Alexandrium catenella cyst distribution and germination in Puget Sound, WA USA

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Abstract
The PS-AHAB (Puget Sound *Alexandrium* Harmful Algal Bloom) program, funded by NOAA/ECOHAB, seeks to understand environmental controls on the benthic (cyst) and planktonic life stages of the toxic dinoflagellate *Alexandrium catenella*, and evaluate the effects of climate change on the timing and location of blooms. This includes detailed mapping of overwintering cysts at 99 stations throughout Puget Sound. Highest surface sediment cyst abundances in 2011 and 2012 were found in Bellingham Bay (north), in bays on the western side of the central main basin and in Quartermaster Harbor (south). While cyst distribution patterns were similar for both years, 2012 cyst abundances were a factor of two lower at most stations. Compared to a 2005 survey, the Bellingham Bay "seed bed" is new, whereas Quartermaster Harbor cyst concentrations have decreased by an order of magnitude. In a related study funded by Washington Sea Grant, cysts from surface sediments at thirty 2012 PS-AHAB stations were evaluated for their germination potential with results ranging from 16% to 66% viability. To date, no relationship between cyst viability and cyst appearance has been detected. These results will be used to inform a model to explore the possibility of providing seasonal *Alexandrium catenella* bloom forecasts.

Keywords: Alexandrium catenella, cysts, sediments, germination, Puget Sound

Introduction

Paralytic shellfish toxins (PSTs) have been present in the Puget Sound region for centuries (Quayle 1969), however, little is known about the local distribution or biology of the causative organism *Alexandrium catenella*. *A. catenella* is a dinoflagellate that spends part of its life cycle as a cyst in the sediment before germinating to become a vegetative cell (Anderson 1998). This species produces a suite of neurotoxins (PSTs), the most potent being saxitoxin (Anderson *et al.* 1990). PSTs can accumulate in the tissues of filter-feeding shellfish, and be lethal in small doses to humans if consumed. *A. catenella* blooms therefore pose significant problems for local human health officials, marine resource managers and shellfish growers.

The Puget Sound Alexandrium Harmful Algal Bloom PS-AHAB study (http://www.tiny.cc/psahab) is a three year project funded by NOAA/ECOHAB designed to 1) map interannual variations in *A. catenella* cyst distribution in Puget Sound, 2) do laboratory experiments to quantify the rates and timing of cyst germination related to exogenous and endogenous factors, 3) integrate the results from the first two objectives into coupled hydrodynamic and climate models to determine current favorable habitat areas for *A. catenella* and evaluate the effects of climate change on these habitats in the future, and 4) establish a time series with sufficient depth to provide seasonal forecasts of harmful algal blooms. In a related study, funded by NOAA Seagrant, we are investigating how cyst distribution in surface sediment changes over the course of a year in one bay. We are also running experiments to determine mandatory dormancy, secondary dormancy and cyst viability for *A. catenella* in Puget Sound. Results from the two years' surveys (2011 and 2012) of *A. catenella* cyst distribution in the surface sediments of Puget Sound are presented here and compared to an earlier surface sediment cyst survey from 2005 (Horner *et al.* 2011). Cyst germination viability experiment results from selected 2012 survey samples are also reported here.

Methods

Field Sampling & Cyst Enumeration

Surface sediment *A. catenella* cyst distribution mapping surveys were completed during winter in 2011 and 2012. Surveys consisted of 99 stations throughout all of Puget Sound, the Strait of Juan de Fuca and the San Juan Islands (Figure 1). Sediment samples from the upper 0-1 cm and 1-3 cm were collected using a Craib corer. (Anderson *et al.* 2003) Sediments were processed for cyst enumeration using the method of Yamaguchi *et al.* (1995), total organic content (loss-on-ignition) and grain size using a particle size analyzer.

