Completion Report
Period: 2/1/2014 - 1/31/2015
Project: R/OCEH-9 - *Understanding dormancy requirements and germination of Alexandrium cysts and evaluating cyst mapping as a tool for early warning of harmful algal blooms*

STUDENTS SUPPORTED

Dunn-Johnson, Alison, adunn235@u.washington.edu, UWT, Environmental Science, status: new, field of study: Environmental Science, advisor: Cheryl Greengrove, degree type: BS, degree date: 2016-06-01, degree completed this period: No
Student Project Title:
Cyst Counting
Involvement with Sea Grant This Period:
capstone
Post-Graduation Plans:
graduate school

Huber, Nannette, nannette.huber@gmail.com, UWT, Environmental Science, status: new, *no field of study*, advisor: Cheryl Greengrove, degree type: BS, degree date: 2012-06-01, degree completed this period: No
Student Project Title:
Mapping history of PSP in Puget Sound using GIS and making a timelapse video
Involvement with Sea Grant This Period:
research assistant
Post-Graduation Plans:
graduate shcool

CONFERENCES / PRESENTATIONS

2014 16th International Conference on Harmful Algae. Wellington, New Zealand 27-31 October 2014. Cheryl Greengrove presented a talk titled “Alexandrium cyst distribution and germination in Puget Sound, WA USA”., public/profession presentation, 600 attendees, 2014-10-27

2014 Salish Sea Ecosystem Conference. Seattle, WA, April 30-May 2, 2014. (I) Stephanie Moore gave a presentation titled “Alexandrium ecology in Puget Sound: bloom transport and climate pathways”, (II) Cheryl Greengrove gave a presentation titled “Alexandrium cyst distribution and germination in Puget Sound”, and (III) Nicholas Schlafer (undergraduate student) presented a poster titled “Quartermaster Harbor Water Properties & Alexandrium catenella”. http: //www.wwu.edu/salishseaconference/, public/profession presentation, 800 attendees, 2014-04-30

2014 4th Annual Northwest Fisheries Science Center Science Symposium. Seattle, WA, March 25-26, 2014. Stephanie Moore gave a presentation titled “Harmful algal blooms of Alexandrium in Puget Sound: cyst dynamics, growth, transport, and climate pathways”., public/profession presentation, 40 attendees, 2014-03-25

2014 American Society of Limnology and Oceanography Ocean Sciences Meeting, Hawaii, 23-28 February 2014. Cheryl Greengrove and Julie Masura gave a poster titled “Alexandrium Bloom Ecology in Puget Sound: Cyst Dynamics, Growth, Transport, and Climate Pathways.”, public/profession presentation, 5000 attendees, 2014-02-23

ADDITIONAL METRICS

P-12 Students Reached:	0	**P-12 Educators Trained:**	0
Participants in Informal Education Programs: Another shipboard education and outreach event was run May 9-10, 2014, on the R/V Barnes in Commencement Bay for multiple college classes and some middle and high school students. Students learned about local oceanography, estuaries and HABs. About 40 students participated in this event. Seattle Aquarium Discover Science Weekends: Julie Masura and students participated in this event again during 8-9 November 2014.	40	**Volunteer Hours:** A large number of students donated a great deal of time to this project	240
Acres of coastal habitat protected, enhanced or restored:	0	**Resource Managers who use Ecosystem-Based Approaches to Management:**	0
Annual Clean Marina Program - certifications:	0	**HACCP - Number of people with new certifications:**	0

ECONOMIC IMPACTS

No Economic Impacts Reported This Period

SEA GRANT PRODUCTS

Description	Developed?	Used?	ELWD?	Number of Managers	Names of Managers
Alexandrium cyst distributions early warning maps to identify potential HAB hotspots in Puget Sound	Yes	Yes	No	10	Washington State Department of Health; Tribes; Shellfish Growers

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Number of stakeholders modifying practices: 3

WA State Department of Health
Tribes
Shellfish Growers

Sustainable Coastal Development

# of coastal communities: 0

PARTNERS

Partner Name: NOAA Northwest Fishery Sciences Center

Partner Name: University of Washington Tacoma

Partner Name: Washington State Department of Health

IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant research compares Alexandrium cyst appearance and viability, seeking a model for predicting harmful blooms**
Type: impact
Relevance, Response, Results:
Relevance: Dinoflagellate Alexandrium toxins can accumulate in shellfish and kill humans. Limited ability to predict Alexandrium blooms creates a significant threat to public health and shellfish aquaculture; information on Alexandrium cell distribution, viability, and germination could improve predictive model accuracy.
Response: Washington Sea Grant-supported researchers investigated Alexandrium's dormancy and germination cycles, seeking key parameters for a bloom risk assessment model. They used image-analysis software to categorize cyst photographs from 23 Puget Sound sites based on "fullness" (size, granular starch accumulation) and the presence of red "eye" spots. Cyst fullness was compared with successful germination rates to determine whether appearance can predict cyst viability. Researchers also measured cyst abundance, sediment composition, vegetative cells, and monthly water column properties to assess whether winter cyst maps represented abundances year-round and to test surface-sediment cysts for secondary dormancy behavior.
Results: Appearance was not a useful predictor: no relationship emerged between fullness and viability. More cysts germinated in spring and summer than in other seasons. However, the trend was not consistent, nor was it clear whether it was a temperature response or secondary dormancy. Cyst abundance in sediments varied inversely with blooms throughout the year, with abundance lower in warm months. Sediment characteristics also changed from lower sand and higher clay content in winter to higher total organic carbon in summer, consistent with enhanced productivity.

Recap:
Recap: Washington Sea Grant research tests the relationship between cyst appearance and viability in harmful Alexandrium and documents seasonal variation in cyst abundance and sediment composition in Central Puget Sound.
Comments:
Primary Focus Area: OCEH (HCE)
Secondary Focus Area: OCEH (SSSS), COCC (HRCC)
Associated Goals: Improve understanding and management of emerging and cumulative threats to ocean and coastal health. (SSSS, Industry)
Improve understanding and management of emerging and cumulative threats to ocean and coastal health. (HCE, Science)
Improve understanding of coastal hazards and environmental change and develop tools and approaches for observation, prediction, planning and adaptation (HRCC, Capacity).
Partners:
Northwest Fisheries Science Center (US DOC, NOAA, NMFS, NWFSC)
University of Washington, Tacoma, School of Interdisciplinary Arts and Sciences (UWT)
Washington State Department of Health
Related Partners: *none*

PUBLICATIONS

Title: **Alexandrium catenella cyst distribution and germination in Puget Sound, WA USA**
Type: Workshops, Proceedings, Symposia Including Highlights/Summaries of (please note: document number reflects the year the proceedin Publication Year: 2015
Uploaded File: *none*
URL: *none*
Abstract:
The Puget Sound Alexandrium Harmful Algal Bloom (PS-AHAB - http://www.tiny.cc/psahab) Program investigated cyst dynamics of the toxic dinoflagellate in the genus Alexandrium. This included mapping overwintering surface sediment cysts at 99 stations throughout Puget Sound in 2011, 2012 and 2013. The distribution patterns of cyst abundances were similar for all three years, but cyst concentrations generally decreased over time. The highest cyst concentrations were found north in Bellingham Bay, west of the Main Basin in Port Madison, Liberty Bay and Port Orchard and centrally in Quartermaster Harbor. Compared to a 2005 survey, the Bellingham Bay “seed bed” is new, whereas Quartermaster Harbor cyst concentrations have decreased by an order of magnitude. A monthly times series in Quartermaster Harbor from 2012-2013 found cyst abundances to vary by a factor of ~6 seasonally over the course of a year. In a related study, surface sediment cysts at thirty 2012 PS-AHAB stations were evaluated for their germination potential with results ranging from 16% to 66% viability. These results indicate that cyst abundances are relatively stable over a winter season, but winter cyst abundances and viability must be taken into account in order to determine the potential for toxic Alexandrium blooms the following season.

