Completion Report

Cattolico, Rose Anne

Period: 2/1/2012 - 1/31/2013

Project: R/OCEH-7 - Troubled sediments: Heterosigma Cyst Formation and Longevity

:: STUDENTS SUPPORTED

Black, Megan, Megan Black , University of Washington, Biology, status:cont, field of study:Biology, advisor:Cattolico, degree type:PhD, degree date:2014-06-01, degree completed this period:No Student Project Title: Diversity in the Raphidophyte, Heterosigma: A Multi-Gene Mitochondrial Study Reveals Phylogenetic Incongruence Involvement with Sea Grant This Period: PhD student Post-Graduation Plans: To form a Center for Continuing Education
Hunsburger, Heather, Heather Hunsperger , University of Washington, Biology, status:cont, field of study:Evolutionary Biology, advisor:R.A. Cattolico, degree type:PhD, degree date:2013-06-01, degree completed this period:No Student Project Title: Evolution of chlorophyll biosynthetic pathways Involvement with Sea Grant This Period: PhD Post-Graduation Plans: Professor
Tobin, Elizabeth, Liz Tobin , University of Washington, Ocean Sciences, status:cont, field of study:Algal biology, advisor:Grunbaum, degree type:PhD, degree date:2014-06-01, degree completed this period:No Student Project Title: Quantification of transitional swimming behaviors in harmful algae and their implications for pelagic and benthic distribution Involvement with Sea Grant This Period: PhD student Post-Graduation Plans: Faculty or government research position

:: CONFERENCES / PRESENTATIONS

Megan Black, Michael Jacobs, Chloe Deodato, Rose Ann Cattolico
BC Examining Algal Bloom Population Diversity in a Salish Sea Heterosigma Bloom using mitochondrial genes., public/profession presentation, 120 attendees, 2012-05-11
Megan Black, Michael Jacobs, Chloe Deodato, Rose Ann Cattolico
Algal Bloom Diversity of Heterosigma akashiwo.

UW Biology Graduate School Symposium, public/profession presentation, 75 attendees, 2012-11-08
Quantification of transitional swimming behaviors in the harmful alga, Heterosigma akashiwo, and their implications for pelagic and benthic distributions."

ASLO/AGU 2012 Ocean Sciences Meeting
*Awarded "Outstanding Student Presentation", public/profession presentation, 300 attendees, 2012-10-29
Life-stage transitions in the harmful alga, Heterosigma akashiwo:
Assessing the role of emergence, swimming and growth in bloom formation.
15th International Conference on Harmful Algae - Changwong, Korea, public/profession presentation, 500 attendees, 2012-02-19

:: ADDITIONAL METRICS

K-12 Students Reached:	0	Acres of degraded ecosystems restored as a result of Sea Grant activities:	0
Curricula Developed:	0	Resource Managers who use Ecosystem-Based Approaches to Management:	0
Volunteer Hours:	0	HACCP - Number of people with new certifications:	0
Cumulative Clean Marina Program - certifications:	0		

:: **PATENTS AND ECONOMIC BENEFITS**

No Benefits Reported This Period

:: TOOLS, TECH, AND INFORMATION SERVICES

Description		Developed	Used	Names of Managers	Number of Managers
New approach for analyzing mitochondrial DNA sequences that will avoid false positives in assessing divergence in algal populations. R/B-48, R/OCEH-7	Actual (2/1/2011 - 1/31/2012) :	1	1		0
	Anticipated (2/1/2012 - 1/31/2013) :	0	1		
Use of data on swimming	Actual (2/1/2011 -	1	1		0

behavior of Heterosigma cells entering resting phase and after survival of the cystic state in model generation. R/B-48, R/OCEH-7	1/31/2012) : Anticipated (2/1/2012 - 1/31/2013) :	0	1	
Tools for measuring lipids by GC/MS to assess impact of lipid stores on Heterosigma cyst survival, activated cell swimming ability. R/B-48, R/OCEH-7	Actual (2/1/2011 - 1/31/2012) : Anticipated (2/1/2012 - 1/31/2013) :	1 0	1 1	0
Optimization and use of molecular toolkit for identifying HAB-forming algal strains of Heterosigma using sequences obtained from mitochondrial DNA to probe Heterosigma population diversity in Puget Sound. R/B-48, R/OCEH-7	Actual (2/1/2011 - 1/31/2012) : Anticipated (2/1/2012 - 1/31/2013) :	1 0	1 1	0

:: HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

:: ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2011 - 1/31/2012) :
Anticipated (2/1/2012 - 1/31/2013) :

Number of fishers using new techniques
Actual (2/1/2011 - 1/31/2012) :
Anticipated (2/1/2012 - 1/31/2013) :

Sustainable Coastal Development
Actual (2/1/2011 - 1/31/2012) :
Anticipated (2/1/2012 - 1/31/2013) :

Coastal Ecosystems
Actual (2/1/2011 - 1/31/2012) :
Anticipated (2/1/2012 - 1/31/2013) :

:: PARTNERS

Partner Name: Gordon and Betty Moore Foundation
Partner Name: Los Alamos National Lab (US DOE)
Partner Name: Northwest Fisheries Science Center (US DOC
Partner Name: Pacific Northwest National Laboratory (US DOE)
Partner Name: University of Washington

:: IMPACTS AND ACCOMPLISHMENTS

Title: Washington Sea Grant research develops tools to assess genetic variability and mechanisms for bloom formation in a widespread toxic alga

Type: accomplishment

Description:

Relevance: Toxic blooms of Heterosigma akashiwo, one of the world's most harmful algal species, can devastate salmon aquaculture operations and also harm oysters, scallops, sea urchins, and other marine organisms. H. akashiwo occurs globally in multiple genetically distinct strains. All form non-motile cysts (resting stages) that can remain in ocean sediments for long periods, then activate and initiate destructive blooms. But key questions remain unanswered: What factors determine cyst activation and bloom formation, and how does bloom formation vary among different strains?
Response: Washington Sea Grant researchers developed a genetic toolkit to "fingerprint" 50 H. akashiwo cultures isolated from blooms worldwide. To assess the role cysts play in bloom development, they developed a new spectroscopic technique to measure cell lipid (fat) energy stores. When dormant cysts were activated, researchers used video to track their swimming from sediment to surface and their aggregation capabilities. Results: Genetic fingerprinting identified significant variation among cultures – even in samples taken from similar locations, such as Puget Sound – and it reduced false positives in the identification of different strains within samples. It also succeeded in identifying cysts in marine sediments and ballast water, a significant technical advance in harmful algae monitoring. The strains identified varied in their survival rates as cysts, their success at activating, and their swimming ability afterward. Cysts with the highest lipid levels outperformed their leaner counterparts. Fat content may be a strong predictor of harmful blooms.

Recap: Washington Sea Grant-sponsored research develops genetic and laboratory techniques that improve the capacity to identify and monitor toxic algal strains and predict the occurrence of blooms posing significant risk to farmed salmon and other marine life.

Comments: Primary Focus Area – OCEH (HCE) Secondary Focus Areas – COCC (HRCC), OCEH (SSSS) Associated Goals: Improve understanding and management of emerging and cumulative threats to ocean and coastal health (HCE Science). Improve understanding and management of

emerging and cumulative threats to ocean and coastal health (SSSS Supply). Improve understanding of coastal hazards and environmental change and develop tools and approaches for observation, prediction, planning and adaptation (HRCC Capacity).

Related Partners:
Gordon and Betty Moore Foundation
Los Alamos National Laboratory (US DOE)
Northwest Fisheries Science Center (US DOC, NOAA, NMFS, NWFSC)
Pacific Northwest National Laboratory (US DOE)
University of Washington, Department of Biology, College of Arts and Sciences (UW)
University of Washington, School of Oceanography, College of the Environment (UW)

:: PUBLICATIONS

Title: Assessment of EvaGreen-based quantitative real-time PCR assay for enumeration of the microalgae Heterosigma and Chattonella (Raphidophyceae)

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2012 Uploaded File: Park.pdf, 372 kb URL: none