Cyst Viability

Additional surface (0-1 cm) sediment samples were collected from 30 stations throughout Puget Sound during the 2012 winter survey using the Craib corer. Stations were chosen where there was greater than 25 cysts/cc sediment from the 2011 survey. Samples were stored at 4^{o}C, in the dark, in nitrogen gas bags until cyst isolation could be performed (10-46 weeks after collection). One cubic centimeter of sediment was diluted to 50 ml with filtered seawater, sonicated and sieved through 90 um and 20 um sieves with the 20-90 um size fraction retained. After settling, sub-samples of this size fraction were placed on a Sedgewick-Rafter slide and individual cysts were picked using a micropipette. The first 60 cysts of *Alexandrium* encountered while scanning through the Sedgwick-Rafter slide were isolated from the 20-90 um size fraction of sediment from each of the 30 stations. Picked cysts were placed in a Palmer-Maloney slide for holding and as a rinsing step, and then one cyst per well was placed into a 96 well plate rack with 200 uL nutrient enriched natural seawater growth media. Well plates were incubated at 4^{o}C with a 14:10 light:dark cycle. Light levels were 70-90 uEM-2s-1 based on in situ data collected at the bottom of Quartermaster Harbor. Each plate was photographed and checked for germinated swimming cells at days 5, 14 and 28 and the number germinated recorded for the first 50 wells. Replicate isolations were conducted using sediment from the Quartermaster Harbor and Bellingham Bay stations at 10 and 20 weeks after collection to test for any effects of storage time on cyst viability.

Results

Highest cyst concentrations were found in Bellingham Bay, Birch Bay and Semiahmoo Bay in the north, Port Madison, Liberty Bay and Port Orchard on the west side of the main basin and Quartermaster Harbor in central Puget Sound (Figure 1). Quartermaster Harbor 2011 cyst concentrations were an order of magnitude less compared to a 2005 survey (Horner et al. 2011), and a new "seed bed" area has developed in Bellingham Bay. 2012 cyst distribution patterns are the same as 2011, but cyst concentrations are generally less in the surface sediment layer. Cyst germination viability ranged between 16-66% with no apparent difference between the surface (0-1cm) and 1-3cm layers (Table 1). To date, no relationship between cyst viability and cyst appearance using image analysis software has been detected.

Table 1. 2012 Puget Sound surface sediment cyst abundances and cyst germination viability.

#	Station	2012 cysts/cc in sediment	Germination
1	Semiahmoo Bay	77	37%
4	Birch Bay	72	34%
8	Bellingham Bay - North (0-1cm)	1070	48%
8	Bellingham Bay - North (0-1cm)	1070	54%
8	Bellingham Bay - North (1-3cm)	3830	44%
9	Bellingham Bay - East	117	52%
10	Bellingham Bay - South	67	44%
11	Bellingham Bay - West	55	48%
12	Padilla Bay	147	30%
15	Lopez Sound - Outer	57	20%
17	Cattle Point (Van Veen)	100	32%
22	Sequim Bay - Center	35	34%
58	Port Madison	300	42%
59	Liberty Bay	500	36%
60	Port Orchard - North	127	46%
61	Port Orchard - South	127	54%
78	Quartermaster Harbor - Center (0-1cm)	708	16%
78	Quartermaster Harbor - Center (0-1cm)	708	38%
78	Quartermaster Harbor - Center (1-3cm)	1022	66%
79	Quartermaster Harbor - Inner	442	42%

Discussion and Future Work

The preliminary maps from the annual cyst distribution surveys are shared with Puget Sound health officials, marine resource managers and shellfish growers in the spring of each year, as part of the PS-AHAB "just-in-time" information dissemination to stakeholders program. One more year of field samples will be collected and analyzed to determine the interannual variability of *A. catenella* cyst distribution in the surface sediments of Puget Sound and provide shellfish growers with an early warning map of potential high bloom areas. Laboratory experiments investigating the rates and timing of cyst germination related to exogenous and endogenous factors are ongoing. In a related study, we are also doing mandatory and secondary dormancy experiments, as well as continuing the viability study. In addition, we are investigating the variation in surface sediment cyst distribution in Quartermaster Harbor monthly for one year. These data will then be used to inform coupled Puget Sound hydrodynamic and climate models to explore the possibility of providing seasonal harmful algal bloom forecasts in the future.

