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Environmental factors influencing the distribution and abundance of *Alexandrium catenella* in Kachemak bay and lower cook inlet, Alaska

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ABSTRACT

Despite the long history of paralytic shellfish poisoning (PSP) events in Alaska, little is known about the seasonal distribution and abundance of the causative organism, *Alexandrium*, or the environmental factors that govern toxic bloom development. To address this issue, a five year study (2012–2017) was undertaken in Kachemak Bay and lower Cook Inlet Alaska to determine how the occurrence of *Alexandrium catenella*, the dominant PSP-causing *Alexandrium* species, was influenced by temperature, salinity, nutrient concentrations, and other environmental factors. Cell concentrations from 572 surface water samples were estimated using quantitative PCR. Monthly sampling revealed a seasonal pattern of *A. catenella* bloom development that was positively correlated with water temperature. Prevailing salinity conditions did not significantly affect abundance, nor was nutrient limitation a direct factor. Elevated cell concentrations were detected in 35 samples from Kachemak Bay (100-3050 cell eq. L^{-1}) while a maximum abundance of 67 cell eq. L^{-1} was detected in samples from lower Cook Inlet sites. Monitoring data showed average water temperatures in Kachemak Bay increased by ~2 °C over the course of the study and were accompanied by an increase in *Alexandrium* abundance. Based on these findings, 7–8 °C appears to represent a temperature threshold for significant bloom development in Kachemak Bay, with the greatest risk of shellfish toxicity occurring when temperatures exceed 10–12 °C. The role of temperature is further supported by time series data from the Alaska Coastal Current (station GAK1), which showed that summertime shellfish toxicity events in Kachemak Bay generally followed periods of anomalously high winter water temperatures. These data indicate monitoring changes in water temperatures may be used as an early warning signal for subsequent development of shellfish toxicity in Kachemak Bay.

1. Introduction

Blooms of the toxic dinoflagellate genus *Alexandrium* occur seasonally in the bays and fjords surrounding the Gulf of Alaska. These dinoflagellates are a serious human health concern because they produce paralytic shellfish toxins (PSTs), a group of > 50 saxitoxin derivatives that accumulate most commonly in clams, mussels, oysters, and other filter feeding invertebrates (Wiese et al., 2010). Ingestion of contaminated shellfish can result in paralytic shellfish poisoning (PSP), a potentially fatal illness associated with a variety of serious neurological and gastrointestinal symptoms (Etheridge, 2010; Cusick and Sayler, 2013). In addition to the human health risks, PSP also reduces access to valuable natural resources by limiting commercial, recreational, and subsistence harvesting of shellfish. Even though episodic PSP events have occurred in Alaska for centuries, until recently there has been uncertainty about the identity of the *Alexandrium* species responsible (Quayle, 1969; Horner et al., 1997; Vandersea et al., 2017). *Alexandrium* species share many morphological similarities with other

Abbreviations: ADEC, Alaska Department of Environmental Conservation; LCI, lower Cook Inlet; PSP, paralytic shellfish poisoning; PSTs, paralytic shellfish toxins; STX eq, saxitoxin equivalents

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thecate dinoflagellates making it difficult to distinguish species using routine light microscopy, especially when these cells comprise a relatively minor component of the phytoplankton community (Eckford-Soper et al., 2013). Identification may be particularly difficult in iodine-preserved samples, a technique commonly used for phytoplankton monitoring (Utermöhl, 1958). To overcome this limitation, Vandersea et al. (2017) used species-specific molecular assays to demonstrate that *Alexandrium catenella* (*A. tamarense* Group I, John et al., 2014) was the primary PSP species in the Gulf of Alaska from Kodiak Island southward to Juneau and the Ketchikan region in southeast Alaska. Natsuike et al. (2013) used similar molecular methods to establish *A. catenella* (Group I) as the primary *Alexandrium* species present in the Bering and Chukchi Seas, thereby confirming *A. catenella* is the dominant *Alexandrium* species throughout Alaska. It should be noted that the nomenclature of *A. tamarense* Group I has been in flux, with use of various species designations including *A. tamarense* (Natsuike et al., 2013, 2017a), *A. fundyense* (Natsuike et al., 2017b; Vandersea et al., 2017) and *A. catenella* (Fraga et al., 2015). A recent decision by the nomenclature committee of the International Code of Nomenclature formally designated *A. tamarense* Group I as *A. catenella* (John et al., 2014; Fraga et al., 2015; Prud'homme van Reine, 2017). Therefore, the dominant *A. tamarense* complex Group I species found throughout Alaska will be referred to as *A. catenella* in this paper.

Though *A. catenella* is found throughout Alaska, relatively little is known about the seasonal abundance patterns of this species, or the environmental factors that most influence bloom formation (Matweyou, 2003). The lack of quantitative *Alexandrium* distribution data in Alaska can be partially attributed to relatively low cell abundances that are difficult to enumerate by light microscopy. In other PSP-endemic regions of North America, *Alexandrium* blooms typically reach 10^4–10^6 cells L^{-1} (Fauchot et al., 2005b; Brosnahan et al., 2014). In contrast, the few data currently available from Alaska indicate *Alexandrium* densities are typically < 1000 cells L^{-1}, even during bloom periods (Matweyou, 2003; Natsuike et al., 2017a). To overcome difficulties in counting cells, a species-specific *A. catenella* quantitative PCR (qPCR) assay capable of accurately measuring cell concentrations from several cells to over 100,000 cell eq. L^{-1} was used (Vandersea et al., 2017).

The assay was used to determine the abundance and distribution of *A. catenella* in relation to environmental conditions in lower Cook Inlet and Kachemak Bay, located in south-central Alaska (Fig. 1). Cook Inlet is of particular interest because it is one of the most intensely used estuarine areas in the state. This waterway supports the majority of the state's population, serving as the only sea-route to the Port of Anchorage, supporting longstanding commercial and noncommercial fishing industries, is a central hub for the petroleum industry, and includes some of the most environmentally sensitive habitats in the area (Brabets et al., 1999; NOAA, 2002; NRPG, 2015). Kachemak Bay, located on the eastern side of lower Cook Inlet, is a highly productive, fjord type estuary characterized by growing fishing, recreation, and tourism pressures with a long history of shellfish harvesting (Brooks, 2001).

The objective of this study was to gain insights into conditions favoring *Alexandrium* bloom development and duration. The ability to quantify very low levels of pre-bloom cell concentrations was especially useful in evaluating the conditions associated with bloom formation. Many of the data used in the study were obtained using sampling opportunities provided by the long-term Gulf Watch Alaska monitoring program (http://www.gulfwatchalaska.org/monitoring) and the phytoplankton monitoring network maintained by the Kachemak Bay National Estuarine Research Reserve (http://accs.uaa.alaska.edu/kbnerr/). Over the course of this study, the Gulf of Alaska and much of the northeast Pacific experienced an abnormal warming trend associated with basin-wide atmospheric and ocean circulation anomalies (Bond et al., 2015b; Hu et al., 2017). This warm water anomaly drove dramatic shifts in ecological processes across the Gulf of Alaska, Bering Sea, and Artic (Bond et al., 2015a; Cavole et al., 2016; Yu-Heng et al., 2017). Coincident with this regional warming trend, *Alexandrium catenella* cell abundances increased in Kachemak Bay, and PST concentrations rose above the Food and Drug Administration's action level of 80 µg saxitoxin (STX) equivalents (eq.) 100 g^{-1} shellfish (Scanlan, 2015; ADFG, 2017; AOOS, 2018).

