Update Report

Period 2/1/2013 - 1/31/2014

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

Hay, Levi, levi.hay@noaa.gov, UWS, no department, status cont, no field of study, advisor Stephanie Moore, degree type BS, degree date 2013-06-01, degree completed this period Yes Student Project Title Seagrant viability study Involvement with Sea Grant This Period Levi was paid work study in charge of laboratory work for Seagrant viability study and secondary dormancy experiment, also volunteered on 2013 PS-AHAB cruise where viability samples were collected Post-Graduation Plans work
Kline, Daryl, djkline@u.washington.edu, UWT, Environmental Science, status new, field of study Environmental Science, advisor Jumile Masura/Cheryl Greengrove, degree type BS, degree date 2014-06-01, degree completed this period No Student Project Title none Involvement with Sea Grant This Period ran TOC and grain size analyses Post-Graduation Plans work
Leftwich, Rachel, rachel.leftwich@noaa.gov, Chatham College, no department, status cont, no field of study, advisor Stephanie Moore, degree type BS, degree date 2014-06-01, degree completed this period No Student Project Title none Involvement with Sea Grant This Period MPN excystment experiments and sampling cruise Post-Graduation Plans none
Rauschl, Elisa, rauschl3@uw.edu, UWT, Environmental Science, status cont, field of study Environmental Science, advisor Cheryl Greengrove, degree type BS, degree date 2012-06-01, degree completed this period No Student Project Title Mandatory Dormancy Experiment Design Involvement with Sea Grant This Period EJ particpated on the monthly sediment sampling crusies and also was in charge of setting up the mandatory dormancy experiment Post-Graduation Plans grad school or work

Schlafer, Nick, schlafer@uw.edu, UWT, Environmental Science, status cont, field of study Environmental Science, advisor Cheryl Greengrove, degree type BS, degree date 2013-06-01, degree completed this period Yes
Student Project Title Water properties in Quartermaster Harbor

Involvement with Sea Grant This Period Nick has participated on all PS-AHAB sediment collection cruises, including on monthy Seagrant sediment coring cruises. Helped process sedments for counting.

Post-Graduation Plans grad school

CONFERENCES / PRESENTATIONS

Presentation and plankton table at Science in Puget Sound Day at Seattle Aquarium., public/profession presentation, 200 attendees, 2013-11-11
R/V Barnes open house at Dockton Dock, Quatermaster Harbor., public/profession presentation, 20 attendees, 2013-10-19
Introduction to oceanography on R/V Barnes for UW oceanography and freshman science classes., public/profession presentation, 70 attendees, 2013-10-17
7th U.S. HABs Meeting - Stephanie Moore gave talk
"ALEXANDRIUM BLOOM ECOLOGY IN PUGET SOUND CYST DYNAMICS, GROWTH, TRANSPORT, AND CLIMATE PATHWAYS", public/profession presentation, 1000 attendees, 2013-10-27
Pacific Estuarine Research Symposium Cheryl Greengrove gave poster
(& brought two students as well)
"Alexandrium cyst distribution and cyst viability in Puget Sound, WA USA"

, public/profession presentation, 200 attendees, 2013-04-05
Science Cafe in Kirkland sponsored by the Pacific Science
"Harmful Algae in Puget Sound" by Cheryl Greengrove, public/profession presentation, 50 attendees, 2013-03-11

ADDITIONAL METRICS

K-12 Students Reached	0	Acres of degraded ecosystems restored as a result of Sea Grant activities	0
Curricula Developed Methods developed, data collected and results will be used to inform Cheryl Greengrove & Julie Masura's teaching of	2	Resource Managers who use Ecosystem-Based Approaches to Management	0

courses such as Oceanography or our Estuarine Field courses at UWT			
Volunteer Hours A large number of students donated a great deal of time on the project.	400	HACCP - Number of people with new certifications	0
Cumulative Clean Marina Program - certifications	0		

PATENTS AND ECONOMIC BENEFITS

Description		Patents	Economic Benefit ($)	Businesses Created	Businesses Retained	Jobs Created	Jobs Retained
Part-time lab position at University of Washington - Tacoma. R/OCEH-9	Actual (2/1/2013 - 1/31/2014)	0	0	0	0	1	0
	Anticipated (2/1/2014 - 1/31/2015)	0	0	0	0	0	0

TOOLS, TECH, AND INFORMATION SERVICES

Description		Developed	Used	Names of Managers	Number of Managers
Alexandrium cysts distribution maps that identify potential HAB hotspots in Puget Sound for development of a HAB forecast tool.	Actual (2/1/2013 - 1/31/2014)	1	1	Anticipate some	0
	Anticipated (2/1/2014 - 1/31/2015)	0	0		

R/OCEH-9

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2013 - 1/31/2014) 3
Anticipated (2/1/2014 - 1/31/2015) 0
WA State Department of Health
Tribes
Shellfish Growers