Citation:
Greengrove, C.L., Masura, J.E., Moore, S.K., Bill, B.D., Hay, K.C. Eldred, L.R., Banas, N.S., Salathé Jr., E.P., Mantua, N.J., J.A. Johnstone, Anderson, D.M., Trainer, V.L., Stein, J.E., Submitted. Alexandrium catenella cyst distribution and germination in Puget Sound, WA USA In: Harmful Algae 2014, Proceedings of the 16th International Conference on Harmful Algae. International Society for the Study of Harmful Algae.
Copyright Restrictions + Other Notes:
Journal Title: Proceedings of the 16th International Conference on Harmful Algae.

Title: **Factors regulating excystment of Alexandrium catenella in Puget Sound, WA USA**
Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2015
Uploaded File: Moore_etal_2015_alex_e....t.pdf, 695 kb
URL: *none*
Abstract:
Factors regulating excystment of a toxic dinoflagellate in the genus Alexandrium were investigated in
cysts from Puget Sound, Washington State, USA. Experiments were carried out in the laboratory using
cysts collected from benthic seedbeds to determine if excystment is controlled by internal or
environmental factors. The results suggest that the timing of germination is not tightly controlled by an
endogenous clock, though there is a suggestion of a cyclical pattern. This was explored using cysts that
had been stored under cold (4 8C), anoxic conditions in the dark and then incubated for 6 weeks at
constant favorable environmental conditions. Excystment occurred during all months of the year, with
variable excystment success ranging from 31–90%. When cysts were isolated directly from freshly
collected sediments every month and incubated at the in situ bottom water temperature, a seasonal
pattern in excystment was observed that was independent of temperature. This pattern may be
consistent with secondary dormancy, an externally modulated pattern that prevents excystment during
periods that are not favorable for sustained vegetative growth. However, observation over more annual
cycles is required and the duration of the mandatory dormancy period of these cysts must be determined
before the seasonality of germination can be fully characterized in Alexandrium from Puget Sound. Both
temperature and light were found to be important environmental factors regulating excystment, with

the highest rates of excystment observed for the warmest temperature treatment (20 8C) and in the light.
Citation:
Moore, S.K., Bill, B.D., Hay, L.R., Emenegger, J., Eldred, K.C., Greengrove, C.L., Masura, J.E., Anderson, D.M., 2015. Factors regulating excystment of Alexandrium catenella in Puget Sound, WA, USA. Harmful Algae 43: 103-110.
Copyright Restrictions + Other Notes:
Journal Title: Harmful Algae

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

Type: influenced Period: 2014-10-23: : 2014-11-02Amount: $5397
Purpose:
Travel to present results at conferences
Source: UWT Travel Funds

COMPLETION NARRATIVE

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Washington Sea Grant Final Report 2012-2015

Understanding dormancy requirements and germination of *Alexandrium* cysts and evaluating cyst mapping as a tool for early warning of harmful algal blooms

Cheryl Greengrove, Stephanie Moore, Julie Masura, and Brian Bill

The Project:

PROJECT SUMMARY

Objectives

Blooms of the harmful alga *Alexandrium* produce potent neurotoxins that accumulate in shellfish and cause gastrointestinal and neurological symptoms if contaminated shellfish are consumed by humans. The limited predictive capacity for *Alexandrium* blooms poses a significant problem for local managers and shellfish growers and threatens the vitality of the $108 million shellfish industry in Washington State. Shellfish closures due to *Alexandrium* blooms in Puget Sound have increased in frequency, duration, and geographic scope since the 1950s. Climate change may increase the bloom season for *Alexandrium* such that toxic events may begin up to 2 months earlier and persist for 1 month longer by the end of the 21st century. The short-term goals of this project were to determine the dormancy requirements and germination characteristics of the benthic cyst life stage of *Alexandrium* and evaluate the effectiveness of traditional cyst mapping as a tool for early warning of bloom events in Puget Sound. The long-term goal is to incorporate this critical information on *Alexandrium* life history characteristics into a model that will provide an unprecedented, powerful risk assessment of toxic blooms in Puget Sound now and in a future warmer climate. While this Sea Grant project stands alone with its own objectives and outcomes, it provides key information for the predictive models that are currently being developed by the NOAA ECOHAB PS-AHAB Program. The two projects are highly complementary and the Seagrant project benefits from synergistic activities, from sample collection to lab work, between the two programs. Note: We requested and received a no cost extension for 2014-2015 to complete this project and present the results.

This project is closely aligned with the WSG changing oceans and coastal communities critical program area and clearly addresses the associated goals identified in the WSG Strategic Plan 2010-2014. In particular, the capacity to manage Puget Sound's valuable shellfish industry under changing climatic conditions will be greatly improved. Shellfish growers will be able to use the information arising from this project to plan and make informed harvesting decisions that will increase their resilience to toxic bloom events and minimize economic losses. The improved understanding of *Alexandrium* bloom events will facilitate the development of management tools and mitigation approaches.

Methodology

The purpose of this project is to provide critical information on the dormancy requirements and germination characteristics of *Alexandrium* cysts that will be used to inform a model that will provide a powerful risk assessment of toxic bloom events in Puget Sound. This project also critically evaluated the premise of using cyst maps to provide early warning of bloom events. The objectives of this project are to (1) evaluate the premise of using winter *Alexandrium* cyst maps to predict summer bloom potential (i.e., spatial consistency), (2) determine the viability (i.e., ability to germinate) of cysts in Puget Sound, (3) determine the mandatory dormancy period of *Alexandrium* cysts, and (4) determine whether *Alexandrium* cysts exhibit secondary dormancy.

To evaluate the effectiveness of using winter cyst maps to predict summer bloom potential (Objective 1), sediment containing *Alexandrium* cysts was obtained in triplicate at two sampling locations in Quartermaster Harbor each month for one year. Cysts were enumerated using traditional staining techniques, sediment characteristics determined and abundances and sediment properties were compared through time. To determine the viability (i.e., ability to germinate) of cysts (Objective 2), sediment containing *Alexandrium* cysts were obtained from 29 sampling locations throughout Puget Sound in 2012. Cysts were isolated live (i.e. unstained) and incubated at conditions that are favorable for germination. The percentage of cysts that germinated from each site provided a measure of viability. Cysts were also photographed to see if there was any visual correlation between cyst fullness and germination. To determine the mandatory dormancy period of *Alexandrium* cysts (Objective 3), fresh seawater was collected during the peak of blooms in Quartermaster Harbor during each year of the project and *Alexandrium* cyst formation was induced. Newly formed cysts were collected for incubation, but unfortunately not enough cysts formed to be able to effectively run the mandatory germination experiment. The time from cyst formation until 50% of the cysts are observed to germinate would have determined the mandatory dormancy period. Finally, to determine whether *Alexandrium* cysts exhibit secondary dormancy (Objective 4), cysts were isolated directly from the natural environment each month for 12 months. Cysts were incubated at the same water temperature as the environment from which they were collected.