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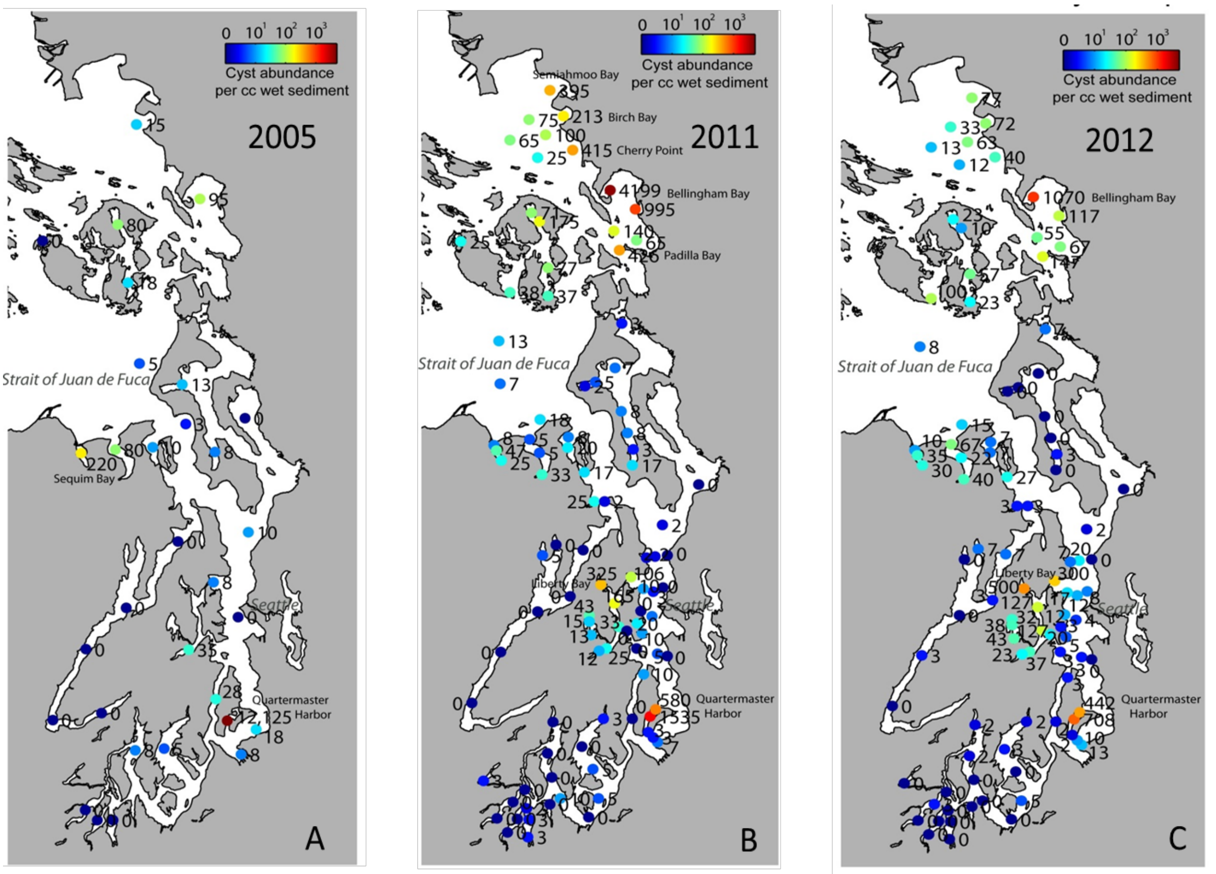

Figure 1. Puget Sound surface sediment cyst abundances per cubic centimeter wet sediment. All surveys were done in the winter of each year. A) 2005; B) 2011 and C) 2012.