Number of fishers using new techniques
Actual (2/1/2013 - 1/31/2014) 0
Anticipated (2/1/2014 - 1/31/2015) 0

Sustainable Coastal Development
Actual (2/1/2013 - 1/31/2014) 0
Anticipated (2/1/2014 - 1/31/2015) 0

Coastal Ecosystems
Actual (2/1/2013 - 1/31/2014) 0
Anticipated (2/1/2014 - 1/31/2015) 0

PARTNERS

Partner Name King County Department of Natural Resources and Parks

Partner Name NOAA Northwest Fishery Sciences Center

Partner Name University of Washington Seattle

Partner Name University of Washington Tacoma

IMPACTS AND ACCOMPLISHMENTS

Title Washington Sea Grant research tests conventional wisdom on harmful algal blooms and helps shellfish growers anticipate them

Type impact

Relevance, Response, Results Relevance The alga Alexandrium catenella produces toxins that can accumulate in shellfish and cause death if ingested by humans. Limited ability to predict A. catenella blooms makes them a significant threat to public health and to Washington’s $100 million-plus shellfish industry. Researchers have tried to predict summer blooms by mapping winter cysts in surface sediments. But this approach relies on the assumption that all cysts are capable of germinating; the share of cysts in “seedbeds” that actually germinate was unknown. Response Washington Sea Grant-supported research tested the assumption that large-scale cyst mapping in winter can fully reveal summer bloom risk. They collected cysts from 100 sites throughout Puget Sound, incubated them to determine germination rates, and used microphotography and image analysis to see if “full” cysts were more likely to germinate. They also conducted experiments to determine the dormancy requirements of cysts and the seasonal regulation of germination. A bloom map was disseminated to the shellfish industry early in the growing season. Results Only 16 to 66 percent of cysts from the 100 sampled stations germinated. Such wide variation suggests that traditional cyst mapping without a viability assessment does not accurately reflect the likelihood of dangerous blooms. By mapping the abundance of viable cysts throughout Puget Sound, this project has produced a more accurate indicator of the potential for toxic blooms. Researchers' outreach helped shellfish producers become aware of areas at high risk of blooms.

Recap Washington Sea Grant-supported research reveals that surface-sediment cyst mapping alone may not reveal the full risk of Alexandrium catenella blooms. Determining the share of cysts capable of germinating helps shellfish growers anticipate toxic blooms.

Comments Primary Focus Area OCEH (HCE) Secondary Focus Area OCEH (SSSS), COCC (HRCC) State 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).

Related Partners , , ,

PUBLICATIONS

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

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year 2014 Uploaded File 2012_GREENGROVE_MASURA....l.pdf URL http //www.issha.org/Welcome-to-ISSHA/Conferences/ICHA-conference-proceedings/ICHA15-Proceedings

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.

Citation 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
Proceedings of the 15th International Conference on Harmful Algae. Changwon, Korea 2012.

Copyright Restrictions + Other Notes

Journal Title none

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

Type influenced Period 2013-01-01 2013-02-28Amount $110000

Purpose Boat time to collect samples - Piggy back on PS-AHAB - 17 plus 5 days of ship time on R/V Barnes for viability samples & some monthly coring

Source NOAA ECOHAB

Type influenced Period 2013-01-01 2013-02-28Amount $14000

Purpose Equipment - Craib corer. NOT REPORTED

Source PS-AHAB NOAA ECOHAB (this does not include match on particle size analyzer maintenance contract and free us of instrument)

Type influenced Period 2013-01-01 2013-02-28Amount $30000

Purpose Ship and lab volunteers time

Source Students, Stephanie Moore and Brian Bill (does not include the Cheryl Greengrove match)

UPDATE NARRATIVE

Uploaded File Moore_3299_update_narr....9.pdf

Seagrant Annual Report 2014

Note 1: We have requested and received a no cost extension to complete this project and present the results.
Note 2: Updates from last year's report are in italics.

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:

The purpose of this project is to provide critical information on the dormancy requirements and germination characteristics of *A. catenella* 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 will also critically evaluate 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 *A. catenella* 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 *A. catenella* cysts, and (4) determine whether *A. catenella* cysts exhibit secondary dormancy. While this Seagrant 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. All results presented here are preliminary.

Cyst Mapping:

To evaluate the effectiveness of winter cyst mapping as a tool to predict *A. catenella* 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 and the inner harbor station 79 (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). The results of the monthly surveys are presented in Figure 2. 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. *All samples have now been counted in triplicate. The TOC and PSA sediment physical characteristic analyses have been completed by UWT students, but data remain to be plotted and analyzed.*

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 *A. catenella* was not observed in the water column of QMH until late June – early July and there was no apparent fall bloom, and though *A. catenella* 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, consisting of more unconsolidated organics in the summer, which disappeared by the winter season.