Rationale

Toxic blooms of *Alexandrium* challenge the management of shellfish resources in Puget Sound. The Washington State Department of Health dedicates significant resources to monitoring shellfish toxicity at more than 70 locations in the Sound at roughly 2 week intervals. In spite of these efforts, health authorities can be caught off guard by bloom events resulting in costly recalls of contaminated product from the market. This considerably reduces consumer confidence in seafood safety and can cause severe economic losses to not only the shellfish industry but the entire seafood industry. This project is important because it will greatly enhance an early warning system for toxic blooms of *Alexandrium* in Puget Sound. Specifically, it will provide critical information on life history characteristics of *Alexandrium* that will inform a predictive model. It is appropriate for WSG support because it will strengthen the ability of coastal communities to adopt economically and environmentally sound management of shellfish resources in Puget Sound in the face of environmental change. The expected users of the results produced by this project include managers at the Washington State Department of Health and shellfish growers (e.g., Taylor Shellfish Farms Inc.). For example, this information will allow managers to better allocate limited resources to monitoring programs and shellfish growers to respond quickly to developing blooms and make decisions related to shellfish harvesting both now and in a future warmer climate.

Please note that the species of *Alexandrium* thought to be responsible for the production of these toxins in Puget Sound has historically been identified as *Alexandrium catenella* (Whedon & Kofoid) Balech. This is synonymous with *Alexandrium tamarense* Group I, a provisional species name proposed by Lilly et al. (2007). However, the name *Alexandrium fundyense* has recently been proposed to replace all Group I strains of the *A. tamarense* species complex that includes *Alexandrium* (John et al., 2014a; 2014b). In light of the recent work by John et al. (2014a) and recognizing alternative recommendations by Wang et al. (2014), we will refer here only to the genus name *Alexandrium*.

Cyst Mapping:

To evaluate the effectiveness of winter cyst mapping as a tool to predict *Alexandrium* summer bloom potential, two stations in Quartermaster Harbor (QMH) were sampled monthly starting with the PS-AHAB cruise in February 2012 and ending with the PS-AHAB February 2013 cruise. Triplicate Craib cores were obtained at the center bay station 78 (13 m) and the inner harbor station 79 (7 m) (Figure 1). Cores were sub-sectioned in the field into 0-1cm and 1-3cm samples and then processed following standard cyst procedures (Anderson et al. 1996) and stained and counted using the methods of Yamaguchi et al. (1995). Sediment samples were also analyzed for total organic carbon (TOC) and grain size (PSA) and are presented in Table 1. The results of the monthly survey cyst counts are presented in Figure 3. Note that we were not able to collect samples in September due to a Captain's family emergency that precluded the use of the ship.

It is interesting to observe the cyst abundance decrease in the spring (April) along with the spring bloom (presumed germination) and then reestablish greater cyst abundance over the course of the summer, until a late fall bloom diminishes the surface sediment cyst abundance again during the fall bloom. While the cyst abundance pattern appears as might be expected, with cysts decreasing during bloom germination times, it does not necessarily correlate with observations in the water column. A bloom of *Alexandrium* was not observed in the water column of QMH until late June – early July and there was no apparent fall bloom, and though *Alexandrium* was occasionally found in the water column throughout the fall, it was never abundant. Over the course of mapping, there were visible changes in the sediment itself (Figure 2), consisting of more unconsolidated organics in the summer, which disappeared by the winter season. This observation is reflected in the measured sediment characteristics, grain size and TOC, in Table 1.

Figure 1. Seagrant project sampling sites in Quartermaster Harbor in central Puget Sound.

Figure 2. Comparison of Craib core samples from June and December in in Quartermaster Harbor.

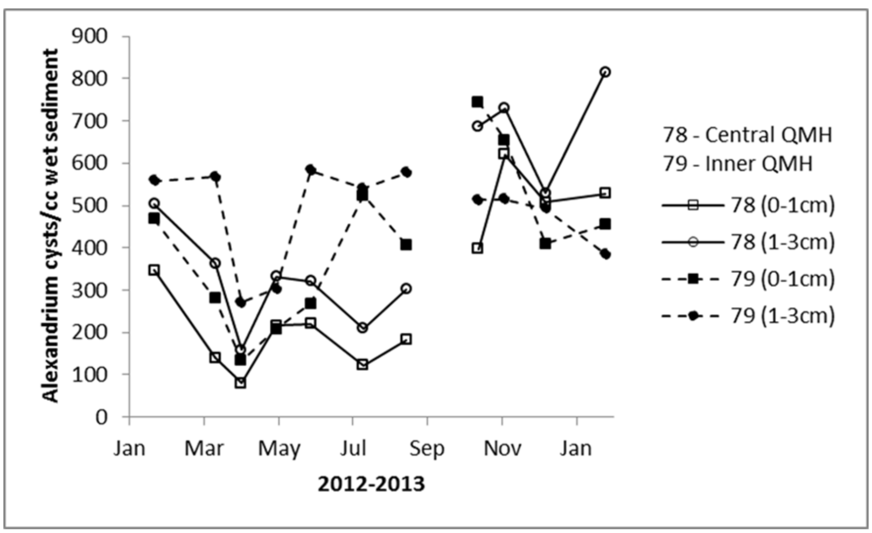

Figure 3. Monthly *Alexandrium* cyst abundances per cc of wet sediment from February 2012- January 2013 in Quartermaster Harbor at two locations, station 78 (solid line) in the central bay and station 79 (dashed line) in the inner bay. The squares are 0-1 cm and circles are 1-3 cm cyst abundances.

Date	SampleID (Station)	Sediment (0-1cm, 1-3cm)	Cysts/cc	% Clay	% Silt	% Sand	% Carbon
2/01/2012	78-1	0-1	520	17.8	72.6	9.6	9.874
2/01/2012	78-1	1-3	215	20.5	71.7	7.7	8.340
2/01/2012	78-2	0-1	305	27.3	67.9	4.8	9.247
2/01/2012	78-2	1-3	860	18.3	74.2	7.5	10.490
2/01/2012	78-3	0-1	265	24.6	69.1	6.2	10.275
2/01/2012	78-3	1-3	385	16.8	74.7	8.5	9.539
2/01/2012	79-1	0-1	500	13.3	78.3	8.4	12.540
2/01/2012	79-1	1-3	420	14.5	76.3	9.3	10.371
2/01/2012	79-2	0-1	485	13.4	79.3	7.3	11.807
2/01/2012	79-2	1-3	695	17.0	76.3	6.7	10.726
2/01/2012	79-3	0-1	440	14.8	77.6	7.5	11.140
2/01/2012	79-3	1-3	540	15.9	75.5	8.6	11.546
3/21/2012	78-1	0-1 cm	100	8.3	74.0	17.7	10.376
3/21/2012	78-1	1-3 cm	350	8.5	76.1	15.4	9.714
3/21/2012	78-2	0-1 cm	175	8.4	73.5	18.0	9.424
3/21/2012	78-2	1-3 cm	370	7.0	74.4	18.7	9.151
3/21/2012	78-3	0-1 cm	145	7.4	73.0	19.7	9.724
3/21/2012	78-3	1-3 cm	370	6.8	72.8	20.4	9.858
3/21/2012	79-1	0-1 cm	145	5.0	78.8	16.2	11.333
3/21/2012	79-1	1-3 cm	610	7.0	76.1	17.0	10.709
3/21/2012	79-2	0-1 cm	155	5.3	79.7	15.0	11.592
3/21/2012	79-2	1-3 cm	390	6.2	71.3	22.5	10.869
3/21/2012	79-3	0-1 cm	540	5.4	76.7	18.0	10.256
3/21/2012	79-3	1-3 cm	700	8.5	76.1	15.5	10.151
4/11/2012	78-1	0-1 cm	115	7.3	75.9	16.8	9.542
4/11/2012	78-1	1-3 cm	180	6.9	69.1	24.0	9.374
4/11/2012	78-2	0-1 cm	20	7.4	76.7	15.9	10.524
4/11/2012	78-2	1-3 cm	140	7.4	74.3	18.3	9.361
4/11/2012	78-3	0-1 cm	105	6.1	73.1	20.9	7.607
4/11/2012	78-3	1-3 cm	155	10.5	68.4	21.2	6.849
4/11/2012	79-1	0-1 cm	80	5.3	80.7	14.0	11.702
4/11/2012	79-1	1-3 cm	110	7.5	76.9	15.7	10.844
4/11/2012	79-2	0-1 cm	180	7.1	78.2	14.7	10.854
4/11/2012	79-2	1-3 cm	150	7.5	76.8	15.8	10.648
4/11/2012	79-3	0-1 cm	140	5.8	76.5	17.7	11.095
4/11/2012	79-3	1-3 cm	550	5.6	73.9	20.6	11.012
5/09/2012	78-1	0-1 cm	205	7.9	75.2	17.0	8.678
5/09/2012	78-1	1-3 cm	380	8.3	73.0	18.7	8.385
5/09/2012	78-2	0-1 cm	270	7.6	70.6	21.8	8.478
5/09/2012	78-2	1-3 cm	345	8.4	71.7	19.9	10.997
5/09/2012	78-3	0-1 cm	175	7.5	75.5	17.1	8.910
5/09/2012	78-3	1-3 cm	270	7.3	72.2	20.5	8.244
5/09/2012	79-1	0-1 cm	185	5.2	79.6	15.3	9.791
5/09/2012	79-1	1-3 cm	240	6.4	76.1	17.5	11.633
5/09/2012	79-2	0-1 cm	180	4.4	71.1	24.6	11.616

