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Mapping the cysts of Alexandrium catenella in the surface sediments of Puget Sound: A comparison of microscopy and molecular methods for enumeration

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Mapping the cysts of *Alexandrium catenella* in the surface sediments of Puget Sound: A comparison of microscopy and molecular methods for enumeration

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Introduction

This newly funded NOAA NCCOS MERHAB project plans to develop new molecular methods for detecting *Alexandrium catenella* cyst concentrations in marine sediments. *A. catenella* is a dinoflagellate that produces saxitoxin, a powerful neurotoxin, that can be concentrated in filter feeding shellfish which, if ingested by humans, can lead to paralytic shellfish poisoning and potentially death. *A. catenella* overwinters as a cyst in the sediment and when environmental conditions are right, in the spring and summer, germinates into the water column as a vegetative cell (Fig. 1). Previous studies (Greengrove et al. 2015; Horner et al. 2011) have mapped the winter distribution of *A. catenella* cysts in Puget Sound sediments as a way of providing shellfish growers with an early warning system of potential hotspots for blooms of this harmful alga (HABs).

The standard method for cyst detection in sediments depends on collecting sediment samples in winter, sieving, preserving, and staining the samples and manually enumerating the cysts using epifluorescence microscopy (Yamaguchi et al. 1995). This method is very labor intensive and requires extensive training to accurately identify the cysts. This new MERHAB project aims to develop new quantitative molecular assays (qPCR and FISH – fluorescent in situ hybridization) that will be compared with the existing standard microscopy protocol using surface sediment samples from Puget Sound, the Gulf of Maine, and Alaska (Fig. 2) to see if these new molecular techniques represent a viable alternative to the existing standard microscopy method. This poster will focus on the preliminary maps of *A. catenella* surface sediment cyst distribution from winter 2020 in Puget Sound. Comparisons with molecular methods are not yet available.

MorACCESs
Molecular *Alexandrium* Cyst Counting and Enumeration Systems

Main Goal - To develop a molecular method to determine cyst abundances in sediments.

Why? – Want to make method of collecting and enumerating cysts more broadly accessible.

Current method requires special surface core sampler, larger boat and is very labor intensive requiring lots of sample preparation and hours of a trained expert in cyst identification counting cysts under an epifluorescent microscope.

Main Hurdle – (besides going out sampling sediments in the winter) breaking open the cyst's outer layer to get access to material inside in order to use molecular methods to identify cyst.

Scientific Objectives
- Obtain sediment samples for method validation
  (Gulf of Maine (GOM); Puget Sound & Alaska)
- Standardize Cyst Counting
- Map *A. catenella* cyst abundance
- Develop a qPCR assay for *A. catenella* resting cysts
- Develop a PNA-FISH probe specific for *A. catenella* cysts
- Validation of Molecular Methods
- Technology Transfer and Communication

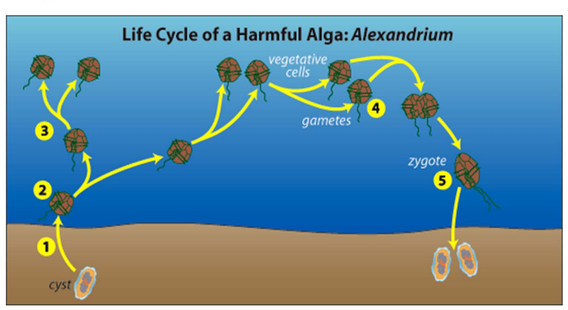


Figure 1. http://www.whoi.edu/redtide/

Methods

Figure 4. Puget Sound field sampling team (left to right) - Wes Parker (Captain), Dan Lomax (1st Mate), Caitlyn McFarland (undergraduate), Julie Masura, Cheryl Greengrove, Gibson French (undergraduate).

Figure 5. Van Veen grab sampler.

- Surface sediment samples were collected using a van veen grab sampler in the GOM (Oct 2019; 54 samples); Puget Sound (Jan-Feb 2020; 47 samples); Alaska Southeast (Aug 2019; 24 samples); Alaska – Chiniak Bay (Feb 2020; 12 samples); Alaska – Kachemak Bay – not collected due to COVID-19 pandemic. Splits of all samples were shipped to UW Tacoma for duplicate cyst counting and to the NOAA Beaufort Laboratory for molecular analyses.

