Completion Report

Period: 2/1/2014 - 1/31/2015

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

STUDENTS SUPPORTED

Hovde, Blake, hovde@u.washington.edu, University of Washington, Genetics, status: cont, field of study: Genetics, advisor: R. Monnet, degree type: PhD, degree date: 2015-11-01, degree completed this period: Yes
Student Project Title:
Genomic analysis of HAB species
Involvement with Sea Grant This Period:
Extensive analyses of both genomic and transcriptomic data for two algal species; instructed other laboratory members in bioinformatics
Post-Graduation Plans:
post doctoral fellow

Hunsburger, Heather, hheather@uw.edu, University of Washington, Biology, status: cont, field of study: Evolutionary Biology, advisor: R.A. Cattolico, degree type: PhD, degree date: 2015-06-01, 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, etobin@u.washington.edu, University of Washington, Oceanography, 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:
Independent NSF grant to pursue HAB work in Alaska

CONFERENCES / PRESENTATIONS

Many paths to chlorophyll: The evolution of protochlorophyllide oxidoreductase in the algae
, public/profession presentation, 3000 attendees, 2014-05-22

Nannochloropsis organellar genomes: novel genetic variations occur in key metabolic genes

Joint meeting:
Society for Fresh Water Science; Phycological Society of America; Association of the

ciences of Limnology and Oceanography; Society of Wetland Scientists, public/profession presentation, 3000 attendees, 2014-05-22

ADDITIONAL METRICS

P-12 Students Reached:	0	**P-12 Educators Trained:**	0
Participants in Informal Education Programs:	0	**Volunteer Hours:**	35
		1) Continued involvement on hand-on workshops for young women in science. 2) Visiting student from Thailand worked in the laboratory for several months learning basic methodologies related to algal curation, algal microbial biome assessment, and application of molecular tools to problems related to harmful algal blooms. 3) Two post-bac students were given projects that allowed them to develop additional bench skills. Each student has now been successfully placed in a research position withinn a Seattle Bio-tech company. Two additional students are progressing through this hands-on learning experience. 4) Presently, there are two Oceanography undergraduates doing cap-stone projects - growing algae in outdoor rafts in Lake Washington. Goal is to demonstrate that algae can be grown in large batches (for sourcing biofuel and other products) in the Pacific North West. They will present their data at the Mary Gates undergraduate symposium this spring. 5) Undergraduate in Microbiology is working with us to look at the bacterial biome of the HAB former Chrysochromulina.	
Acres of coastal habitat protected, enhanced or restored:	0	**Resource Managers who use Ecosystem-Based**	0

		Approaches to Management:	
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
Chysochromulina trasncriptomic and genomic libraries	Yes	Yes	No	0	
Heterosigma cyst transcriptome	Yes	Yes	No	0	
Dye for measuring DNA lipid levels in harmful algal species	Yes	No	No	0	

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Number of stakeholders modifying practices:	Sustainable Coastal Development
	# of coastal communities:

PARTNERS

Partner Name: Gordon and Betty Moore Foundation
Partner Name: Los Alamos National Laboratory (US DOE)
Partner Name: Pacific Northwest National Laboratory (US DOE)
Partner Name: University of Washington Commercial Endeavors, type: Academic Institution, scale: Local

IMPACTS AND ACCOMPLISHMENTS

Title: **The double life of Heterosigma: Washington Sea Grant research explores the behavioral, metabolic, and genetic mysteries of a fish-killing toxic alga**