5/09/2012	79-2	1-3 cm	285	5.4	77.3	17.3	11.203
5/09/2012	79-3	0-1 cm	260	5.1	78.5	16.4	11.357
5/09/2012	79-3	1-3 cm	385	6.1	75.0	18.9	10.728
6/06/2012	78-1	0-1 cm	295	6.8	74.0	19.2	10.118
6/06/2012	78-1	1-3 cm	355	7.0	74.0	19.0	9.223
6/06/2012	78-2	0-1 cm	140	5.2	75.1	19.7	9.732
6/06/2012	78-2	1-3 cm	340	8.8	74.5	16.7	8.763
6/06/2012	78-3	0-1 cm	225	7.1	73.2	19.7	9.326
6/06/2012	78-3	1-3 cm	270	9.7	72.7	17.7	8.774
6/06/2012	79-1	0-1 cm	375	5.6	79.0	15.5	11.616
6/06/2012	79-1	1-3 cm	275	6.7	76.3	17.1	11.583
6/06/2012	79-2	0-1 cm	200	4.7	78.5	16.8	12.166
6/06/2012	79-2	1-3 cm	640	6.6	74.7	18.7	11.036
6/06/2012	79-3	0-1 cm	230	5.7	79.6	14.8	11.509
6/06/2012	79-3	1-3 cm	830	6.4	75.5	18.1	10.733
6/06/2012	79 test	Floc Layer		6.4	77.3	16.3	11.192
6/06/2012	79 test	1-3 cm		5.5	74.5	20.0	10.873
7/18/2012	78-1	0-1 cm	55	8.0	76.0	16.1	13.961
7/18/2012	78-1	1-3 cm	155	6.8	74.0	19.2	13.320
7/18/2012	78-2	0-1 cm	130	7.4	72.7	19.9	19.000
7/18/2012	78-2	1-3 cm	445	6.5	74.9	18.6	13.204
7/18/2012	78-3	0-1 cm	180	9.4	73.9	16.8	NA
7/18/2012	78-3	1-3 cm	30	7.5	81.2	11.3	20.149
7/18/2012	79-1	0-1 cm	735	6.4	75.4	18.2	14.879
7/18/2012	79-1	1-3 cm	400	6.6	77.8	15.6	13.189
7/18/2012	79-2	0-1 cm	330	6.8	75.7	17.5	14.180
7/18/2012	79-2	1-3 cm	600	6.0	77.9	16.2	13.875
7/18/2012	79-3	0-1 cm	505	6.5	76.1	17.4	13.824
7/18/2012	79-3	1-3 cm	620	6.1	76.1	17.8	13.142
8/22/2012	78-1	0-1 cm	265	9.0	77.2	13.8	15.582
8/22/2012	78-1	1-3 cm	175	8.2	79.4	12.4	14.601
8/22/2012	78-2	0-1 cm	195	8.2	73.4	18.5	13.130
8/22/2012	78-2	1-3 cm	495	7.6	79.5	12.9	11.132
8/22/2012	78-3	0-1 cm	85	9.7	72.7	17.7	16.519
8/22/2012	78-3	1-3 cm	235	9.1	78.3	12.6	13.605
8/22/2012	79-1	0-1 cm	335	5.6	81.5	12.9	16.667
8/22/2012	79-1	1-3 cm	595	5.9	76.2	17.9	13.581
8/22/2012	79-2	0-1 cm	540	7.1	78.5	14.5	15.411
8/22/2012	79-2	1-3 cm	660	6.4	77.5	16.1	13.392
8/22/2012	79-3	0-1 cm	345	7.2	79.2	13.6	14.000
8/22/2012	79-3	1-3 cm	475	6.8	78.0	15.2	13.252
10/19/2012	78-1	0-1 cm	220	7.3	70.5	22.2	9.937
10/19/2012	78-1	1-3 cm	455	8.1	66.7	25.2	9.906
10/19/2012	78-2	0-1 cm	525	10.5	68.5	21.1	10.147
10/19/2012	78-2	1-3 cm	1005	7.1	66.6	26.4	9.816
10/19/2012	78-3	0-1 cm	445	7.2	66.3	26.6	10.764

10/19/2012	78-3	1-3 cm	600	7.0	65.0	28.0	9.902
10/19/2012	79-1	0-1 cm	710	5.9	72.1	22.0	12.820
10/19/2012	79-1	1-3 cm	580	6.2	69.9	23.9	11.593
10/19/2012	79-2	0-1 cm	965	5.5	68.4	26.1	12.519
10/19/2012	79-2	1-3 cm	605	6.1	70.5	23.4	12.010
10/19/2012	79-3	0-1 cm	560	5.1	66.8	28.2	12.632
10/19/2012	79-3	1-3 cm	355	5.6	69.3	25.1	12.654
11/09/2012	78-1	0-1 cm	220	8.5	65.6	25.9	10.428
11/09/2012	78-1	1-3 cm	725	9.8	68.7	21.5	9.636
11/09/2012	78-2	0-1 cm	510	8.2	69.9	21.9	11.580
11/09/2012	78-2	1-3 cm	330	8.2	69.5	22.3	12.035
11/09/2012	78-3	0-1 cm	1130	8.2	66.6	25.3	10.248
11/09/2012	78-3	1-3 cm	1135	7.2	69.3	23.5	9.785
11/09/2012	79-1	0-1 cm	655	7.8	76.9	15.3	11.680
11/09/2012	79-1	1-3 cm	365	7.9	74.2	17.9	11.589
11/09/2012	79-2	0-1 cm	535	6.3	73.4	20.3	12.308
11/09/2012	79-2	1-3 cm	550	8.5	76.7	14.9	12.018
11/09/2012	79-3	0-1 cm	775	6.4	73.5	20.1	12.211
11/09/2012	79-3	1-3 cm	630	7.9	75.2	16.9	11.512
12/12/2012	78-1	0-1 cm	445	10.6	72.9	16.5	8.979
12/12/2012	78-1	1-3 cm	420	9.2	72.7	18.2	8.843
12/12/2012	78-2	0-1 cm	490	6.0	68.6	25.4	9.382
12/12/2012	78-2	1-3 cm	465	8.3	74.0	17.7	9.080
12/12/2012	78-3	0-1 cm	585	8.0	73.0	19.1	8.843
12/12/2012	78-3	1-3 cm	705	10.3	70.1	19.6	8.832
12/12/2012	79-1	0-1 cm	330	6.5	77.7	15.8	11.716
12/12/2012	79-1	1-3 cm	425	5.7	76.0	18.3	11.711
12/12/2012	79-2	0-1 cm	460	6.3	76.3	17.4	11.557
12/12/2012	79-2	1-3 cm	390	8.5	75.9	15.6	11.139
12/12/2012	79-3	0-1 cm	440	6.3	77.5	16.3	11.256
12/12/2012	79-3	1-3 cm	660	7.0	74.6	18.5	10.629
1/29/2013	78-1	0-1	695	17.8	76.3	5.8	10.806
1/29/2013	78-1	1-3	545	19.1	72.3	8.6	10.087
1/29/2013	78-2	0-1	345	16.2	75.0	8.9	10.748
1/29/2013	78-2	1-3	820	19.2	73.1	7.8	10.156
1/29/2013	78-3	0-1	755	19.7	72.1	8.2	9.846
1/29/2013	78-3	1-3	870	20.5	70.7	8.8	9.194
1/29/2013	79	0-1	455	13.1	76.9	9.9	12.732
1/29/2013	79	1-3	385	14.3	77.0	8.7	11.934