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

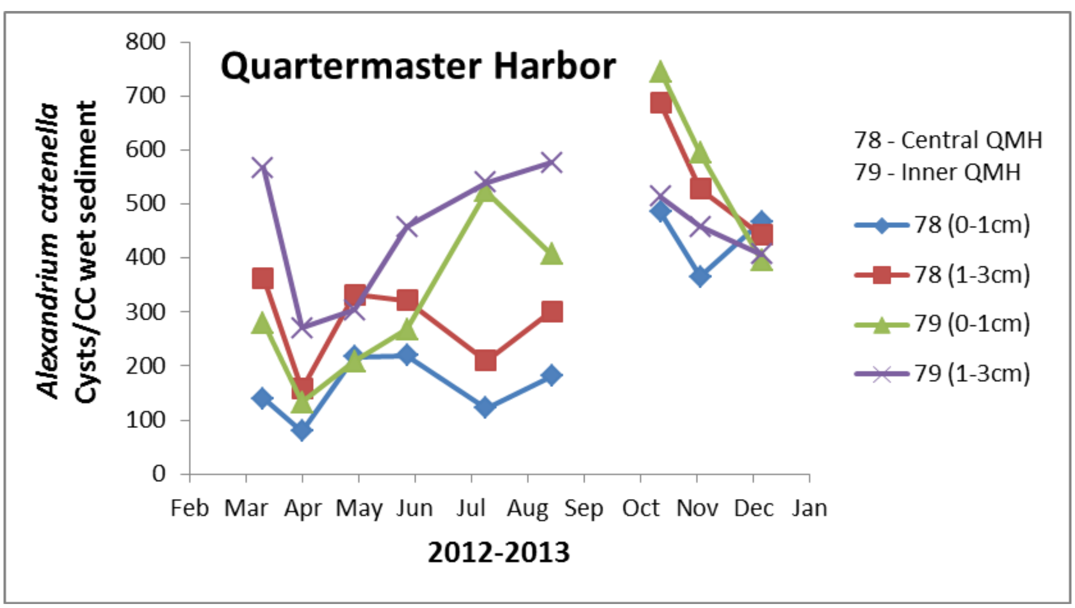

Figure 2. *Alexandrium catenella* cyst abundances per cm^3 of wet sediment in upper (0-1cm) and (1-3cm) sediment layers at the central (78) and inner (79) stations in Quartermaster Harbor from February 2012 –January 2013.

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 A. catenella 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 A. catenella cysts is the only part of our original proposed work that we were not able to complete again this year. 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 have saved some student money to complete the experiment this year on the spring or fall bloom and received our no cost extension for a year to complete this task.

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 3). 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.

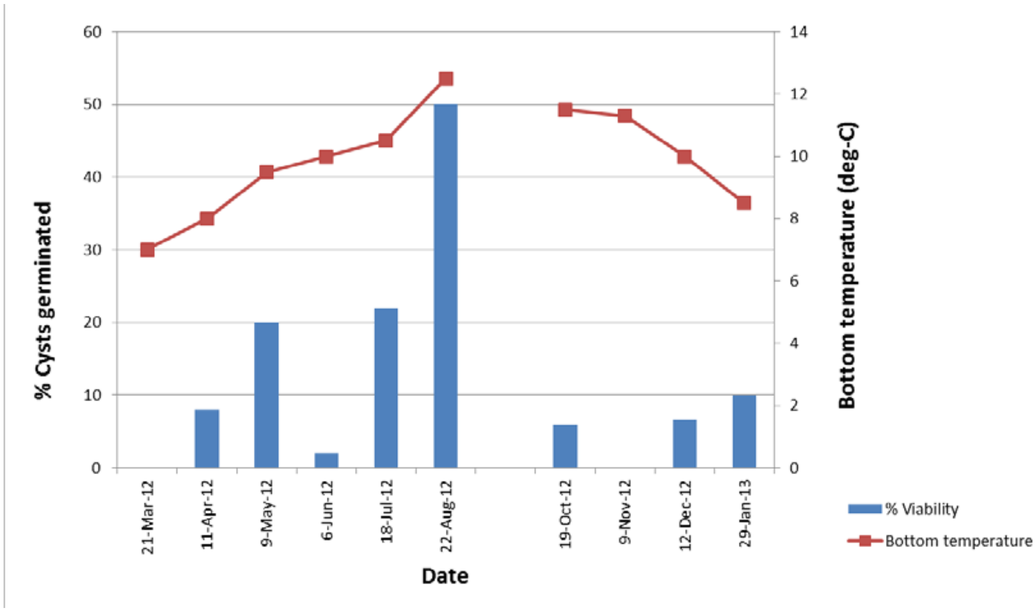

Figure 3. Excystment of cysts (left axis) and water temperature at the bottom of Quartermaster Harbor (right axis) for each month that sediment was collected and cysts isolated to determine secondary dormancy.