Type: impact
Relevance, Response, Results:
Relevance: The single-celled alga Heterosigma akashiwo forms massive toxic blooms that have killed farmed salmon worth millions of dollars and, in 2014, ravaged a wild chum salmon run near Sequim, Washington. Heterosigma is enigmatic and resilient, resting for months in deep, cold waters until growth conditions improve. It then suddenly becomes active, swims back toward the surface, and blooms. Heterosigma distribution and bloom frequency appear to be increasing; understanding and anticipating toxic blooms is key to mitigating Heterosigma's effects.
Response: Washington Sea Grant researchers used diverse techniques to analyze Heterosigma changes between active and resting states. These included high-resolution videography to track swimming behavior and a suite of instruments and biochemical procedures to measure lipid quantity and quality and examine transitions in metabolic pathways. The team partially sequenced the genome of another algal species as a proxy for the larger Heterosigma genome and used the database developed to explore its transcriptome.
Results: The only integrated study of the behavioral and metabolic responses of Heterosigma, this effort shows that lipid levels are key to swimming capacity. Genetic sequencing uncovered several novel biochemical factors controlling the metabolic shifts that insulate this alga from unfavorable conditions. Data, which suggest a sexual cycle in Heterosigma, can be used to build a testable model relying on cell-activation responses to predict bloom formation.
Recap:
Recap: Washington Sea Grant researchers develop a toolbox of diverse techniques—from gene sequencing to videography—to probe the elusive toxic alga Heterosigma akashiwo and uncover a metabolic key to predicting harmful blooms.
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)
Partners:
Gordon and Betty Moore Foundation
Los Alamos National Laboratory (US DOE)
Pacific Northwest National Laboratory (US DOE)
University of Washington Commercial Endeavors
Related Partners: *none*

PUBLICATIONS

Title: **Genome sequence and transcriptome analyses of Chrysochromulina:**

metabolic tools for enhanced algal fitness in the prominent order Prymnesiales (Haptophyceae)

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2015
Uploaded File: *none*
URL: *none*

Abstract:
Background: Haptophytes are newly recognized as seminal players in aquatic ecosystem function. These organisms are important in global carbon sequestration, form destructive harmful algal blooms, and given their rich fatty acid content, serve as a highly nutritive food source to a broad range of eco-cohorts. Haptophyte dominance in both fresh and marine waters is supported by the mixotrophic nature of many taxa. Given their position as keystone members in aquatic communities, it is surprising that the nuclear genome sequence of only a single representative (Emiliania huxleyi: Isochrysidales) is available. Here we report the complete genome sequence of Chrysochromulina tobin (Prymnesiales). Preliminary transcriptome data collected at seven time points from a synchronized culture over a 24-hour light/dark cycle are also reported.

Results: The nuclear genome of C. tobin is small (59 Mb), compact (~40% of the genome is protein coding) and encodes an estimated 16,977 genes. The organism is haploid but contains a full complement of meiosis-related genes. Genes important to fatty acid synthesis, modification, and catabolism show distinct patterns of expression that are related to the ER and chloroplast localization of these processes when monitored over the circadian photoperiod. The C. tobin genome provides evidence of the first hybrid polyketide synthase/non-ribosomal peptide synthase gene complex reported for an alga; reveals the presence of anti-microbial peptides; gives evidence of macrolide biochemistry (e.g., tylosin synthesis, erythromycin esterase); shows the presence of multidrug and toxic compound extrusion proteins (MATE proteins); identifies a new xanthorhodopsin in haptophytes and reveals not only the presence of two “red” RuBisCO activases (with the chloroplast and nucleus each harboring one copy), but also shows that this gene duality is found across additional algal lineages. All aforementioned genes are expressed over the light/dark photoperiod, and several are shown to be products of lateral gene transfer.

Conclusions: The Chrysochromulina tobin genome sequence provides new information on the evolutionary history, ecology and economic importance of haptophytes.

Citation:
Hovde B., Starkenburg S.R., Deodato C., Chertkov O., Monnat R. and R.A. Cattolico (2015) Genome sequence and transcriptome analyses of Chrysochromulina: metabolic tools for enhanced algal fitness in the prominent order Prymnesiales (Haptophyceae). PLoS Genetics (in review)

This paper represents only the second haptophyte genome to be sequenced.

