Harmful Algae 77 (2018) 81–92

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

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

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ARTICLE INFO

Keywords:
Bloom
Nutrients
Paralytic shellfish poisoning
Salinity
Saxitoxin
Temperature
Dinoflagellate

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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https://doi.org/10.1016/j.hal.2018.06.008
Received 12 March 2018; Received in revised form 14 June 2018; Accepted 16 June 2018
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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

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