2. Materials and methods

2.1. Site description

Cook Inlet is a large embayment in south-central Alaska extending over 300 km from the city of Anchorage at its northern end and opening into the Gulf of Alaska to the south (Fig. 1A; Evans et al., 1972). The lower portion of Cook Inlet is bordered by the Aleutian Mountain Range to the west and Kenai Peninsula to the east. Kachemak Bay is located on the eastern side of lower Cook Inlet (LCI), approximately 200 km south of Anchorage (Fig.1B). The Bay is ~35 km wide at its mouth and ~57 km in length with a southern shore characterized by steep mountains and a series of deep fjords and shallower bays. In contrast, the north side of Kachemak Bay is more shallow and includes a glacial moraine (Homer Spit) extending 6–7 km outward from the city of Homer, dividing the bay into distinct inner and outer portions (Fig. 1C; Field and Walker, 2003). Freshwater input to the bay is derived from the meltwater of seven area glaciers and surrounding snowpack, as well as many smaller streams. In summer, glacial meltwater contributes ~70,000 m^3 d^{-1} of fresh water to the inner bay, and carries a substantial load of suspended sediment. The freshwater input in the inner bay and inflow of seawater from LCI and the Gulf of Alaska cause salinities to vary from ~0 near the head to ~35 at the bay mouth (DOC/NOAA/DOI/BLM, 1978; Abookire et al., 2000; Okkonen et al., 2009).

The overall circulation pattern of Kachemak Bay is characterized by outflow of surface water along the northeast shore of the outer bay, a counterclockwise gyre in the inner bay, and a clockwise gyre in the outer bay (Burbank, 1977; Field and Walker, 2003). Most of the inflow occurs along the southern side of the bay below a depth of 30 m within a > 100 m deep trench, consistent with the Bay's character as a positive, partially mixed estuary. The vertical structure of the water column varies seasonally, with stratification during the summer, followed by relaxation and vertical mixing in winter. The complexity of the bay's circulation is further increased by the ~8 m semidiurnal tidal amplitude that generates very strong tidal currents (Abookire et al., 2000).

2.2. Phytoplankton samples

Sampling transects were conducted in LCI quarterly during 2012–2017 (Fig. 1B; Transects 3, 6, and 7), most frequently from the RV *Pandalus* (State of Alaska) as part of the Alaska Gulf Watch Monitoring Program (http://www.gulfwatchalaska.org/monitoring/). Monthly sampling cruises were also conducted during the same period in Kachemak Bay (Transects 4 and 9; Fig. 1C) using the National Oceanic and Atmospheric Administration (NOAA) Research Vessels *Edgecombe* or *Barnacle*. During each cruise, surface water was collected at three stations along each transect and corresponding water temperature and conductivity were measured using a CTD (conductivity, temperature, depth; model SBE 19plus V2, Sea-Bird Scientific, Bellevue, Washington, USA). Ten to forty liters of surface water containing live phytoplankton were collected using a bucket and concentrated with a 20 µm mesh plankton net. The cod end from each sample was shaken gently to homogenize the sample and a 125 mL aliquot was preserved in neutral Lugol's iodine solution for *Alexandrium catenella* qPCR assays (Andersen and Throndsen, 2003; Vandersea et al., 2017). The nearshore samples were collected at the docks in Jakolof Bay and Kasitsna Bay (NOAA Kasitsna Bay Laboratory; Fig. 1C). The water samples were processed and preserved as described above. All samples were stored at 4 °C in the dark until they could be processed for DNA extraction and qPCR assay. Lugol's preserved water samples were shipped to the NOAA Beaufort

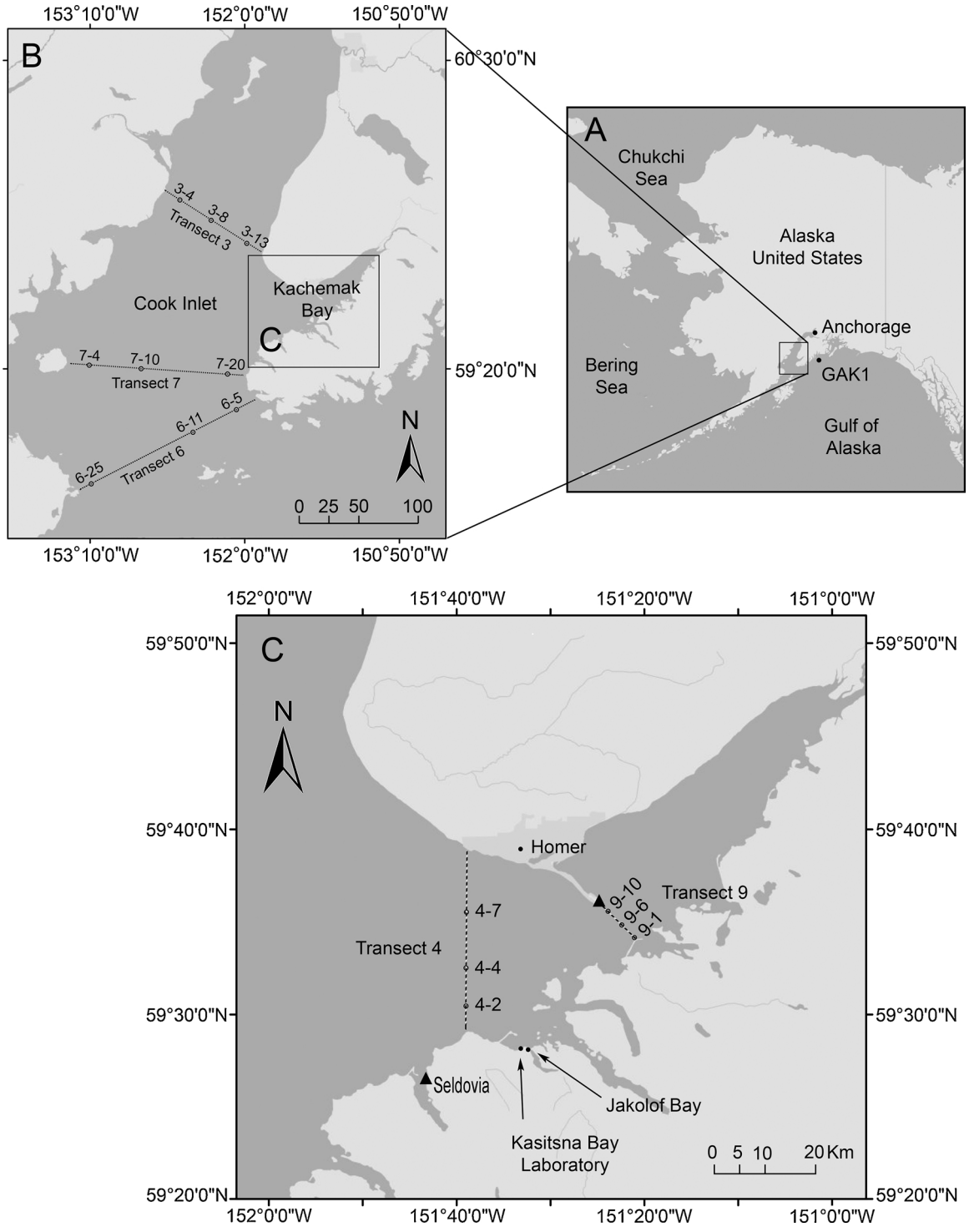

Fig. 1. Maps of study area: (A) Location of study area in Alaska, (B) Lower Cook Inlet and Kachemak Bay with transects 3, 6 and 7 and sampling stations, (C) Detail of Kachemak Bay with sampling stations on transects 4 and 9, the Kasitsna Bay Laboratory and Jakolof Bay. Filled triangles (▲) denotes location of monitoring stations at Homer Spit and Seldovia.

Laboratory, in Beaufort, NC, USA for qPCR analysis.

2.3. Nutrient and chlorophyll measurements

Nutrient measurement data for phosphate, ammonium, nitrate + nitrite and chlorophyll *a* (Chl*a*) for Kachemak Bay were obtained from the National Estuarine Research Reserve System Centralized Data Management Office website (http://cdmo.baruch.sc.edu/get/export.cfm). Nutrient and chlorophyll samples were collected monthly from the Homer environmental monitoring station (59.4409 °N, 151.7209 °W) maintained by the Kachemak Bay National Estuarine Research Reserve (KBNERR; triangle, Fig. 1C). The nutrient and chlorophyll samples were collected independent of the Lugol's preserved samples for qPCR analysis (section 2.2). Each month, triplicate samples were collected from one meter below the surface and one meter above the bottom during both the tidal flood stage and within 3 h of low tide using a Niskin bottle. All samples were transferred to wide-mouth Nalgene™ sample bottles (Thermo Fisher Scientific, Inc.,

Waltham, Massachusetts, USA) that were previously acid washed (10% HCl), rinsed (5x) with distilled-deionized water, dried and followed by rinsing (5x) with ambient water prior to collection of the sample. Samples were immediately placed on ice, in the dark, and returned to the laboratory. Once in the laboratory, samples were shaken and processed for nutrient and Chl*a* analysis as described by the National Estuarine Research Reserve System Centralized Data Management Office manuals and standard operating procedures website (http://cdmo.baruch.sc.edu/request-manuals-admin/pdfs/NUTCHLA_SOPv1.8.pdf).