Journal Title: PLoS Genetics

Title: **Extensive horizontal gene transfer, duplication, and loss of chlorophyll synthesis genes in the algae.**
Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2015
Uploaded File: *none*
URL: http: //www.biomedcentral.com/1471-2148/15/16
Abstract:
Background
Two non-homologous, isofunctional enzymes catalyze the penultimate step of chlorophyll a synthesis in oxygenic photosynthetic organisms such as cyanobacteria, eukaryotic algae and land plants: the light-independent (LIPOR) and light-dependent (POR) protochlorophyllide oxidoreductases. Whereas the distribution of these enzymes in cyanobacteria and land plants is well understood, the presence, loss, duplication, and replacement of these genes have not been surveyed in the polyphyletic and remarkably diverse eukaryotic algal lineages.

Results
A phylogenetic reconstruction of the history of the POR enzyme (encoded by the por gene in nuclei) in eukaryotic algae reveals replacement and supplementation of ancestral por genes in several taxa with horizontally transferred por genes from other eukaryotic algae. For example, stramenopiles and haptophytes share por gene duplicates of prasinophytic origin, although their plastid ancestry predicts a rhodophytic por signal. Phylogenetically, stramenopile pors appear ancestral to those found in haptophytes, suggesting transfer from stramenopiles to haptophytes by either horizontal or endosymbiotic gene transfer. In dinoflagellates whose plastids have been replaced by those of a haptophyte or diatom, the ancestral por genes seem to have been lost whereas those of the new symbiotic partner are present. Furthermore, many chlorarachniophytes and peridinin-containing dinoflagellates also possess por gene duplicates.

In contrast to the retention, gain, and frequent duplication of algal por genes, the LIPOR gene complement (chloroplast-encoded chlL, chlN, and chlB genes) is often absent. LIPOR genes have been lost from haptophytes and potentially from the euglenid and chlorarachniophyte lineages. Within the chlorophytes, rhodophytes, cryptophytes, heterokonts, and chromerids, some taxa possess both POR and LIPOR genes while others lack LIPOR. The gradual process of LIPOR gene loss is evidenced in taxa possessing pseudogenes or partial LIPOR gene compliments. No horizontal gene transfer of LIPOR genes was detected.

Conclusions
We document a pattern of por gene acquisition and expansion as well as loss of LIPOR genes from many algal taxa, paralleling the presence of multiple por genes and lack of LIPOR genes in the angiosperms. These studies present an opportunity to compare the regulation and function of por gene families that have been acquired and expanded in patterns unique to each of various algal taxa.
Citation:

Hunsperger, HM, Randhawa T, Cattolico RA (2015) Extensive horizontal gene transfer, duplication, and loss of chlorophyll synthesis genes in the algae. BMC Evolutionary Biology 2015 15: 16.
Copyright Restrictions + Other Notes:
NB: I can not upload the pdf of this file....
Journal Title: BMC Evolutionary Biology

Title: **The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): Illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing**
Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2014
Uploaded File: MMETSP.pdf, 1174 kb
URL: http: //journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001889
Abstract:
"Community Page " presentation - overview of a large assemblage of co-workers on a targeted project.
Citation:
Keeling PJ, Burki F, Allam B, Allen E, Amaral-Zettler L, Armbrust V, Archibald J, Bell C, Beszteri B, Bidle K, Cameron CT, Campbell L, Caron DA, Cattolico RA, et al. (2014) The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. PLoS Biology 12(6): e10018889.
Copyright Restrictions + Other Notes:
Journal Title: PLoS Biology

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

Type: influenced Period: 2015-02-10: : 2015-12-31Amount: $55000
Purpose:
Development of a DNA-staining dye that would replace the toxic ethidium bromide conventionally used in the laboratory
Source: University of Washington

Type: influenced Period: 2014-09-01: : 2015-08-31Amount: $50000
Purpose:
Sequencing algal transcriptome and measuring DOC accumulation
Source: PNNL: award ONLY covers costs of sequencing samples and running DOC analysis