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 30 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 30 stations where additional surface sediment was collected, viability experiments have been completed on 17 stations. The remaining stations had too few cysts to feasibly isolate 50 cysts within the timeframe necessary to conduct the experiment. The percentage of viable cysts ranges from 16 to 66% (Table 1). To our knowledge, no other studies have examined the viability of *Alexandrium* cysts on such a large scale. Therefore, it is difficult to put these findings into context. However, 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 may not be useful in Puget Sound for modeling and prediction of toxic blooms.

Table 1. *Alexandrium* cyst viability from surface sediments in Puget Sound.

Site #	**Site name**	**Depth (m)**	**Cysts/cc**	**% Viability[**}**
1	Semiahmoo Bay	16	77	37[30]
4	Birch Bay	9	72	34
5	Georgia Strait - SE	50	63	24
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
22	Seqium Bay - Center	27	35	34
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
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

** Superscript in brackets indicates where fewer than 50 cysts were isolated to determine the % viability.

Each cyst that was isolated for the viability experiments was photographed. The fullness of cysts will be 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 4). 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.

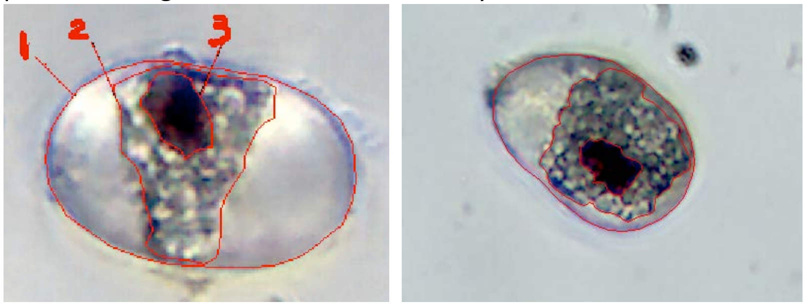

Figure 4. Photographs of *Alexandrium* cysts showing the metrics calculated in ImageJ to determine if any relationship exists between cyst fullness and viability.

To date, no relationship between cyst viability and cyst appearance using image analysis software has been detected (Figure 5). *However, not all cyst photographs have been analyzed. A UWT undergraduate will finish this analysis this summer.*

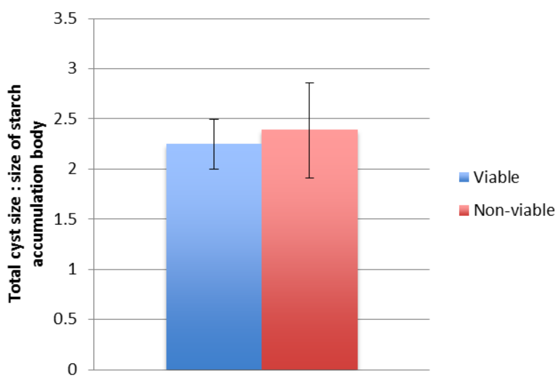

Figure 5. Ratio of the total cyst size to the size of the granulated starch accumulation body for viable and non-viable cysts.

References:

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

Anderson, D. M., and B. A. Keafer. 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*. Nature **325:** 616-617.

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Binder, J. B., and D. M. Anderson. 1990. Biochemical composition and metabolic activity of *Scripsiella trochoidea* (Dinophyceae) resting cysts. J Phycol **26:** 289-298.

Dale, B. 1977. Cysts of the toxic red-tide dinoflagellate *Gonyaulax excavata* (Braarud) Balech from Oslofjorfen, Norway. Sarsia **63:** 29-34.

Genovesi, B., M. Laabir, E. Masseret, Y. Collos, A. Vaquer, and D. Grzebyk. 2009. Dormancy and germination features in resting cysts of *Alexandrium tamarense* species complex (Dinophyceae) can facilitate bloom formation in a shallow lagoon (Thau, southern France). J Plankton Res **31:** 1209-1224.

Matrai, P., B. Thompson, and M. Keller. 2005. Circannual excystment of resting cysts of *Alexandrium* spp. from eastern Gulf of Maine populations. Deep-Sea Res Pt II **52:** 2560-2568.

Rathaille, A. N., and R. Raine. 2011. Seasonality in the excystment of *Alexandrium minutum* and *Alexandrium tamarense* in Isish coastal waters. Harmful Algae **10:** 629-635.

Vleeshouwers, L. M., H. J. Bouwmeester, and C. M. Karssen. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. J Ecol **83:** 1031-1037.

Yamaguchi, M., S. Itakura, I. Imai, and Y. Ishida. 1995. A rapid and precise technique for enumeration of resting cysts of *Alexandrium* spp. (Dinophyceae) in natural sediments. Phycologia **34:** 207-214.