2.4. Oceanographic and meteorological data

Water quality data (2012–2017) from KBNERR's Homer and Seldovia monitoring stations (Triangles, Fig. 1C) consisting of water temperature, salinity, and solar radiation were obtained from the NERR website (http://cdmo.baruch.sc.edu/get/export.cfm). Precipitation data for Homer were obtained from The Alaska Climate Research Center (http://akclimate.org/acis_data). A monthly average, interannual stratification index was calculated using CTD measurements of temperature and salinity obtained from the middle of Kachemak Bay at Transect 9, station 6 (Fig. 1C). The stratification index was calculated as the slope of the vertical density gradient from the surface to 30 m depth.

To examine how regional and local warming trends affected shellfish toxicity associated with *Alexandrium* blooms in LCI, monthly temperature anomalies (°C) were determined using CTD profile data at GAK1 and Seldovia, AK (1998–2016). The GAK1 average daily water temperature data from the ocean surface layer (≤ 30 m) in the Alaska Coastal Current were downloaded from the Time Series website maintained by the University of Alaska Fairbanks (http://www.ims.uaf.edu/gak1/). The station is located at the mouth of Resurrection Bay near Seward, Alaska, (59.8352 °N, 149.4667 °W Fig. 1A). The Seldovia temperature data were obtained from the NERR website. GAK1 and Seldovia water temperature anomalies were calculated as the difference from the long-term mean for each corresponding month and compared with shellfish toxicity data (1998–2016) from the Alaska Department of Environmental Conservation (ADEC) and from NOAA.

2.5. DNA extraction and qPCR assay

Molecular analyses were performed using reagents and methods described in Vandersea et al. (2017). The Lugol's-preserved phytoplankton samples described above were concentrated by vacuum filtration onto 47 mm, 8 µm pore size polycarbonate filters (Whatman Nucleopore™, GE Healthcare Bio-sciences, Pittsburgh, Pennsylvania, USA). DNA was extracted from each filter using the Mo Bio Laboratories Power® Soil DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, California, USA) following the manufacturer's protocol except that 350 µL of cell lysate rather than the prescribed 450 µL was processed. The DNA extracts were eluted from the mini columns using 50 µL of elution buffer. Spectrophotometric analysis (260 nm/280 nm) was conducted to assess DNA concentration and purity. The DNA extracts were stored at 4 °C pending *Alexandrium catenella* qPCR assay analyses.

The qPCR assay was conducted using an Eppendorf Mastercycler® ep realplex 4 system with white Eppendorf real-time tube strips (Eppendorf North America, Inc., Westbury, New York, USA) and a total reaction volume of 10.5 µL per tube. Each PCR reaction mixture contained 4.5 µL of 5 Prime RealMasterMix SYBR ROX 2.5x [0.05 units μL^{-1} Taq DNA polymerase, 10 mM $Mg(CH_3COO)_2$, 1.0 mM dNTPs, 20X SYBR® Green solution], each primer at a concentration of 0.15 µM, 4.7 µL of sterile deionized water, and 1 µL of template DNA [*Alexandrium catenella* forward primer (5′-GATAAGTCTCCTGTGGG GGG-3′) reverse primer (5′-AAGCACAGGAACACACACATA-3′)].

Thermal cycling conditions included denaturation at 95 °C for 2 min followed by 40 cycles at 95 °C for 10 s, annealing for 15 s at 60 °C with a subsequent extension at 68 °C for 20 s. The fluorescence threshold was determined by the Eppendorf realplex 4 analytical software and the PCR cycle during which fluorescence crossed the threshold was designated the quantification cycle (Cq) as defined by (Bustin et al., 2009). A melting curve analysis was performed following thermal cycling to check the specificity of the PCR reactions. A limit of ± 0.5 °C for melting temperature peak shift was set as the cutoff for species-specific amplifications.

2.6. qPCR standard curves

Standard curves were constructed following methods described in Vandersea et al. (2017). The DNA concentration of purified amplicons, containing the qPCR assay target site, was estimated spectrophotometrically by measuring UV absorbance at 260 nm. The number of target copies was determined as described in Vandersea et al. (2012). To construct standard curves, the purified amplicons were serially diluted 1:10 in dH_2O and encompassed six orders of magnitude. Each serial dilution was qPCR amplified for 40 cycles using the *Alexandrium catenella* primers followed by a melting curve analysis to check for secondary qPCR products and primer dimers. Only Cq values from qPCR reactions that produced a specific product within 35 amplification cycles were included in the standard curves. For each curve, the Cq values generated from the tenfold serial dilutions were plotted against the log transformed copy numbers to obtain regression equations. Regression analyses were performed to calculate the slope values and confirm the linearity of the standard curves.

2.7. Estimating cell numbers

Methods described in Vandersea et al. (2017) were used to obtain *Alexandrium catenella* qPCR cell number estimates. First, the number of extractable PCR amplicons per environmental sample was calculated by solving the regression equations derived from diluted PCR amplicon-based standard curves using Cq values acquired from qPCR assay of environmental samples. This value was divided by the number of *Alexandrium catenella* extractable rDNA copies per cell (11,107 copies $cell^{-1}$). The sample volume (10–40 L) and the volume removed from the cod end (125 mL) was used to calculate the number of cells per liter in each sample and abundance was expressed as *A. catenella* cell equivalents per liter of seawater (cell eq. L^{-1}).

2.8. qPCR assay controls

To assess potential DNA contamination and PCR inhibitors in the extracted field samples, each qPCR assay included a positive control, a negative DNA control, a blank extraction control, and two spiked DNA controls. The positive control contained a known amount of target DNA in the qPCR reaction mixture and ensured that the qPCR reagents were properly assembled. The negative control included the addition of 1 µL of reaction buffer to a subset of reaction mixes in order to test for contaminated reagents or cross-contamination between samples. The blank controls were incorporated during the DNA extractions of the environmental samples to test for potential DNA contamination during the extraction process. Spiked DNA controls consisted of adding target DNA to a subset of the environmental samples to determine if PCR inhibitors were present.

2.9. Statistical analyses

Environmental data and qPCR abundance estimates for *A. catenella* were processed and analyzed using SigmaPlot software (version 11, Systat Software, Inc., San Jose, California, USA). Neither the environmental data nor the *A. catenella* qPCR cell concentration estimates were normally distributed. Non-parametric Spearman rank correlations were used to evaluate the degree of association between surface water temperature, salinity, and *A. catenella* abundance. Differences in *A. catenella*