COMPLETION NARRATIVE

Uploaded File: Cattolico_6375_complet....5.pdf, 2753 kb

Objectives: *Heterosigma* forms massive blooms in marine and brackish waters world-wide. This organism has the ability to change from an actively swimming cell that resides in the photic zone to a non-motile resting cell that resides in coastal sediments (Fig.1). *Heterosigma* resting cells can exist in the sediments for long time periods. Similar to terrestrial plant seed, or fungal spores, these resting cells act as the progenitors of a new generation of cell that potentiate new HAB bloom events. Our ongoing studies with respect to *Heterosigma* life history transitions probe three questions: (a.) what physiological cues cause a cell change from one state to another; (b.) how does metabolism change to allow survival under stressed conditions; and (c.) why are some strains of this alga potentially a greater risk as bloom formers. In past studies, we determined a method in our laboratory to induce actively swimming vegetative cells to become non-motile resting cells, then activate back to the vegetative state. Our present study has provided the first analyses of shifts in cellular behavior and metabolic parameters that support successful *Heterosigma* life history transitions. Briefly, results are summarized below.

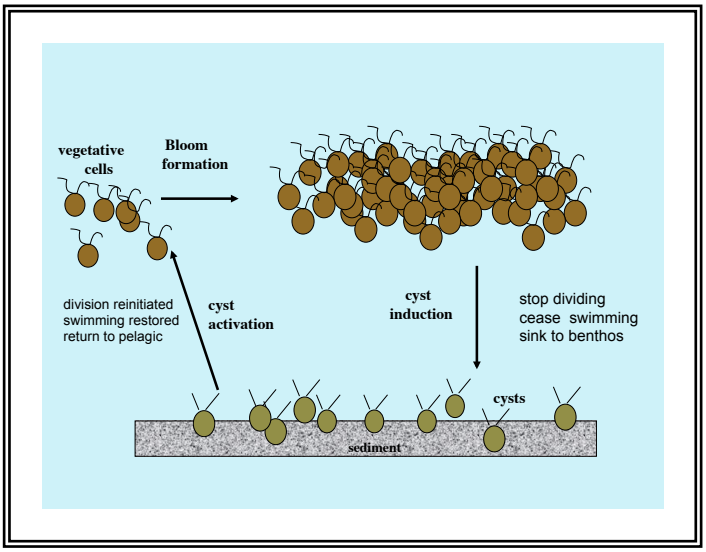


Fig. 1. *H.akashiwo* life cycle. Vegetative cells swim up-wards. If physical conditions are conducive, a high-density bloom forms. Stress conditions cause cyst induction. Cysts can be lost to physical processes, metabolic exhaustion or predation. Both vegetative and cyst life stages can act as a genetic sieve.

Methods: Samples for cells counts, swimming behavior, lipid analysis, and transcriptome assessment were recovered at select times during the dual life history transitions of *Heterosigma*.

Cyst induction: To induce cyst formation, vegetative cells harvested at the 6th hour of a 12 hour ligh:12 hour dark photoperiod, were placed in total darkness (10 ^{0}C) for 18 days, then returned to a 12 hour light:12 hour dark photoperiod. Temperature differences in vegetative cell maintenance before induction are reported in: Han et al, 2002; Tobin et al 2011 and Tobin et al 2013.

Details of methods that were used in this study are found in our recently published manuscripts: *cells swimming behavior* (Tobin et al, 2011; Tobin et al 2013); *lipid analysis* (Bigelow et al, 2011; Bigelow et al 2013); *Molecular analysis:* DNA isolation, sequencing and phylogentic analysis as well as RNA isolation and transcriptomic analysis (Starkenburge et al, 2014; Hovde et al, 2014; Hovde et al, 2015; Hunsperger et al, 2015). These studies were accomplished with laboratory- maintained *Heterosigma* cultures. *Heterosigma fingerprinting*: An extensive collection of *Heterosigma* ecotypes were obtained from the culture collection that is maintained at the University of Washington for this algal genus, or directly from field samples in the Salish Sea. Fingerprinting studies, that targeted 5 mitochondrial genes (*nad 2,4,5,7* and *cox*1), are in preparation (also see Karol et al, 2010).