Table 1. All *Alexandrium* cyst enumeration and sediment characteristic data including grain size and total organic carbon at stations 78 and 79 in Quartermaster Harbor from February 2012 – January 2013.

Mandatory Dormancy:

Alexandrium cysts have a mandatory dormancy period after cyst formation (Anderson 1980; Dale 1977). This time period is typically thought of as time for the cysts to "mature". Germination cannot be induced during the dormancy period, even if cysts are presented with appropriate environmental conditions, and metabolic activity is low (Binder and Anderson 1990). The length of the mandatory dormancy period varies among *Alexandrium* species and has been documented to last from <15 to 180 days (Anderson 1980). The duration of the mandatory dormancy period may also be affected by temperature (Rathaille and Raine 2011). The mandatory dormancy period of *Alexandrium* in Puget Sound is unknown, but it is likely to be an important parameter that influences the timing of toxic blooms.

Evaluating the mandatory dormancy of *Alexandrium* cysts is the only part of our original proposed work that we were not able to complete. In 2012, we were able to test our protocol with a spring bloom and all appeared to work well, but then a big fall bloom did not materialize in Quartermaster Harbor that year. In 2013, we did a trial run again in the spring and then captured a large fall bloom in August, but still did not get enough cysts to encyst in order to run the experiment. We went over our protocol with Don Anderson (WHOI) who has done this is the past and he suggested using F/100 solution instead of F/20 and incubating in the light rather than the dark. We collected a fall bloom in September again in Quartermaster Harbor in 2014, but once again did not get enough cysts to encyst in order to run the experiment. We plan to continue on our own to try and capture a bloom and determine the mandatory dormancy for *Alexandrium*.

Secondary dormancy:

Knowledge of the factors that govern the dormancy of *Alexandrium* cysts will likely be a key component of any HAB forecast. A lesser known mechanism that could produce a seasonal cycle in cyst germination is referred to as secondary dormancy. In higher plants, secondary dormancy prevents germination during periods that are actually favorable for germination but not for growth and survival or the production of offspring (Vleeshouwers et al. 1995). This mechanism results in seeds alternately germinating or remaining unresponsive in the quiescent state when exposed to the same environmental conditions. Secondary dormancy (or some other as yet undescribed process that produces a similar result) was recently reported in two species of *Alexandrium* from Cork Harbor, Ireland (Rathaille and Raine 2011). We conducted a 12-month experiment to determine if *Alexandrium* cysts in Puget Sound displayed evidence of secondary dormancy.

Each month beginning in March 2012, surface sediment was collected from station 78 in Quartermaster Harbor using the Craib corer. The sediment was stored at 4°C until it could be transported to the laboratory (less than 8 hours), at which point it was transferred to an incubator set at the same temperature that was measured at the bottom of Quartermaster Harbor that same day. The next morning, 1 cm^3 of sediment was diluted to 50 ml with filtered seawater, sonicated and sieved with the 20-90 µm 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 50 cysts of *Alexandrium* encountered while scanning through the Sedgwick-Rafter slide were isolated from the 20-90 µm size fraction of sediment. 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 µL nutrient enriched natural seawater growth media. Well plates were incubated at the Quartermaster Harbor bottom water temperature on the day of sample collection with a 14:10 light:dark cycle. Light levels were 70-90 $\mu EM^{-2}s^{-1}$ 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.

Water temperature at the bottom temperature of Quartermaster Harbor varied from 7-12.5°C and the percentage of cysts that germinated each month varied from 0-52% (Figure 4). In general, there appears to be a tendency for higher percentages of cysts germinating in spring and summer compared to fall and winter; however, no consistent trend was evident from the results and it is not clear if this is a temperature response or evidence of secondary dormancy. Unfortunately, we were not able to collect samples in September. We recommend repeating this experiment to determine if Puget Sound cysts display secondary dormancy – an endogenous mechanism that could produce a seasonal cycle in cyst germination.