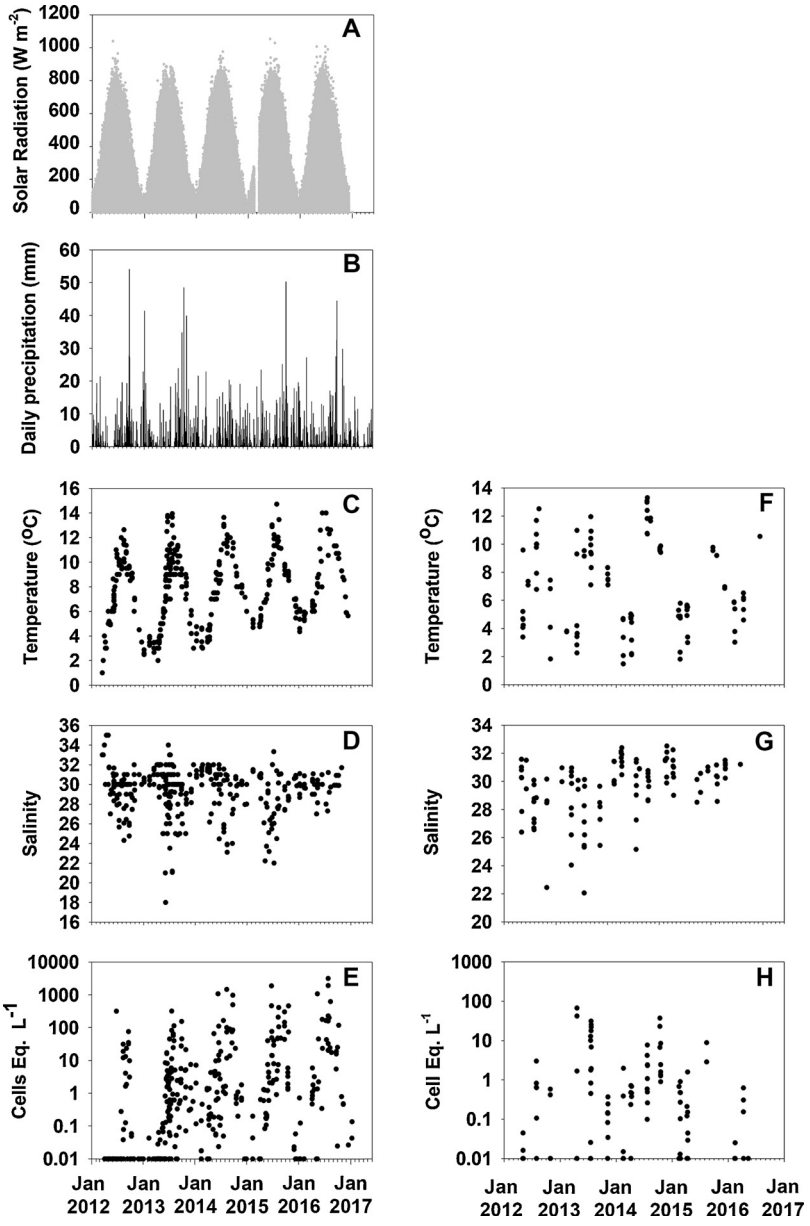

Fig. 2. 2012–2017 Time series of *Alexandrium catenella* cell abundances, meteorological and hydrographic monitoring data from Kachemak Bay, Homer, and lower Cook Inlet, Alaska. Panel (A) Daily solar radiation (W m^{-2}) recorded at the Homer airport, (B) Daily total precipitation (mm) measured at Homer, Alaska (C) Kachemak Bay surface water temperature measured at stations along Transect 4 (4-2, 4-4, 4–7), Transect 9 (9-1, 9-6, 9–10), Kasitsna Bay Laboratory, and Jakolof Bay, (D) Surface salinity measured at the stations listed in (C), (E) *A. catenella* cell abundances observed in Kachemak Bay at the stations listed in (C), (F) Lower Cook Inlet surface water temperature measured at stations along Transect 3 (3–4, 3–8, 3–13), Transect 6 (6-5, 6–11, 6–25), and Transect 7 (7-4, 7–10, 7–20), (G) Surface salinity measured at lower Cook Inlet stations listed in (C), (H) *A. catenella* cell abundances detected at Lower Cook Inlet stations listed in (C).

abundance among sampling locations were analyzed using a Kruskal-Wallis rank ANOVA (Zar, 1996).

3. Results

3.1. Effect of temperature and salinity on A. catenella abundance

Environmental data, collected in conjunction with water sampling from LCI, Kachemak Bay as well as from the KBNERR's Homer monitoring station during 2012–2017, illustrate the regular seasonal patterns of water temperature, salinity, and precipitation coincident with increasing daily levels of solar radiation (Fig. 2). Water temperatures in Kachemak Bay reached annual maxima (14–16 °C) in late July-August, during periods of the highest daily solar radiation (800-950 W m^{-2}, Figs. 2A and 2C). Salinity was 26–32 during most of each year, reaching annual maxima (32–33) during March-April and minima (< 25) during August-October (Fig. 2D). In comparison, water temperatures in LCI reached annual maxima (10–13 °C) in late July (Fig. 2F). Salinity was 29–32 during most of each year with annual maxima (31–32.5) occurring February-May and minima (< 26) occurring July-October (Fig. 2G). The results of a Spearman rank correlation showed a significant negative association between monthly average water temperature and salinity during the 2012–2017 study period ($r = -0.743$, $p < 0.0001$, $n = 53$). These data are consistent with previous observations that salinity in Kachemak Bay is largely governed by freshwater input from snow and ice melt, rather than the more variable periods of precipitation (Fig. 2B and D; (Savard and Scully, 1984; Okkonen and Howell, 2003).

qPCR cell abundance data from surface water samples (n = 572) collected from Kachemak Bay (Transects 4, 9, Jakolof Bay, and Kasitsna Bay) and LCI (Transects 3, 6, and 7; Fig. 1) indicated that the majority (92%) of the phytoplankton samples contained fewer than 100 *A. catenella* cell eq. L^{-1}. Abundance of *A. catenella* cells exhibited a yearly bloom cycle with a clear dependence on seasonal water temperatures (Figs. 2C, E, F, H). Cell concentrations were near zero in the winter and exhibited a single annual peak of 200–3050 cell eq. L^{-1} in late summer (Fig. 2E). To demonstrate the annual bloom cycle more clearly, *Alexandrium* abundances during 2015 and 2016 are shown at a higher resolution in Fig. 3 along with temperature, salinity, and Chl*a*. The data show *A. catenella* concentrations started increasing in May-June, when

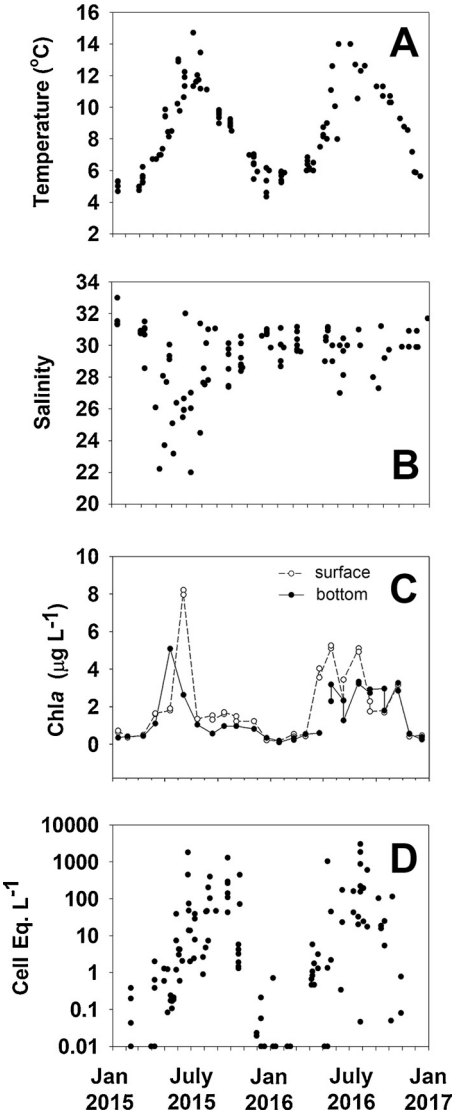

Fig. 3. Seasonal *Alexandrium catenella* cell abundances, meteorological, and hydrographic monitoring data collected during 2015–2016 from Kachemak Bay. Panel (A) Kachemak Bay surface water temperature measured at stations along Transect 4 (4-2, 4-4, 4–7), Transect 9 (9-1, 9-6, 9–10), Kasitsna Bay Laboratory, and Jakolof Bay, (B) surface salinity measured at the stations in (A), (C) chlorophyll *a* measured at Homer Spit (D), *A. catenella* cell abundances observed in Kachemak Bay listed in (A).

water temperatures were > 6.5 °C. Cell concentrations peaked during July–September, after the Chl*a* maxima, when temperatures were 14–15 °C. Cell concentrations started decreasing in September-October as temperatures declined (Figs. 3A, C, D). There was little apparent effect of seasonal salinity on abundance (Figs. 3B and D). Interestingly, *A. catenella* cells were detected in surface samples in all months of the year, with < 1 cell eq. L^{-1} during Dec-March (Figs. 2E and D). It is not known if these low abundances reflect flagellated cells, or dormant resting stages suspended in the water column.