Major Findings

I. Lipid biogenesis vs swimming behavior: Lipids serve as the metabolic currency of a cell, providing an energy sink for a variety of metabolic demands. Our data show the following:

(a.) *Lipid quantity* : Importantly, different *Heterosigma* ecotypes not only significantly vary in lipid content, but also have different programs of lipid utilization during their life history phase transitions. Dependent on induction conditions, *Heterosigma* cells lose lipids as they transition from vegetative to resting cell life history phase (Fig.2). For example, although *Heterosigma* ecotype UWC 13.03 had almost 3 times as much fatty acid in the vegetative state as *Heterosigma* ecotype CCMP 452, both ecotypes had a similar amount of fatty acid at the end of the cyst induction phase. Cyst survivorship for *Heterosigma* ecotype UWC 13.03 is exceedingly low when compared to *Heterosigma* ecotype CCMP 452.

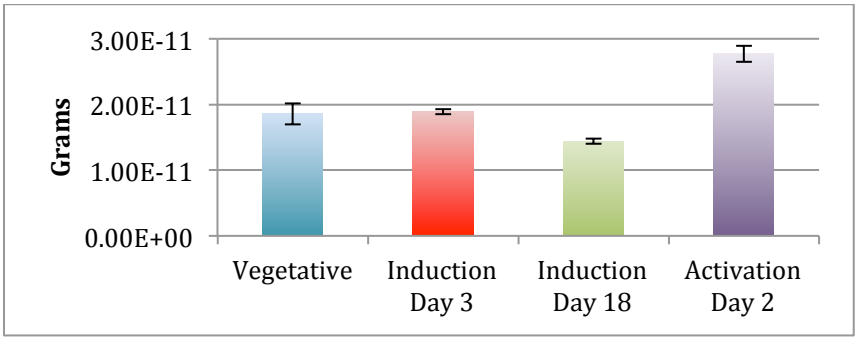


Fig. 2: Differences in the amount of total lipids per cell as *Heterosigma* (ecotype 452) transitions through phases of its life history.

(b.) *Lipid identity*: The types of lipids found in vegetative and resting cells differ (Fig. 3). GC/MS analysis show that resting cells have a greater quantity of long chain fatty acids (e.g., C18:4/5 and C20:5) that are needed to maintain membranes (e.g., unlike other algae in stress, *Heterosigma* maintain chloroplast integrity).