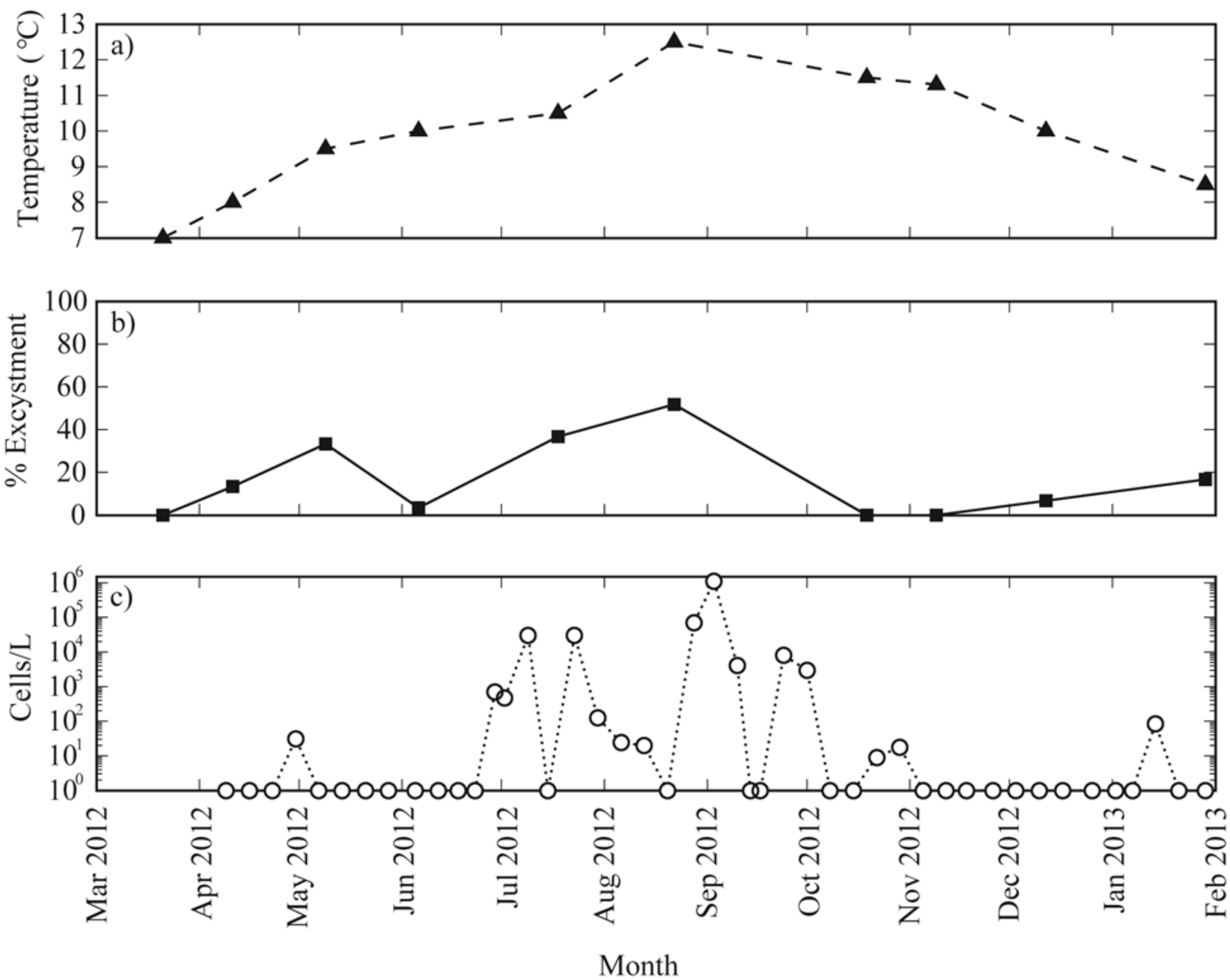

Figure 4. Monthly variation in (a) water temperature at the seafloor of Quartermaster Harbor, (b) *Alexandrium* excystment using freshly collected sediment from Quartermaster Harbor, and (c) the abundance of *Alexandrium* spp. vegetative cells in the surface water at dockside location in Quartermaster Harbor over the period March 2012 to February 2013. Vegetative cell abundance data are provided by the SoundToxins program. Isolated cysts (n = 50) were germinated in 96-well plates at in situ water temperatures measured at the seafloor at the time of sediment collection. The x-axis location of markers for excystment represents the day that the sediment was collected, and the value for excystment is the cumulative result from the three checks over the 28 days that plates were observed.

Viability:
Newly formed cysts of *Alexandrium* are densely granulated and have a brownish colored cytoplasm. As the cysts age they become less densely granulated, the cytoplasm shrinks away from the outer cell wall, and their ability to germinate decreases (Genovesi et al. 2009). The primulin staining technique of Yamaguchi et al. (1995) that is used to determine abundances of cysts mapped over large areas does not discriminate between viable and non-viable cysts. Furthermore, the staining procedure shrinks the cytoplasm away from the cell wall, thereby obscuring the ability to determine viability. This means that cyst seed beds identified using traditional cyst abundance mapping techniques (i.e., staining) may not accurately reflect their true potential to initiate blooms.

In order to determine if the standard mapping techniques used to determine cyst abundances in Puget Sound by the PS-AHAB project (www.tiny.cc/psahab) were an accurate indication of bloom potential, additional surface (0-1 cm) sediment samples were collected from 29 stations throughout Puget Sound during the PS-AHAB 2012 winter survey using the Craib corer. Stations were chosen where there was greater than 25 cysts/cc sediment from the PS-AHAB 2011 survey. Samples were stored at 4°C, in the dark, in nitrogen gas bags until cyst isolation could be performed (10-52 weeks after collection). Sediment was processed and cysts were isolated according to the procedure described above for the secondary dormancy experiment. For some stations with low cyst abundances, it was not possible to isolate the full 50 cysts from the 20-90 µm size fraction within the necessary time frame and percentages of germinated cysts were calculated using fewer cysts (i.e., 25-40). 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.

Of the 29 stations where additional surface sediment was collected, viability experiments were successfully run on 23 of them. Stations 7, 13, 14, 16, 27,and 31 had too few cysts to feasibly isolate 50 cysts within the timeframe necessary to conduct the experiment. The percentage of viable cysts in the remaining 23 stations ranged from 16 to 66% (Table 2). A cyst mapping and viability study in the St. Lawrence Estuary in eastern Canada using a SYTOX Green nucleic acid stain and flow cytometry method to determine cyst viability found average viability to be low (28%) with a range of 0-80% (Gracia et al. 2013). It is hard to do a direct comparison with this mapping study given the difference in methods used. For reference, studies of *A. fundyense* cyst germination from surface sediment at single locations in the Gulf of Maine report 90-100% viability (Anderson and Keafer 1987; Matrai et al. 2005). If our results for *Alexandrium* cyst viability in 2012 are typical of other years, then large-scale cyst mapping using traditional staining techniques alone may not be useful in Puget Sound for modeling and prediction of toxic blooms.

Table 2. *Alexandrium* cyst viability from surface sediments in Puget Sound.

Site #	**Site name**	**Depth (m)**	**Cysts/cc**	**% Viability**	**Notes**
1	Semiahmoo Bay	16	77	37	only 30 isolated (time constraints led to % out of 30 cysts due to low concentration/inability to locate cysts)
2	Georgia Strait - NE	63	33	16	Van Veen
3	Georgia Strait - NW	223	13	24	only 25 isolated (time constraints) rocks/sand CRAIB
4	Birch Bay	9	72	34	
5	Georgia Strait - SE	50	63	24	Van Veen
6	Georgia Stait - SW	137	12	24	only 25 isolated (time constraints) sand VV
8 (0-1 cm)	Bellingham Bay - North	9	1070	48	
8 (0-1 cm)	Bellingham Bay - North	9	1070	54	
8 (1-3 cm)	Bellingham Bay - North	9	1070	44	
9	Bellingham Bay - East	24	117	52	
10	Bellingham Bay - South	18	67	44	
11	Bellingham Bay - West	55	55	48	
12	Padilla Bay	26	147	30	
15	Lopez Sound - Outer	22	52	20	
17	Cattle Point	26	160	32	Van Veen
22	Seqium Bay - Center	27	35	34	
23	Sequim Bay - Inner	13	30	38	only 40 isolated (time constraints)
58	Port Madison	36	320	42	
59	Liberty Bay	4	545	36	
60	Port Orchard - North	21	130	46	
61	Port Orchard - South	25	175	54	
62	Sinclair Inlet - Outer	17	37	33	only 40 isolated (time constraints)
64	Dyes Inlet - Inner	12	32	31	only 35 isolated (time constraints)
78 (0-1 cm)	Quartermaster Harbor - Center	13	708	16	
78 (0-1 cm)	Quartermaster Harbor - Center	13	708	38	
78 (1-3 cm)	Quartermaster Harbor - Center	13	708	66	
79	Quartermaster Harbor - Inner	7	500	42	

Each cyst that was isolated for the viability experiments was also photographed. The fullness of cysts was determined using image analysis techniques in the program ImageJ. The protocol for this technique was established using photographs of cysts from a prior experiment we conducted. In short, after setting the scale in ImageJ, 3 metrics are calculated for each photographed cyst; (1) the total size of the cyst, (2) the granular starch accumulation body, and (3) the red "eye spot" (Figure 5). The ratio of the total size of the cyst to the size of the starch accumulation body was examined to determine if there was a cut-off point that might determine whether a cyst was viable or not.