Environmental data recorded during *Alexandrium* cell sample collection were used to quantify the effect of temperature and salinity on abundance (Fig. 4). The data firmly support the dependency of *Alexandrium* abundance on temperature (Fig. 4A). Furthermore, the results of a Spearman rank correlation showed *A. catenella* cell concentrations were positively correlated with water temperature at sites within Kachemak Bay ($r = 0.61$, $p < 0.001$, $n = 445$). Although salinity was negatively correlated with cell abundance (Fig. 4B), the relationship was weak ($r = -0.2$, $p < 0.001$, $n = 445$). At stations outside of the Bay, Spearman rank correlation results showed *A. catenella* abundance was similarly affected by temperature ($r = 0.38$, $p < 0.001$, $n = 127$) and there was no apparent correlation with salinity ($p > 0.05$, $n = 127$).

3.2. Site differences in abundance

Overall, the highest *A. catenella* cell concentrations detected throughout the course of this study were consistently observed in samples collected within Kachemak Bay (Transects 4 and 9, Jakolof Bay, Kasitsna Bay) rather than LCI (Transects 3, 6, 7) (Figs. 1, 2E, 2 H). The highest *Alexandrium* cell concentration was observed in a sample collected during July 2016 from Transect 9, station 6 and contained ~3050 cell eq. L^{-1}. In comparison, qPCR cell concentration estimates for stations in LCI ($n = 127$) revealed that concentrations were all less than 100 cell eq. L^{-1}. The highest cell concentration estimate from LCI was observed in a water sample collected in April of 2013 from transect 7, station 10 (67 cell eq. L^{-1}). When data from all stations were pooled across all years, the results of a Kruskal-Wallis rank ANOVA showed *A. catenella* abundances fell into two distinct groups according to location ($H = 16.83$, $p < 0.001$, $n = 572$). The first group included Kachemak Bay and sub-bay locations with highest abundances (Transect 4, Transect 9, Kasitsna Bay, Jakolof Bay; Fig. 5). The second group included the LCI stations (Transects 3, 7, 6; Fig. 5) with the lowest abundances. Dunn's follow-up tests showed differences in *A. catenella* abundance between stations within each of the two groups were not significant ($p > 0.05$).

3.3. Nutrients, chlorophyll, stratification, and Alexandrium abundance

Monitoring data from Kachemak Bay showed a regular cycle of surface nutrient depletion with the annual phytoplankton bloom. Surface and subsurface nutrient and Chl*a* concentrations from the Homer Spit monitoring station during 2012–2017 are shown in Fig. 6. The data indicate both surface and subsurface nitrate + nitrite concentrations ($NO_2^- + NO_3^-$), which were > 0.2 mg L^{-1} during the winter months, began to decline in April-May as chlorophyll *a* concentrations increased. $NO_2^- + NO_3^-$ concentrations continued to decline during summer months (< 0.06 mg L^{-1}) and then began increasing in autumn (Figs. 6A and 6D). Dissolved surface and subsurface phosphate (PO_4^{3-}) exhibited a similar pattern, with concentrations of 0.03-0.04 mg L^{-1} in winter that declined to 0.01-0.025 mg L^{-1} during the summer phytoplankton bloom (Figs. 6C and 6D). Both surface and subsurface $NO_2^- + NO_3$- and PO_4^{3-} levels exhibited similar seasonal patterns, indicative of nutrient drawdown during the phytoplankton bloom. Relative to subsurface concentrations, the drawdown of surface PO_4^{3-} levels was more apparent than for $NO_2^- + NO_3^-$. Surface concentrations of ammonium (NH_4^+) were generally at their highest from October-March (0.03-0.05 mg L^{-1}) and decreased to ≤ 0.02 mg L^{-1} during June-August. Differences between surface and subsurface nutrients were also evident in NH_4^+ concentrations. Near bottom NH_4^+ concentrations were similar to surface levels in the winter months, but then increased sharply during May-September (0.03-0.08 mg L^{-1}, Fig. 6B). This pattern likely reflects utilization by phytoplankton at the surface during the summer coupled with greater N regeneration in subsurface waters.

Seasonal trends in particulate chlorophyll and qPCR data indicated spring diatom bloom and subsequent *A. catenella* blooms were influenced by onset of surface stratification in Kachemak Bay. Chlorophyll *a* concentrations at Homer generally started increasing in March-April with the initiation of the spring diatom bloom (KBNERR, 2014). Peak annual Chl*a* concentrations occurred during these blooms and ranged from 0.16 to 12.5 µg L^{-1} (Fig. 6D). The diatom blooms declined as the water column became stratified during the June-August period. Onset of stratification was accompanied by an increase in *A. catenella* cell abundances, with the highest cell densities occurring during periods of

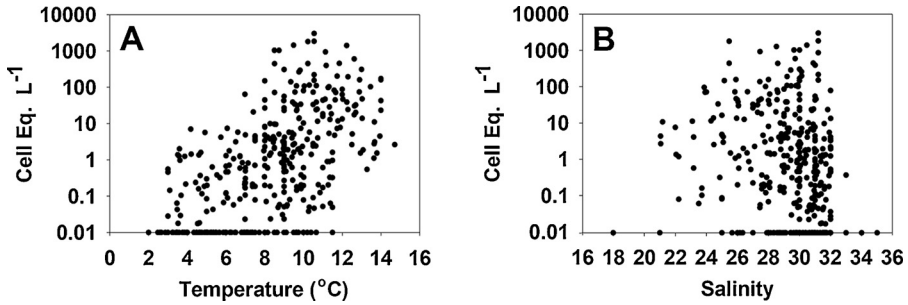

Fig. 4. *Alexandrium catenella* cell abundances in Kachemak Bay as a function of surface (A) temperature and (B) salinity.

strongest stratification (Figs. 6E and 6 F). *Alexandrium catenella* abundances remained relatively high through late September or early October when temperatures rapidly declined and the water column became mixed from mid-fall to early spring (Fig. 6E). The residual nutrient levels present during the bloom were above those that commonly limit phytoplankton growth indicating the *A. catenella* populations were not nutrient-limited (Figs. 6A-6C).

4. Discussion

This is the first quantitative study to characterize *Alexandrium catenella* blooms in the Cook Inlet region of Alaska (Fig. 1). A qPCR assay developed by Vandersea et al. (2017) was employed as a monitoring tool to elucidate the distribution and abundance of *A. catenella* cells, the seasonal development of toxic blooms, and potential linkage to environmental drivers. The results showed a cyclic, seasonal pattern of *A. catenella* cell growth that was positively correlated with increasing water temperatures. Furthermore, the cell abundance data indicated that *A. catenella* blooms ($>$ 100 cell eq. L^{-1}) developed within Kachemak Bay rather than lower Cook Inlet.

Most of the samples collected in this study contained low *A. catenella* cell concentrations ($\leq$ 10 cell eq. L^{-1}, Figs. 2 and 3), consistent with the historically low occurrence of PSP in the Cook Inlet region (Allen and Pauls, 1977; Castrodale, 2015). Samples with the highest cell abundances were only present at sites within Kachemak Bay with no real differences among sampling stations (Fig. 5). Bloom concentrations of *A. catenella* in Kachemak Bay ranged from 100 to 3050 cell eq. L^{-1} (Figs. 2E and 3). In comparison, the highest cell concentration detected at LCI sites was only 67 cell eq. L^{-1} (Fig. 2H). The greater abundances of *Alexandrium* cells within Kachemak Bay are mirrored in other systems with seasonal dinoflagellate blooms, where sheltered, stratified conditions promote dinoflagellate growth, reduce advection of cells from the system, and allow accumulation of benthic resting cysts to initiate blooms during following years (Margalef, 1978; Kim et al., 2002; Cloern et al., 2005; Faust et al., 2005; Glibert, 2016).