Abstract: Abstract Heterosigma akashiwo and Chattonella species (Raphidophyceae) are difficult to detect and quantify in environmental samples because of their pleomorphic and fragile cell nature. In this study, we developed a quantitative real-time polymerase chain reaction (qRT-PCR) assay for the enumeration of these algal taxa using a new DNA- binding dye, EvaGreen. Species-specific qRT PCR primers to H. akashiwo, Chattonella antiqua, Chattonella marina, Chattonella ovata, and Chattonella subsalsa were designed to target the ITS2 rRNA gene intergenic region. Primer specificities were tested via BLAST searches. In addition, specificity was verified using empirical tests, including competitive PCR. The qRT PCR assay analyzing Ct value and the log of cell number showed a significant linear relationship ($r2 \geq 0.997$). When light microscopy was used to monitor the population dynamics of targeted Raphido- phyceae from Lake Shihwa, H. akashiwo was detected in ten samples and no Chattonella spp. were detected (70 samples collected from May, 2007 to January, 2008). In contrast, when the qRT-PCR assay was used, H. akashiwo was detected in 41 samples. C. antiqua, C. marina, and C. ovata were detected in eight samples. Most of the samples analyzed using qRT-PCR assays showed higher algal numb- ers than did those assayed via microscopy, suggesting that the enumeration of Raphidophyceae via classic microscopic methods most likely underestimates true algal concentration.

Citation: Park, B.S., S.H. Baek, J.S. Ki, R.S. Cattolico, and M.S. Han. 2012. Assessment of EvaGreen-based quantitative real-time PCR assay for enumeration of the microalgae Heterosigma and Chattonella (Raphidophyceae). Journal of Applied Phycology 24:1555–1567. DOI: 10.1007/s10811-012-9816-2

Journal Title: Journal of Applied Phycology

:: OTHER DOCUMENTS

No Documents Reported This Period

:: LEVERAGED FUNDS

No Leveraged Funds Reported This Period

Results summary:

Identification of *Heterosigma akashiwo* strain types: Algae commonly form cryptic species complexes. These complexes are comprised of representatives that may be genetically and physiologically diverse but cannot be readily differentiated from one another due to their small size or plastic morphology. The cosmopolitan raphidophyte, *Heterosigma akashiwo*, is a particularly intractable morpho-species. Controversy with respect to the taxonomy of this organism has been long-standing. This marine alga, which causes toxic and destructive blooms, has a world-wide distribution.

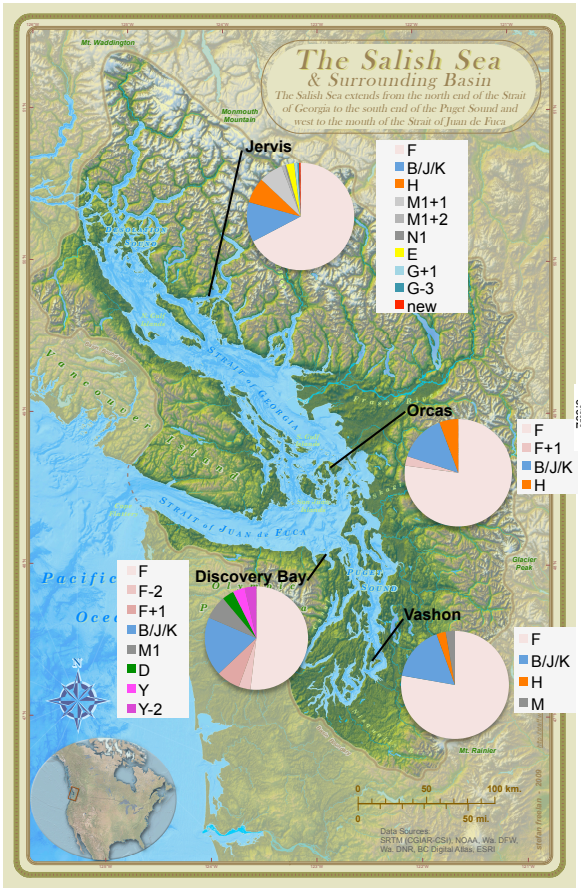
To determine if *H. akashiwo* forms a cryptic species complex, isolates from 50 cultures, initiated from bloom events occurring both in Pacific and Atlantic Ocean coastal regions were examined. Comparative gene sequence analysis of the mitochondrial genes *cox*1, *nad*2, *nad*4, *nad*5 and *nad*7 was used. Twenty-three distinct mitotypes were identified. Mitochondrial genes were shown to have differing evolutionary rates; *nad*5 (3.6%) has the highest divergence, *nad*2 (1.7%) & *nad*7 (1.8%) the lowest. *H. akashiwo*'s *nad*5 gene offers the highest resolution and can be used to differentiate 18 of 23 mitotypes.