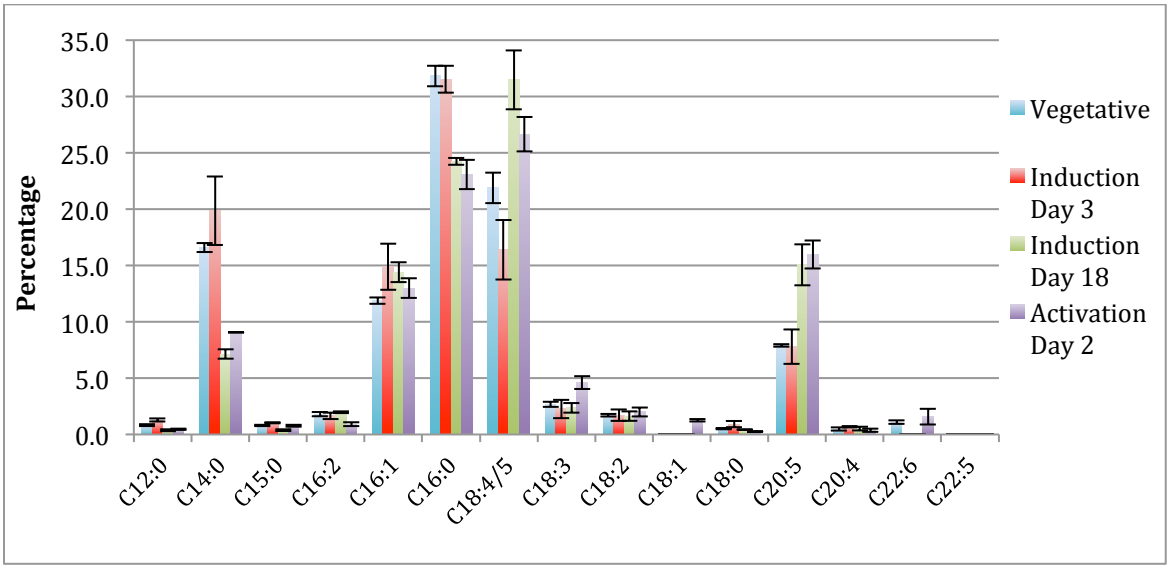


Fig. 3: GC/MS analysis of lipid content in cells as cultures progress through different life history phases. Each data point is a replicate of 4 samples (From Tobin et al, 2013)

(c.) *RNA abundance:* Transcriptome studies (see below) nicely correlate observations seen in lipid production. As lipid stores change, genes regulating fatty acid biosynthesis and catabolism are differentially regulated. For example, genes that encode enzymes responsible for the formation of unsaturated fatty acids (e.g., delta 5 fatty acid desaturase catalyzes C20:5 formation) are upregulated, while a gene important to lipid catabolism (e.g., 2,4 dienol CoA reductase produces trans-3-enoyl-CoA) is repressed (Fig.4).

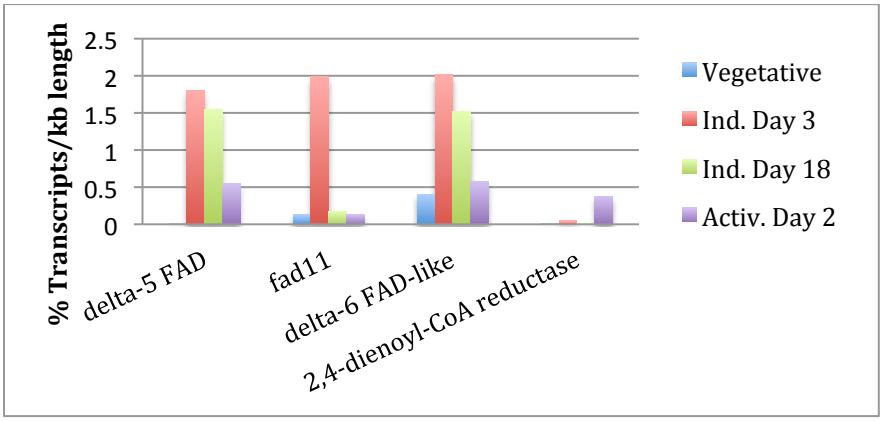
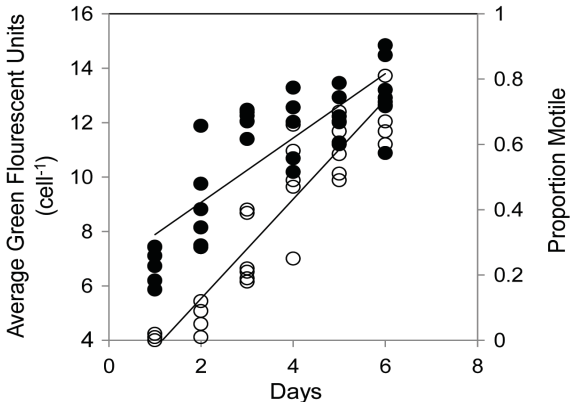


Fig. 4: Differential regulation of fatty acid biosynthesis genes in response to *Heterosigma* life cycle progression.

(d.) *Cell swimming behavior*: It has been suggested (Tobin et al, 2013) that the ability of an alga to swim is directly correlated with amount of lipid per cell. Our data suggests that the availability of fatty acids serves as a driver of swimming behavior. That is, as more cells enter the activated (i.e., swimming) state, lipid content per cell increases (Fig. 5). These data demonstrate the importance of lipid metabolism to the critical phase in *Heterosigma* survival – return to the photic zone. This laboratory has shown that *Heterosigma* cells can activate in the dark – but can not successfully divide without light (Han et al, 2002).