4.1. Water temperature and seasonal A. catenella blooms

Environmental monitoring data indicated seasonal *A. catenella* blooms and shellfish toxicity in Kachemak Bay are driven largely by water temperature, rather than salinity or nutrients. Peak surface water temperatures occurred in Kachemak Bay in late July-August (12–15 °C) following periods of maximum solar radiation (Fig. 2). The high water temperatures promoted *Alexandrium* growth, and abundances reached their highest levels during the same period. Throughout 2012–2017 cell concentrations never exceeded 10 cell eq. L^{-1} when water temperatures were $<$ 6–7 °C and concentrations $>$ 100 cell eq. L^{-1} only occurred at temperatures above 8 °C. Based on these findings, 7–8 °C appears to represent the temperature threshold for *A. catenella* bloom development in Kachemak Bay. Cells were present throughout the spring and summer, with highest abundances ($>$ 500 cell eq. L^{-1}) occurring at temperatures above 10–12 °C. It is also important to note that every sample collected when temperatures were $\geq$ 12 °C contained *A. catenella* cells, indicating this species is ubiquitous in the water column at these temperatures waiting to respond to environmental conditions permissive of bloom formation.

The effect of temperature on *A. catenella* blooms was also evident in the inter-annual trend, with high temperatures prompting a greater risk for PST accumulation in shellfish. During the course of this study, the Gulf of Alaska and much of the subarctic experienced a multi-year warming trend characterized by elevated ocean temperatures and a cascade of physical, chemical and biological perturbations (Bond et al., 2015b; Cavole et al., 2016; Di Lorenzo and Mantua, 2016). The effect of this regional warming event on *A. catenella* abundance was most apparent in rising winter minimum water temperatures but also in summer maximum water temperatures. In Kachemak Bay, annual minimum water temperatures rose from ~2 °C in February 2012 to ~6 °C in February 2016 while the maximum summer temperatures

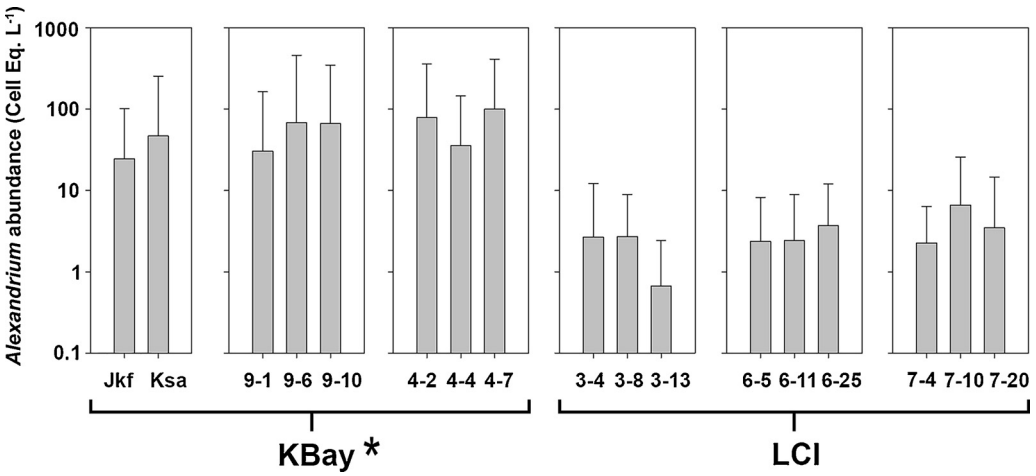

Fig. 5. Distribution of *Alexandrium catenella* cells (mean cell eq. L^{-1} ± std) at sub-bay sites in Kachemak Bay, Jakolof Bay (Jkf), Kasitsna Bay (Ksa), at stations on Transect 9 (9-1, 9-6, 9–10), stations on Transect 4 (4-2, 4-4, 4–7), and at lower Cook Inlet stations on Transects 3 (3–4, 3–8, 3–13), 6 (6-5, 6–11, 6–25) and 7 (7-4, 7–10, 7–20). See Fig. 1 for site locations. The asterisk denotes significant difference in cell abundances between Kachemak Bay and LCI sampling sites (Kruskal-Wallis rank ANOVA, $H = 16.83$, $p < 0.001$).

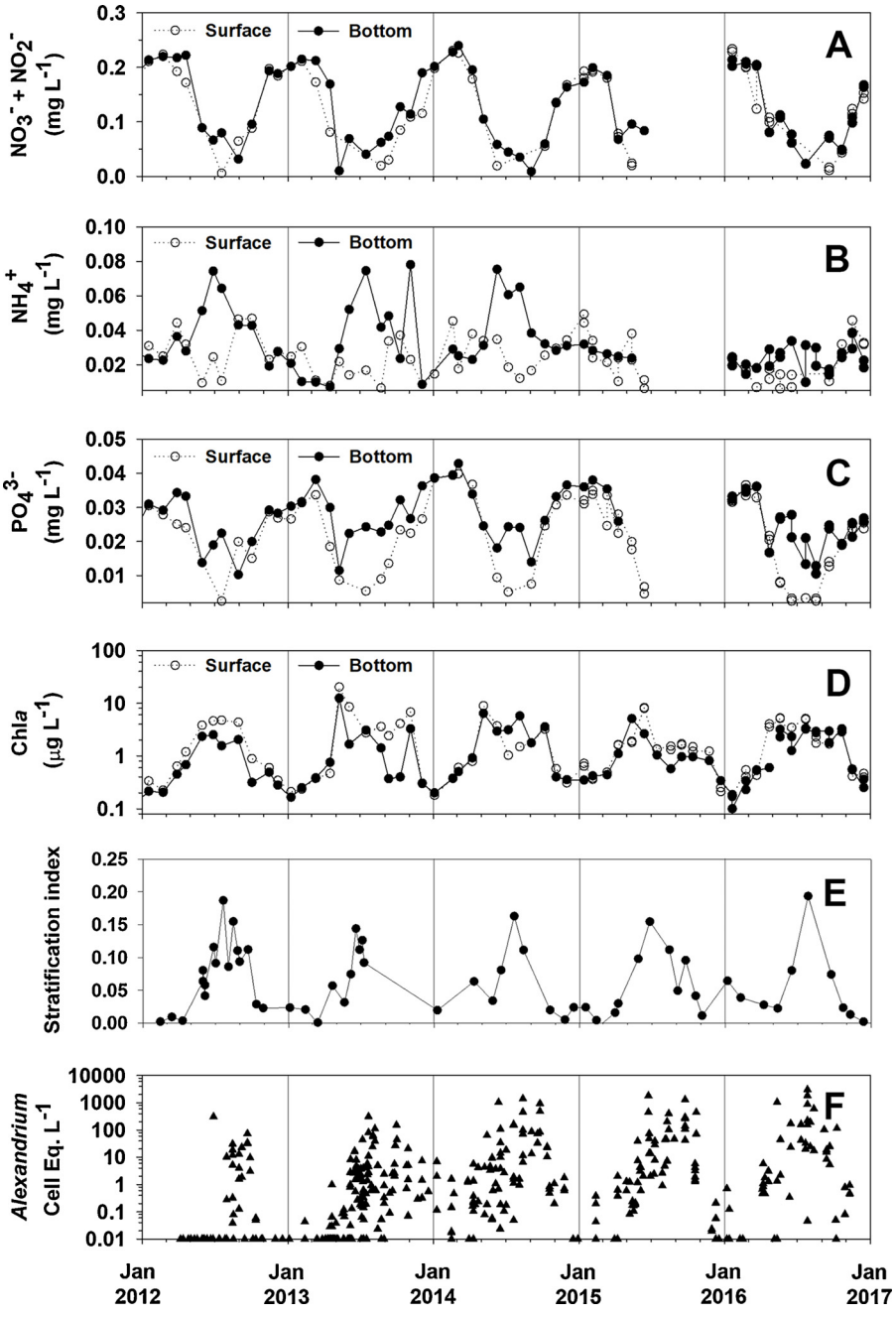

Fig. 6. Time series of surface (○) and bottom (●) dissolved (A) nitrate + nitrite (NO_3^- + NO_2^-, mg L^{-1}), (B) ammonium (NH_4^+, mg L^{-1}), (C) phosphate (PO_4^{-3}, mg L^{-1}) and (D) chlorophyll-*a* (Chl*a* μg L^{-1}) concentrations in comparison with (E) water column stratification index and (F) estimated *Alexandrium catenella* abundance (cell eq. L^{-1}). Nutrient concentrations were measured at the monitoring station on Homer Spit (Fig. 1C). The water column stratification index was calculated using CTD data from Transect 9, station 6 for the upper 30 m. *Alexandrium* abundance was determined using surface water samples at all sampling stations within Kachemak Bay. See Fig. 1 for site locations.