- Sampling in Puget Sound was done in three legs: northern (24-27 Jan), central (7-8 Feb) and southern (17-20 Jan). A van veen surface sediment grab sample, secchi, CTD profile and 20 micron mesh vertical phytoplankton net tow 10 meters to the surface was done at each station. The net tow is done to insure that there are no vegetative cells of *A. catenella* in the water column.

- A calibration meeting was held at UW Tacoma to align the standard microscopy technique used by cyst enumerators for GOM (Steve Kibler), Puget Sound (Julie Masura), Alaska SE (Courtney Hart) and Alaska Bays (Julie Matweyou). Julie Masura will count samples from all locations as a check on standardization of the microscopy method.

- Cyst monitoring capability in Alaska is being expanded as part of this project by Julie Matweyou setting up a lab for cyst enumeration using microscopy at Sea Grant on Kodiak Island.

- Two molecular methods (qPCR (Fig. 7) and FISH (Fig. 8) will be explored and developed for determining relative *A. catenella* cyst abundances. All molecular work will be done at the NOAA Beaufort Laboratory by Steve Kibler.

NOTE: Currently all work on this project in halted due to the COVID-19 pandemic. We are only partially done with the microscopy cyst counts and molecular methods are still in the development phase.

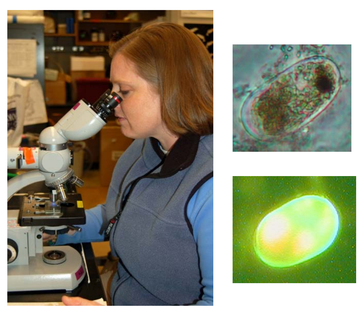


Figure 6. Julie Masura counting cysts using the standard microscopy method of Yamaguchi and others (1995) with insets of *A. catenella* cysts unstained and stained.

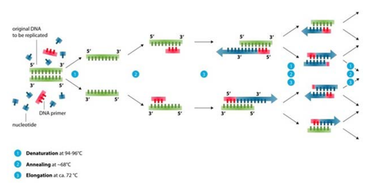
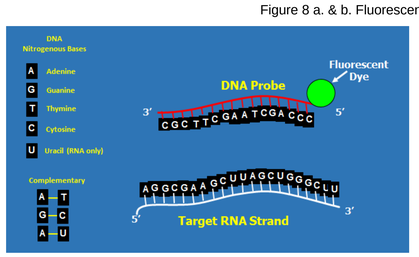


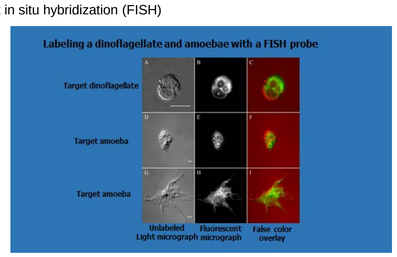
Figure 7. qPCR

PCR – Polymerase Chain Reaction: Makes many copies of a particular gene sequence.
Heating & cooling cycle in the presence of enzymes
The gene sequence is duplicated 1x during each heating-cooling cycle
Proceeds at a constant reaction rate
1 copy-2 copies-4 copies-8-16-32-64.....

qPCR
Incorporate a fluorescent dye during the PCR reaction to label the gene sequence being copied
Monitor the amount of fluorescence during each PCR cycle
After a known number of reaction cycles, this allows the number of copies in the original sample to be estimated.
Need to establish the number of gene copies per cell
Quantify the number of cells at the start of the reaction

Figure 8 a. & b. Fluorescent in situ hybridization (FISH)





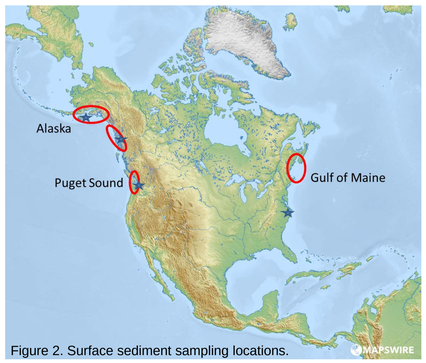


Figure 2. Surface sediment sampling locations.