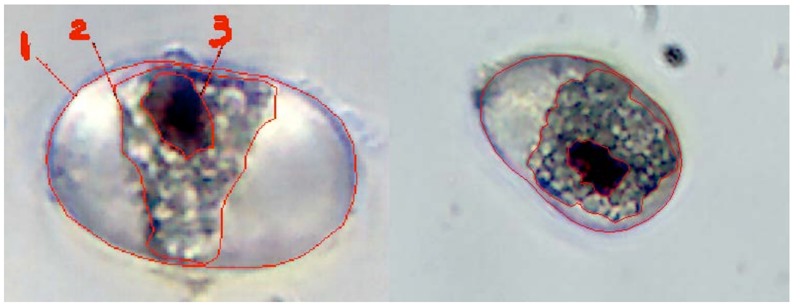

Figure 5. Photographs of *Alexandrium* cysts showing the metrics calculated in ImageJ to determine if any relationship exists between cyst fullness and viability.

No relationship between cyst viability and cyst appearance using image analysis software has been detected (Figure 6).

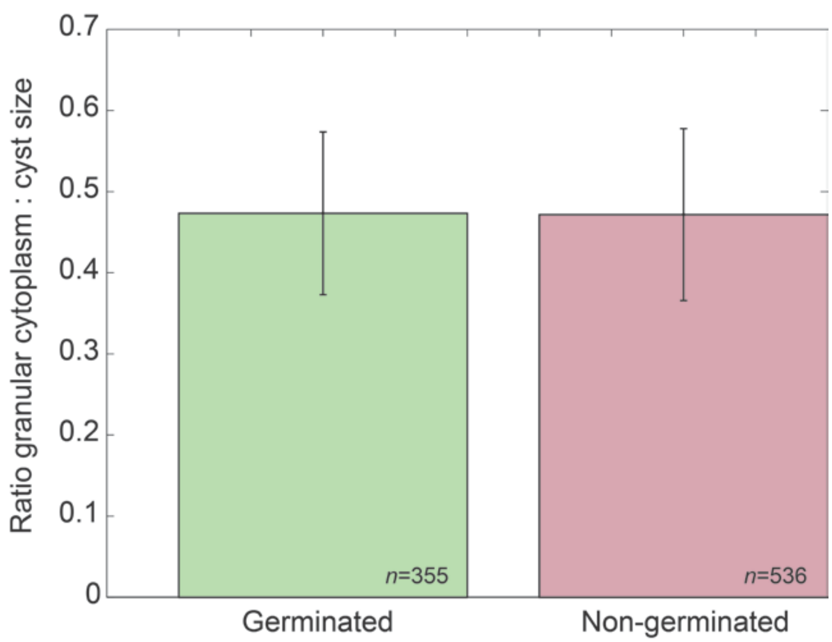

Figure 6. Ratio of the total cyst size to the size of the granulated starch accumulation body for viable and non-viable cysts.

Education and Outreach:

Student training: Fourteen undergraduate students were trained as part of this project (listed below). Students participated in cyst surveys conducted from the R/V Barnes and learned to collect and process samples onboard, learned lab techniques for identifying and isolating live cysts as well as enumerating stained cysts, methods for examining cyst germination characteristics such as mandatory dormancy, processing and analyzing sediment samples for TOC and grain size. Some students presented their related undergraduate research projects at the regional Salish Sea Ecosystem Conference, PERS and the annual UW Undergraduate Research Symposiums.

Alex Gipe	Nick Schlafer	Caitlin Olive
Jessica Maves	Dana Olsen	Kiara Eldred
Daryl Kline	Elisa Rauschel	Levi Hay
Rachel Leftwich	Jennifer Emenegger	Nannette Huber
Troy Albom	Alison Dunn-Johnson	

R/V Barnes Education and Outreach Days: October 17-18, 2013, Cheryl Greengrove and Julie Masura, hosted three classes of about 70 college students on the R/V Barnes to sample water properties, phytoplankton, sediments and plastics in Puget Sound's Commencement Bay. Students learned about local oceanography, estuaries and HABs. On October 19th, Cheryl and Julie hosted an open house aboard the R/V Barnes for the community at Dockton Dock in Quartermaster Harbor on Vashon-Maury Island in Central Puget Sound. With the help of students, Elisa Rauschl, Nick Schlafer and Alex Gipe, and the crew of the R/V Barnes they showcased Sea Grant and PS-AHAB HABs work in Quartermaster Harbor and Puget Sound more broadly, as well as Greengrove/Masura's EPA Quartermaster Harbor Nitrogen Management Study done in collaboration with King County and Washington State Department of Ecology. Over 20 local community members participated in this event. Another shipboard education and outreach event was run May 9-10, 2014, on the R/V Barnes in Commencement Bay for multiple college classes and some middle and high school students. Students learned about local oceanography, estuaries and HABs. About 40 students participated in this event.

Seattle Aquarium Discover Science Weekends: Cheryl Greengrove and undergraduate students Elisa Rauschl and Nick Schlafer, participated in “Discover Science Weekend” at the Seattle Aquarium. The Aquarium had 8500+ guests come through its doors over the three day event. On November 11th, 2013, Sea Grant researchers and students hosted a table with microscopes and live phytoplankton samples from Puget Sound for children and adults. Children learned how to use a microscope, what phytoplankton are and how to identify them. Through videos and picture ID guides, they learned about Harmful Algae in Puget Sound and about local, state and regional marine toxin monitoring programs, such as “SoundToxins”. Cheryl was the featured “Meet a Local Scientist” for the day and gave a brief talk on HABs in Puget Sound. Julie Masura and students participated in this event again during 8-9 November 2014.



Media Coverage:
Glenn Farley from King 5 news and Ashley Ahearn from KUOW joined the PS-AHAB and Sea Grant science crew aboard the R/V Barnes during the 2012 *Alexandrium* cyst survey . The stories can be accessed using the links below. The KUOW story was subsequently featured on NPR’s “All Things Considered”, reaching a national audience.
http://www.king5.com/news/Early-warning-system-against-shell-fish-toxins-138447799.html
http://earthfix.opb.org/water/article/cruising-for-toxic-algae/

Public Presentations:
2013 Eastside Science Café March 11, 2013. Cheryl Greengrove gave a public presentation titled “Harmful Algae in Puget Sound” as part of the Puget Sound region Science Café program sponsored by the Pacific Science Center and KCTS 9. http://kcts9.org/education/science-cafe/harmful-algae

Conference Presentations:
2012 19th Conference for Shellfish Growers. Alderbrook Resort, WA, 5-6 March 2012. Stephanie Moore gave a presentation titled "Modeling favorable habitat areas for *Alexandrium catenella* in Puget Sound and evaluating the effects of climate change".

2012 GEOHAB Open Science Meeting on HABs in Fjords and Coastal Embayments: Progress in Interpreting Life History and Growth Dynamics of Harmful Algal Blooms in Fjords and Coastal Environments. University of Victoria, Victoria BC, 29-30 May 2012. Cheryl Greengrove gave a presentation titled "*Alexandrium catenella* in Puget Sound, WA USA: Cyst Distribution and Germination and Cell Growth Rates and Toxicity".

2012 15th International Conference on Harmful Algae. Changwon, Korea, 29 October -5 November 2012. (I) Stephanie Moore gave a presentation titled "Modeling favorable habitat for Alexandrium blooms in Puget Sound, WA, USA: present-day and future climate pathways and transport patterns", and (II) Cheryl Greengrove and Julie Masura presented a poster titled "*Alexandrium* cyst distribution and germination in Puget Sound, WA USA".