Mitochondrial genes display incongruent phylogenetic topologies among cultures. Twelve new *H. akashiwo* subspecies were designated according to translated amino acid sequences. Sequence modeling predicts the degree to which specific amino acid substitutions affect steric function of electron transport proteins. No distinct biogeographic identity was seen among mitotypes or subspecies indicating this genus has undergone extensive dispersal, and that multiple subspecies may coexist at the same location. Conversely, some mito-types have remained endemic to a specific geographic local for fifty-years.

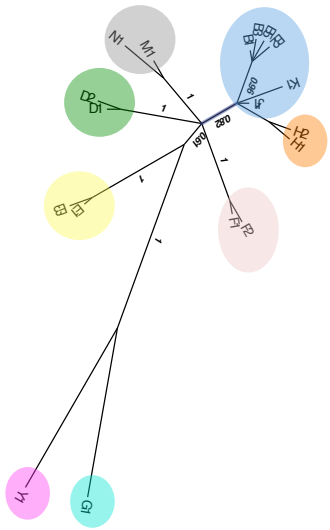
To compare the effect of DNA polymerase on the error rates of the PCR-cloning-sequencing process, a cloned plasmid containing a *H. akashiwo's nad*5 gene was used as control template DNA. Tsg+ (high fidelity TAQ) generated twenty unique DNA Sequences for the seventy clones sampled, while Phusion (the ultra-high fidelity DNA polymerase) produced the correct sequence for every sample tested. An important consideration is the fact that different algal strains may have different levels of the taget gene present in their genome. To test this possibility, qPCR was done using *nad* 5 as the target gene. *Heterosigma akashiwo* strain NIES 293, has 2.0 X *nad* 5 gene copy number /cell than UWC 13.03and ~ 1.66 X more that strain 13.03. This observation will be critical when looking at *Heterosigma* distribution in field populations.

Our findings show: (a) extensive genetic variation among *H. akashiwo* cultures exist, (b) little biogeographical fidelity is observed among the populations examined, (c) that the canonical assumptions about mitochondrial DNA inheritance are not upheld in this genus, and (d) because of identified mitochondrial disparities among cultures tested, subspecies are designated according to mitotypes.

Future work: We are looking at the distribution of algal strain types within *Heterosigma* populations in Puget Sound. This work, though preliminary is showing interesting correlations as seen in the following Figure.



Map of the Salish Sea & Surrounding Basin, Stefan Freelan, WWU, 2009



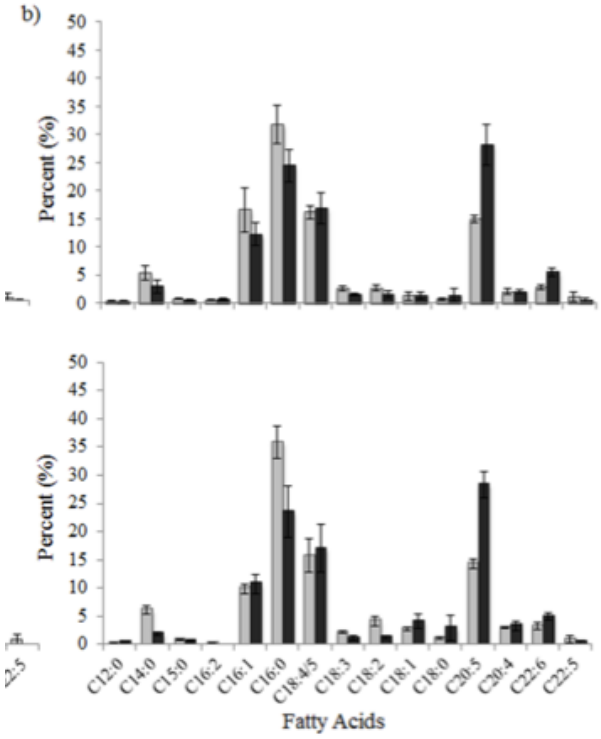
*H. akashiwo nad*5 gene
Color key

Pie charts indicate the population distribution of *H. akashiwo* genotypes in each 2011 bloom. All blooms had multiple genotypes, but the contained bays of Vashon and Orcas Island had fewer types than areas of high flow in Jervis Inlet and Discovery Bay on the Strait of Juan de Fuca. The Bayesian gene tree for mitochondrial *nad*5 has been colored to show the sensitivity of the gene region used in this analysis and corresponds to the pie chart colors. When a novel genotype was sequenced, it was designated by the closest relative +/- the number of nucleotide changes.