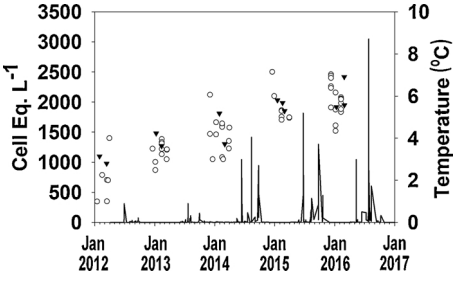

Fig. 7. Effect of rising winter temperatures on *Alexandrium catenella* abundances. Average winter minimum surface water temperatures recorded at sampling sites inside Kachemak Bay (○) and at station GAK1 (▼, Fig. 1A) versus *A. catenella* abundance in Kachemak Bay (—).

increased from 10 °C in 2012 and 2013 to between 11 °C and 12 °C for 2014 through 2016 (circles, Fig. 7, red arrows and black dashed lines in Fig. 8A). Higher winter temperatures also were observed within the Alaskan Coastal Current (ACC) at oceanographic monitoring station GAK1 (triangles, Fig. 7) indicating the increase in water temperatures occurred throughout the region. From an ecological standpoint, the gradual rise in winter temperatures allowed *A. catenella* cells a longer growing season. This likely provided cells more time to grow and may have contributed to the increasing annual peak abundances (Fig. 7) as well as elevated PSTs in shellfish during the same periods (Fig. 8). Interestingly, the *A. catenella* peak abundances were not shifted earlier in the annual cycle. Comparison of Figs. 6D and 6 F reveals that peak Chl*a* concentrations, corresponding to the annual diatom bloom, were observed during April-June (2012–2016). Peak *A. catenella* concentrations occurred after the annual diatom bloom and occurred annually from June-October. Given that a few high cell concentrations were detected in October of 2015 and 2016, this suggests that nutrients were not

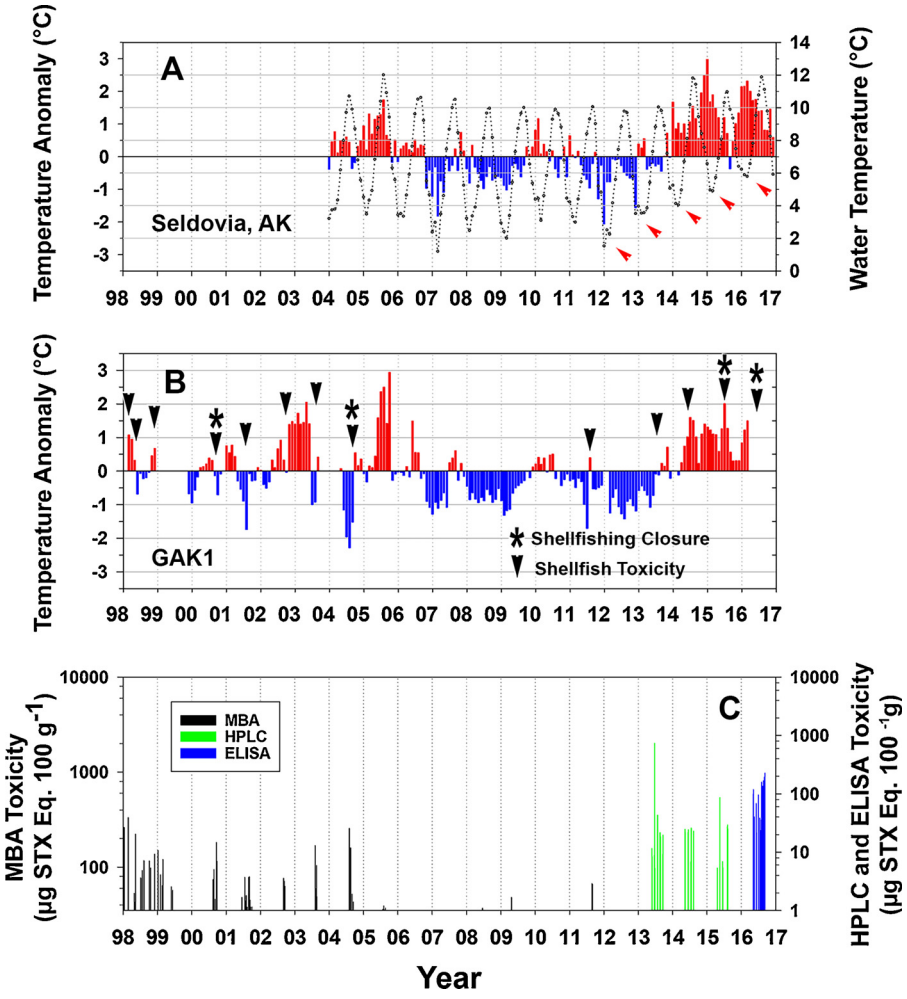

Fig. 8. Correlation of water temperature anomalies at Seldovia, AK, oceanographic station GAK1, and shellfish toxicity in Kachemak Bay (1998–2016, See Fig. 1 for site locations). (A) Near-bottom (~10 m) water temperature and water temperature anomalies (°C, 2004–2016) at Seldovia monitoring station. Red arrowheads denote increasing winter minimum temperatures. (B) Near-surface (≤ 30 m) water temperature anomalies (°C, 1998–2016) at GAK1. Temperature anomalies were calculated relative to the long-term average (see methods). Red bars indicate a positive anomaly while blue bars indicate a negative anomaly. Arrowheads denote measurable shellfish toxicity as shown in panel C. Asterisks denote shellfishing closures in Kachemak Bay. (C) Shellfish toxicity testing (µg STX Eq. 100 g^{-1}) was performed by the State of Alaska Department of Environmental Conservation using mouse bioassay (MBA, 1998–2011, left axis) or high performance liquid chromatography (HPLC, 1999–2016, right axis), and by the National Oceanic and Atmospheric Administration's Beaufort Lab by Enzyme-linked immunoassay (ELISA, 2016 only, right axis). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

limiting during the extended growth seasons but probably water temperature, light, and water column stability played more of a role in diminishing cell abundances.

4.2. Relationship between Alexandrium abundance and shellfish toxicity

Shellfish toxicity data collected in Kachemak Bay during this study enabled estimation of a threshold *A. catenella* cell concentration above which the risk of PSP should be of concern to state regulators and shellfish harvesters. Specifically, shellfish monitoring data showed PST concentrations in Kachemak Bay exceeded the 80 µg STX eq. 100 g^{-1} FDA action level during summer blooms in June-July 2015, and August 2016 (ADEC, 2015; HOMER-NEWS, 2016; AOOS, 2018). Cell estimates obtained from May to September 2015–2016 from Transects 4, 9, Kasitsna Bay Laboratory, and Jakolof Bay revealed average *A. catenella* concentrations of ~350 cell eq. L^{-1}. From these data, it was estimated that ~300-400 *A. catenella* cell eq. L^{-1} are sufficient to elicit PST accumulation in excess of the FDA action level. A literature review confirmed that this estimate is comparable to data from the Gulf of St. Lawrence, the California coast, the Okhotsk Sea, and the Bay of Fundy, where *Alexandrium* concentrations of 100–1000 cells L^{-1} were reported to increase shellfish toxicity above the FDA limit (Blasco et al., 2003; Jester et al., 2009; Shimada et al., 2012; Martin et al., 2014).