2013 Pacific Estuarine Research Society (PERS) Meeting, Vancouver BC Canada, 4-7 April 2013. (I) Cheryl Greengrove and Julie Masura presented a poster titled "*Alexandrium* cyst distribution and germination in Puget Sound, WA USA", (II) Nick Schlafer (undergraduate student) presented a poster titled "Water Properties in Quartermaster Harbor Puget Sound"

2013 7th Symposium on Harmful Algae in the U.S. Sarasota, FL, 27-31 October 2013. Stephanie Moore gave a presentation titled "*Alexandrium* bloom ecology in Puget Sound: cyst dynamics, growth, transport, and climate pathways".

2014 American Society of Limnology and Oceanography Ocean Sciences Meeting, Hawaii, 23-28 February 2014. Cheryl Greengrove and Julie Masura gave a poster titled "Alexandrium Bloom Ecology in Puget Sound: Cyst Dynamics, Growth, Transport, and Climate Pathways."

2014 4th Annual Northwest Fisheries Science Center Science Symposium. Seattle, WA, March 25-26, 2014. Stephanie Moore gave a presentation titled "Harmful algal blooms of *Alexandrium* in Puget Sound: cyst dynamics, growth, transport, and climate pathways".

2014 Salish Sea Ecosystem Conference. Seattle, WA, April 30-May 2, 2014. (I) Stephanie Moore gave a presentation titled "*Alexandrium* ecology in Puget Sound: bloom transport and climate pathways", (II) Cheryl Greengrove gave a presentation titled "*Alexandrium* cyst distribution and germination in Puget Sound", and (III) Nicholas Schlafer (undergraduate student) presented a poster titled "Quartermaster Harbor Water Properties & *Alexandrium catenella*". http://www.wwu.edu/salishseaconference/

2014 16th International Conference on Harmful Algae. Wellington, New Zealand 27-31 October 2014. Cheryl Greengrove presented a talk titled "*Alexandrium* cyst distribution and germination in Puget Sound, WA USA".

2015 22nd Conference for Shellfish Growers. Alderbrook Resort, WA, 2-3 March 2015. Cheryl Greengrove gave a presentation "Emergency Response Mapping of *Alexandrium* cysts in the surface sediments of Quilcene and Dabob Bays".

2015 Pacific Estuarine Research Society (PERS) Meeting, Seattle WA, 19-21 March 2015. Cheryl Greengrove gave a presentation on "Emergency Response Mapping of *Alexandrium* cysts in the surface sediments of Quilcene and Dabob Bays".

Papers and Publications:

Greengrove, C.L., Masura, J.E., Moore, S.K., Bill, B.D., Hay, K.C. Eldred, L.R., Banas, N.S., Salathé Jr., E.P., Mantua, N.J., J.A. Johnstone, Anderson, D.M., Trainer, V.L., Stein, J.E., Submitted. *Alexandrium catenella* cyst distribution and germination in Puget Sound, WA USA In: Harmful Algae 2014, Proceedings of the 16th International Conference on Harmful Algae. International Society for the Study of Harmful Algae.

Moore, S.K., Bill, B.D., Hay, L.R., Emenegger, J., Eldred, K.C., Greengrove, C.L., Masura, J.E., Anderson, D.M., 2015. Factors regulating excystment of *Alexandrium catenella* in Puget Sound, WA, USA. Harmful Algae 43:103-110.

Greengrove, C.L., Masura, J.E., Moore, S.K., Bill, B.D., Hay, L.R., Banas, N.S., Salathé Jr., E.P., Mantua, N.J., Anderson, D.M., Trainer, V.L., Stein, J.E., 2014. *Alexandrium catenella* cyst distribution and germination in Puget Sound, WA USA In: Kim, H.G., Reguera, B., Hallegraeff, G.M., Lee, C.K., Han, M.S., Choi., J.K. (Eds.), Harmful Algae 2012, Proceedings of the 15th International Conference on Harmful Algae. International Society for the Study of Harmful Algae.

Workshops, Partnerships, Collaborations and Related Projects:

Sea Grant workshops – Cheryl Greengrove participated in Sea Grant National Site Visit Workshops in March 2012 and May 2015.

PS-AHAB Workshop – All principal investigators participated in final PS-AHAB workshop on 26 June 2014 in Seattle.

Annual Review of Puget Sound Marine Waters - Cheryl Greengrove and Stephanie Moore are members of the Marine Waters Workgroup (commissioned by the Puget Sound Partnership's Puget Sound Ecosystem Monitoring Program). This group assembles an Annual Review of Puget Sound Marine Waters which includes the final copies of the PS-AHAB surface sediment cyst map. The goal of the report is to connect the drivers of ecological variability in Puget Sound marine waters and to disseminate this information more broadly to interested parties and the general public. The annual report is distributed via websites, email, and print to over 500 recipients. The annual cyst maps have been featured in two subsequent reports.
http://www.psp.wa.gov/downloads/psemp/PSmarinewaters_2011_overview.pdf
http://www.psp.wa.gov/downloads/psemp/PSmarinewaters_2012_overview.pdf
http://www.psp.wa.gov/downloads/psemp/PSmarinewaters_2013_overview.pdf

Elizabeth D. Tobin – We trained Liz Tobin PhD and recent UW graduate on how to use the Craib Corer so she could do some *Alexandrium* surface sediment cyst mapping in Alaska as part of her NSF SEES Postdoctoral Fellow project with the School of Fisheries & Ocean Sciences at University of Alaska Fairbanks. We shipped the core to Alaska this spring and Liz will be sampling over the next two years. This is the first sampling of its kind in Alaska.

Washington State Department of Health has provided us with their historical shellfish PST data set and student Nannette Huber has mapped all occurrences of High PSTs on a series of GIS maps and assembled them into a time series video for Puget Sound.

Emergency Response Mapping of *Alexandrium* cysts in the surface sediments of Quilcene and Dabob Bays – collaboration with PS-AHAB and Shellfish Growers - In September and October 2014, there was an unprecedented bloom of *Alexandrium* in Dabob and Quilcene Bays in Hood Canal. The area where the bloom took place has historically been biotoxin free. At the peak of the event, shellfish were extremely toxic with values of up to 12,688 ug STX equiv./100 g shellfish tissue detected in blue mussels. Sea Grant/PS-AHAB researchers as well as the Washington State Department of Health and local shellfish growers were extremely concerned that the large bloom may have resulted in a new seed bed forming that could increase bloom risk in the area during the 2015 season and beyond. Even though no cyst mapping was planned for either Sea Grant or PS-AHAB beyond 2013, ECOHAB Program Managers permitted us to use leftover funds from unused weather days on this project to conduct focused cyst mapping in Hood Canal.

Mapping was conducted on the University of Washington's R/V Barnes from January 17-20, 2015. Ian Jefferds from Penn Cove Shellfish contributed funds to purchase lab consumables needed to do the cyst mapping. UWT undergraduate students, initially trained through this Sea Grant project, participated on the cruise and processed the samples. This project will serve as one undergraduate's capstone work. A videographer from NOAA Fisheries Ocean Media Center, Paul Hillman, joined the cruise to document the effort and obtained great underwater footage of the Craib corer in action (available on the PS-AHAB website: www.tiny.cc/psahab). Our previous cyst mapping efforts found zero or very low concentrations of cysts in the area; the highest concentration observed was 10 cysts per cc wet sediment in Quilcene Bay in 2013. In January 2015, we found an order of magnitude greater concentration of cysts; up to 120 and 180 cysts per cc wet sediment in Quilcene Bay and Dabob Bay, respectively. These results warrant increased vigilance for monitoring cells and toxins during the 2015 season and beyond in this area.

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