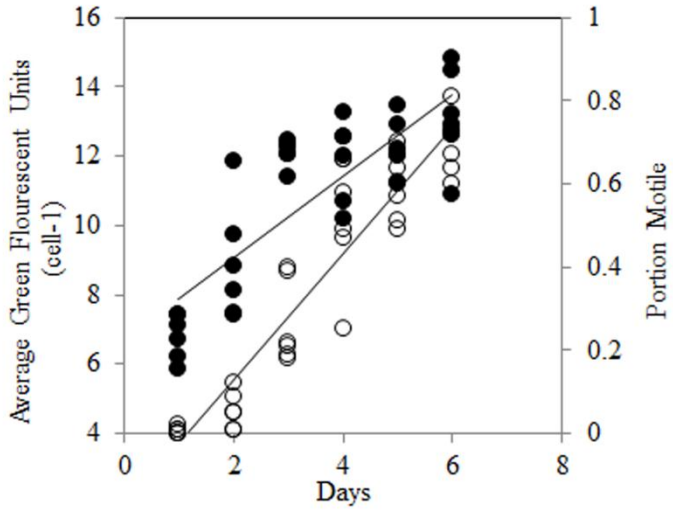
Analysis of cellular mechanisms that drive *Heterosigma* cyst survival and activation: Many HAB-forming species exhibit a dual-stage life history by transitioning between a pelagic vegetative stage and a benthic resting stage. Despite having potentially important impacts on bloom formation and termination, transitions between pelagic and benthic stages are among the least understood aspects of HAB dynamics. Our study presents a multifaceted approach to understand the biochemistry that drives cyst survival, resting cell activation and the re-establishment of a bloom when cells swim upward to the water surface. We now know that cyst survivorship is extremely strain dependent. In this study we showed *Heterosigma* CCMP 452 (West Atalntic) and UWC 13.03(East Pacific; Puget Sound) strains had 94% and 51% resting cell viability, respectively.

Stored lipids are the energy currency of the cell. By analyzing this storage commodity we will better understand how an obligately autotrophic organism can survive stasis in the dark cold sediments of coastal regions. Using the neutral lipid staining dye BODIPY 505/515 and flow cytometry, relative neutral lipid content of *Heterosigma* cells was measured as cells transitioned between benthic and pelagic life stages. This laboratory has devised a new GC/MS method to quantitate total cellular fatty acids and to assess the lipid products that requires only 250 ug of dried biomass. This new technique has allowed us to compare the lipid profiles of two *Heterosigma* strains in the vegetative and resting state.

Similar to the BODIPY505/515 neutral lipid signal, the fatty acid analysis indicated a significant change in the total amount of fatty acid per cell between the vegetative and resting life stages. The total fatty acid content for vegetative cells of CCMP 452 was only 1/3 that of UWC 13.03. As these strains progress through the 18 days required for cyst induction, UWC 13.03 looses more lipids/unit time than CCMP 452 (92% vs 67% respectively). Additionally, cells shift in fatty acid profiles – with significant lipid restructuring occurring. Data showed excellent correlation between lipid quantity per cell and swimming capacity that was measured by our video capture experiments. The ability for a *Heterosigma* cell to resume swimming is crucial for survivorship. Our previous studies show that the cell division rate of newly activated cells to be extremely light dependent for this up-swimming organism.



Changes in lipid profiles in cell vegetative (gray bars) and cyst (black bars). Upper graph CCMP 452; Lower UWC13.03



Changes in neutral lipid content cell-1 (BODIPY 505/515 signal) and motility following restoration of growth supporting conditions. The scatter plot shows the association of average neutral lipid content cell-1 (black circles) and the portion of motile cells (open circles) with time under activation conditions (in days). A Spearman's rank correlation indicates that both mean neutral lipid content ($r = 0.676$) and population motility ($r = 0.824$) have a significant, positive correlation with time under activation conditions ($N = 65$, $p = < 0.001$).