4.3. PSP risk versus temperature anomalies and implications for the Bering and Chukchi Seas

The regional warming trend that occurred in the Gulf of Alaska during this study also impacted the nearshore waters of the ACC (Bond et al., 2015a, b; Cavole et al., 2016). The similarity of warming in the ACC and Kachemak Bay is born out in a comparison of temperature anomalies measured at GAK1 and the KBNERR monitoring station at Seldovia (Fig. 8). Warm and cold anomalies measured at both locations exhibited very similar temporal patterns (Figs. 8A and B), which corroborated the extent of the regional warming trend and suggested that *A. catenella* blooms in Southcentral Alaska likely responded to warming conditions in similar ways. Evidence of the linkage between rising regional water temperatures and increased PSP risks comes from further comparison of the water temperature anomalies measured in outer Kachemak Bay and the ACC with shellfish toxicity data from LCI. Monthly surface temperature data from Seldovia and GAK1 oceanographic stations showed that periods of anomalously warm conditions (≥ 0.5 °C) occurred repeatedly between 1998 and 2016 (Figs. 8A and B). Although State of Alaska shellfish toxicity monitoring data were not collected systematically from sites across LCI (Fig. 8C), the existing data show shellfish toxicities exceeded 80 µg STX Eq. 100 g^{-1} following many of the warm anomalies (arrowheads Fig. 8B). Historical records from the region also indicate toxicity was high enough to prompt shellfishing closures in Kachemak Bay during three such events in 2004, 2015, and 2016 (ADEC, 2015; HOMER-NEWS, 2016; AOOS, 2018). Despite a substantial warm anomaly (1–3 °C) during 2005, however, there was little indication of corresponding shellfish toxicity suggesting other factors than water temperature may have been involved. During periods with cold anomalies, shellfish toxicity was generally low (e.g., 2007–2012). Taken together, the analysis indicates regional water temperature data may be used as an early indicator of *A. catenella*

bloom formation and PSP risk in the LCI region, and potentially, statewide. Similar conclusions were reached by researchers in other PSP-prone locations in North America (Erickson and Nishitani, 1985; Moore et al., 2015b; Wells et al., 2015; FOC, 2017). If the temperature-PSP linkage is robust, then greater availability of near real time temperature data may be invaluable for estimating PSP risks in Alaska and other coastal areas. Consequently, there is a need to expand temperature monitoring capabilities in more remote parts of Alaska where PSP is prevalent, such as the Kodiak, Aleutian and Pribilof Islands, and the southeastern region of Alaska. This need for increased temperature monitoring is likely to grow given rising ocean temperature conditions in the Bering and Chukchi Seas which has resulted in record low winter sea ice (Graham et al., 2017; NSIDC, 2018). Our findings indicate these increased temperatures will promote *Alexandrium* blooms resulting in accumulation of PSTs in marine mammals and seabirds, and increased risk to human populations dependent on these resources (Lefebvre et al., 2016; Dobbyn, 2017; Haecker, 2018; Natsuike et al., 2017a; Eisner, 2018).

4.4. Salinity and A. catenella abundance

Salinity is another environmental factor potentially governing *A. catenella* abundance and distribution in Kachemak Bay and LCI. Monitoring data showed lower surface salinities followed periods of maximum solar radiation and water temperature during late summer months (Fig. 2) that were largely attributable to snowpack and glacial meltwater (Field and Walker, 2003). The relationship between salinity and *A. catenella* cell concentrations from Kachemak Bay revealed that bloom concentrations of cells were highest (> 100 cell L^{-1}) at salinities ranging from 24 to 32 (Fig. 4B), indicating the effect of seasonal pulses of freshwater had little direct impact on *Alexandrium* abundance. This is supported by a weak negative correlation between cell concentrations and salinity ($r^2 = -0.2$, $p < 0.001$, $n = 572$). A similar pattern was reported at other locations in North America, where high *Alexandrium* abundance was generally associated with mesohaline, stratified portions of estuaries (Anderson et al., 2005a; Fauchot et al., 2005b; Townsend et al., 2005a) and by Moore et al. (2015a) who assessed *A. catenella* bloom risk for Puget Sound based on temperature and salinity and noted that cells were euryhaline and insensitive to changes in salinities except for those < 20. In this study, *A. catenella* cell concentration were also low ($\leq$ 10 cell eq. L^{-1}) at salinities below 24 (Fig. 4B).

4.5. Seasonal bloom dynamics, nutrients, and stratification

Diatom-dominated phytoplankton blooms occurred in April and May and were identified by an annual increase in Chl*a* that coincided with warming water temperatures and increasing light availability (KBNERR, 2014). Peak Chl*a* concentrations were generally observed in April-June, and coincided with a sharp decline in NH_4, $NO_2^- + NO_3^-$, and PO_4^{-3} in surface waters (Fig. 6A-D). The decline of the diatom bloom occurred in June-July and coincided with increasing water column stratification (Figs. 6D-6E). Stratified conditions persisted through August and were accompanied by an increase in *A. catenella* abundances that peaked in late summer, and in some years continued into October (Figs. 6E-6 F). During this period, nutrient concentrations in the stratified surface layer remained lower compared to the underlying waters (Fig. 6A-6C). The combined stratified surface waters and reduced nutrient concentrations limited diatoms and other phytoplankton but favored vertically migrating dinoflagellate species better able to utilize sub-surface nutrient pools (Fauchot et al., 2005a; Townsend et al., 2005b; Ralston et al., 2007). This niche strategy enables *Alexandrium* and other relatively slow-growing dinoflagellates to reach high abundance levels despite their inability to compete with smaller, faster growing diatoms for nutrients (Margalef, 1978; Love et al., 2005; Klais et al., 2011; Signorini et al., 2012; Gettings et al., 2014; Lin et al., 2016). Further, a very similar pattern of stratification and *A. catenella* bloom development has also been described in the Gulf of Maine (Townsend et al., 2005a). This pattern of summertime stratification and nutrient drawdown is common throughout much of the northern Gulf of Alaska which may account for the widespread occurrence of *A. catenella* in the region (Abookire et al., 2000; Fiechter et al., 2009; Okkonen et al., 2009; Vandersea et al., 2017). In September-October, *Alexandrium catenella* populations declined as stratification relaxed and water temperatures and light availability decreased (Figs. 6E-6 F).

5. Conclusions

Periods of elevated water temperatures from 2012 to 2017 promoted greater development of *A. catenella* blooms in Kachemak Bay with greater PSP risks. Increased winter minimum and summer maximum water temperatures resulted in longer growing seasons, allowing peak *A. catenella* abundances to increase during 2014-2016. While temperatures between 7–8 °C appear to represent the temperature threshold for *A. catenella* bloom development in Kachemak Bay, the highest cell abundances (> 500 cell eq. L^{-1}) occurred at temperatures above 10–12 °C. From coincident toxicity data, it was estimated that in Kachemak Bay, ~300-400 *A. catenella* cell eq. L^{-1} were sufficient to elicit PST accumulation in shellfish in excess of the FDA action level of 80 μg 100 g^{-1}. The monitoring data collected over the course of this study coincided with a regional warming event that drove dramatic shifts in ecological processes across the Gulf of Alaska, Bering Sea and Arctic (Bond et al., 2015a; Cavole et al., 2016; Yu-Heng et al., 2017). The clear connection between regional warming events and shellfish toxicity in Kachemak Bay suggest rising temperatures tied to climatic processes may be expected to drive greater PSP risks in the coming decades. Such an increase in HAB risks was projected recently by (Gobler et al., 2017), who predicted increases in *A. catenella* bloom-season length and growth rates for coastal regions of Alaska. Based upon the data presented here, annual winter minimum temperatures may prove an effective monitoring tool for PSP risks in Kachemak Bay and Lower Cook Inlet during the following summer. More substantial analysis is needed to judge the utility of such an approach as a forecasting tool for PSP across the northeastern Pacific.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.hal.2018.06.008.

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