Figure 3. Project Principal Investigators (left to right) – Julie Masura, Julie Matweyou, Courtney Hart (graduate student), Cheryl Greengrove & Steve Kibler

Results & Future Work

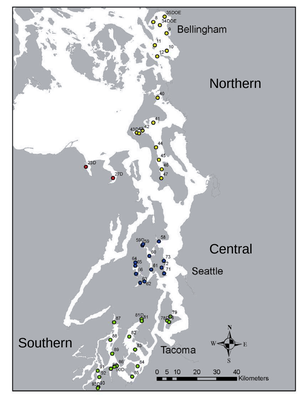
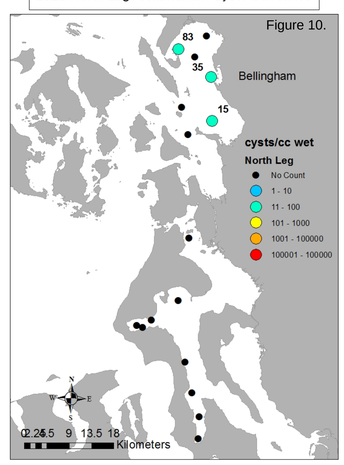


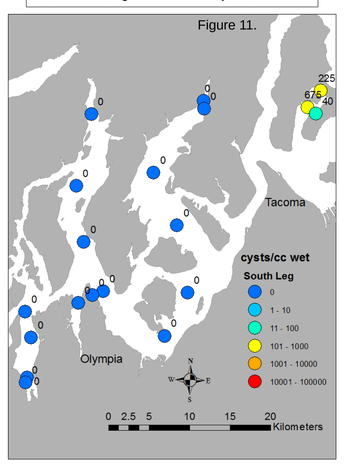
Figure 9. Station sampling locations in Puget Sound winter 2020.

As noted earlier, all work on this project is on hold due to the COVID-19 pandemic. As such we are only reporting the surface sediment cyst counts done to date in Puget Sound which include the southern leg (Fig. 11) and part of the northern leg (Fig. 10).
- There were no cysts found in the southern basin of Puget Sound.
- Cyst abundances were high (up to 675 cysts/cc wet sediment) in Quartermaster Harbor.
- Stations in Bellingham Bay counted to date indicate cysts are present, but lower that Quartermaster Harbor.
- These results are consistent with previously observed cyst distribution patterns in Puget Sound ((Greengrove et al. 2015; Horner et al. 2011).

2020 North Leg Alexandrium Cyst Abundance



2020 South Leg Alexandrium Cyst Abundance



Future Work:
- Cyst counting using microscopy needs to be completed for all sample areas.
- qPCR and FISH molecular techniques need to be developed and tested.
- Surface sediment samples will be collected again next year to further develop and test these methods before disseminating the results.

References

- Greengrove, C.L., Masura, J.E., Moore, S.K., Bill, B.D., Hay, L.R., Eldred, K.C., Banas, N.S., Salathé Jr., E.P., Mantua, N.J., Johnstone, J.A., Anderson, D.M., Trainer, V.L., Stein, J.E., 2015. Alexandrium cyst distribution and germination in Puget Sound, WA USA In: A. Lincoln MacKenzie (Ed.) 2015. Marine and Freshwater Harmful Algae. Proceedings of the 16 International Conference on Harmful Algae, Wellington, New Zealand 27th-31st October 2014. Cawthron Institute, Nelson, New Zealand and International Society for the Study of Harmful Algae.

- Horner, R.A., Greengrove, C.L., Davies-Vollum, K.S., Gawel, J.E., Postel, J.R., Cox, A., 2011. Spatial distribution of benthic cysts of Alexandrium catenella in surface sediments of Puget Sound, Washington, USA. Harmful Algae 11, 96-105.
- 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

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