonia (Argentina). J Plankton Res 23: 463–468

Genovesi B, Mouillot D, Laugier T, Fiandrino A, Laabir M, Vaquer A, Grzebyk D (2013) Influences of sedimentation and hydrodynamics on the spatial distribution of *Alexandrium catenella/tamarense* resting cysts in a shellfish farming lagoon impacted by toxic blooms. Harmful Algae 25:15–25

Godhe A, McQuoid MR (2003) Influence of benthic and pelagic environmental factors on the distribution of dinoflagellate cysts in surface sediments along the Swedish west coast. Aquat Microb Ecol 32:185–201

Gu H, Zeng N, Xie Z, Wang D, Wang W, Yang W (2013) Morphology, phylogeny, and toxicity of Atama complex (Dinophyceae) from the Chukchi Sea. Polar Biol 36: 427–436

Gudfinnsson HG, Eydal A, Gunnarsson K, Gudmundsson K, Valsdóttir K (2010) Monitoring of toxic phytoplankton in three Icelandic fjords. ICES theme session N, ICES CM 2010/N:12

Guillard RRL (1973) Division rates. In: Stein JR (ed) Handbook of phycological methods: culture methods & growth measurements. Cambridge University Press, Cambridge, p 289–312

Guillard RR, Ryther JH (1962) Studies of marine diatoms. I. *Cyclotella nana* Husdedt and *Detonula confervacea* Gran. Can J Microbiol 8:229–239

Heide-Jørgensen MP, Laidre KL, Logsdon ML, Nielsen TG (2007) Springtime coupling between chlorophyll *a*, sea ice and sea surface temperature in Disko Bay, West Greenland. Prog Oceanogr 73:79–95

Holinde L, Zielinski O (2016) Bio-optical characterization and light availability parameterization in Uummannaq Fjord and Vaigat–Disko Bay (West Greenland). Ocean Sci 12:117–128, doi:10.5194/os-12-117-2016

Horner RA, Greengrove CL, Davies-Vollum KS, Gawel JE, Postel JR, Cox AM (2011) Spatial distribution of benthic cysts of *Alexandrium catenella* in surface sediments of Puget Sound, Washington, USA. Harmful Algae 11: 96–105

John U, Litaker RW, Montresor M, Murray S, Brosnahan ML, Anderson DM (2014a) Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. Protist 165:779–804

John U, Litaker W, Montresor M, Murray S, Brosnahan ML,

Anderson DM (2014b) (2302) Proposal to reject the name *Gonyaulax catenella* (*Alexandrium catenella*) (Dinophyceae). Taxon 63:932–933

Kamykowski D, Reed RE, Kirkpatrick GJ (1992) Comparison of sinking velocity, swimming velocity, rotation and path characteristics among six marine dinoflagellate species. Mar Biol 113:319–328

Kremp A, Anderson DM (2000) Factors regulating germination of resting cysts of the spring bloom dinoflagellate *Scrippsiella hangoei* from the northern Baltic Sea. J Plankton Res 22:1311–1327

Lilly EL, Halanych KM, Anderson DM (2007) Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). J Phycol 43:1329–1338

Lund-Hansen LC, Andersen TJ, Nielsen MH, Pejrup M (2010) Suspended matter, chl-a, CDOM, grain sizes, and optical properties in the Arctic fjord-type estuary, Kangerlussuaq, West Greenland during summer. Estuaries Coasts 33:1442–1451

Madsen SD, Nielsen TG, Hansen BW (2001) Annual population development and production by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* in Disko Bay, western Greenland. Mar Biol 139:75–93

McGillicuddy DJ Jr, Brosnahan ML, Couture DA, He R and others (2014) A red tide of *Alexandrium fundyense* in the Gulf of Maine. Deep-Sea Res II 103:174–184

Moore SK, Mantua NJ, Kellogg JP, Newton JA (2008) Local and large-scale climate forcing of Puget Sound oceanographic properties on seasonal to interdecadal timescales. Limnol Oceanogr 53:1746–1758

Natsuike M, Nagai S, Matsuno K, Saito R, Tsukazaki C, Yamaguchi A, Imai I (2013) Abundance and distribution of toxic *Alexandrium tamarense* resting cysts in the sediments of the Chukchi Sea and the eastern Bering Sea. Harmful Algae 27:52–59

Nehring S (1994) Spatial distribution of dinoflagellate resting cysts in recent sediments of Kiel Bight, Germany (Baltic Sea). Ophelia 39:137–158

Okolodkov YB (2005) The global distributional patterns of toxic, bloom dinoflagellates recorded from the Eurasian Arctic. Harmful Algae 4:351–369

Preston-Thomas H (1990) The International Temperature Scale of 1990 (ITS-90). Metrologia 27:3–10

Rengefors K, Anderson DM (1998) Environmental and endogenous regulation of cyst germination in two freshwater dinoflagellates. J Phycol 34:568–577

Scholin CA (1998) Morphological, genetic and biogeographic relationships of toxic dinoflagellates *Alexandrium tamarense*, *A. catenella* and *A. fundyense*. In: Anderson DM, Hallegraeff GM, Cembella AD (eds) Physiological ecology of harmful algal blooms. NATO Advanced Study Institute Series G: Ecological Sciences, Vol 41. Springer-Verlag, Heidelberg, p 13–28

Schwinghamer P, Anderson DM, Kulis DM (1991) Separation and concentration of living dinoflagellate resting cysts from marine sediments via density-gradient centrifugation. Limnol Oceanogr 36:588–592

Tillmann U, Krock B, Alpermann TJ, Cembella A (2016) Bioactive compounds of marine dinoflagellate isolates from western Greenland and their phylogenetic association within the genus *Alexandrium.* Harmful Algae 51: 67–80

Vahtera E, Crespo BG, McGillicuddy DJ, Olli K, Anderson DM (2014) *Alexandrium fundyense* cyst viability and germling survival in light vs. dark at a constant low temperature. Deep-Sea Res II 103:112–119

Watras CJ, Chisholm SW, Anderson DM (1982) Regulation of growth in an estuarine clone of *Gonyaulax tamarensis* Lebour: salinity-dependent temperature responses. J Exp Mar Biol Ecol 62:25–37

Wood AM, Everroad R, Wingard L (2005) Measuring growth rates in microalgal cultures. In: Anderson RA (ed) Algal culturing techniques. Elsevier Academic Press, Burlington, MA, p 269–286

Yamaguchi M, Itakura S, Nagasaki K, Kotani Y (2002) Distribution and abundance of resting cysts of the toxic *Alexandrium* spp. (Dinophyceae) in sediments of the western Seto Inland Sea, Japan. Fish Sci 68:1012–1019

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