A model generated wherein observed values of resting cell viability, activation rate and cell division potential were used as variables has been used to probe the potential time scale of *Heterosigma* bloom formation (Tobin et al 2013). This model suggested that the size of the cell inoculum into the water column, a function of benthic viable resting cell density and activation rate, most strongly determines the time scales over which blooms can potentially form.

Fig. 5. Changes in neutral lipid content cell-1 and cell motility following exposure to activation conditions. The scatter plot shows the association of average neutral lipid content cell-1 (BODIPY 505/515 signal; black circles) and the proportion of motile cells (open circles) with time under activation conditions (in days) for *H. akashiwo* strains CCMP452 and UWC 13.01. A Spearman's rank correlation indicates that mean neutral lipid content ($r = 0.676$) and population motility ($r = 0.824$) both have significant, positive correlations with time under activation conditions ($N = 65$, $p = < 0.001$). From Tobin et al, 2013.

II. Metabolic control: Transcriptome analysis

I successfully competed and received 4 transcriptome libraries for *Heterosigma* as the alga moves from the vegetative to the resting cell and back to vegetative state through the Moore Foundation MMETSP program (Keeling et al, 2014). We have just begun to analyze metabolic pathways impacted by the transitional programs in the shift from vegetative cell to resting cells and back to vegetative life history phases. We are specifically targeting assessment of those genes important to the regulation of the Calvin Cycle, nitrogen processing and photosynthesis. This newly acquired data set is providing an exciting and completely new insight to *Heterosigma* life history changes. Interesting data has already been observed. Among the highlights that will be published:

(a.) *Mitosis and meiosis*

As anticipated, virtually all cell division was halted as vegetative cells progressed into stasis, and reassumed on resting cell activation. Significant to these observations is the depression, then up-regulation of genes that encode both alpha and beta tubulin (Fig. 6). Tubulin synthesis would be needed to support mitotic (and possibly meiotic – see below) events, flagellar function and intracellular transport activity.

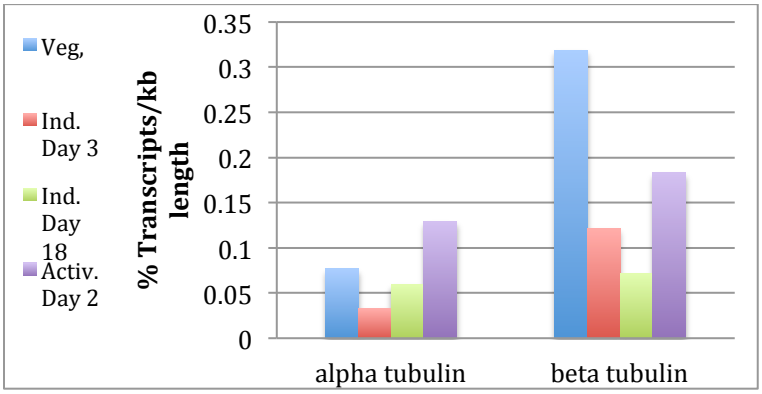


Fig 6: Differential regulation of tubulin genes as *Heterosigma* cells progress through life history phases.

A long-standing question in the *Heterosigma* literature has been whether this alga has a sexual cycle or is dependent solely on vegetative reproduction. Our earlier experiments suggested that a subset of resting cells and newly activated cells had higher ploidy levels – but this factor could result from to cells having duplicated their DNA, but not having successfully divided. Notably, our transcriptome data mining has allowed us to identify the presence of meiosis-specific genes in *Heterosigma* (Fig. 7). These genes include: *dmc*1, *mer*3, *spo*1, *msh*4 as well as several DNA recombination and repair genes (e.g., *msh*2, *msh*6, smc 2 to 5). Though this bioinformatic assessment is preliminary, these data are quite exciting. If verified, our observations open a completely new aspect of *Heterosigma* life history for study.

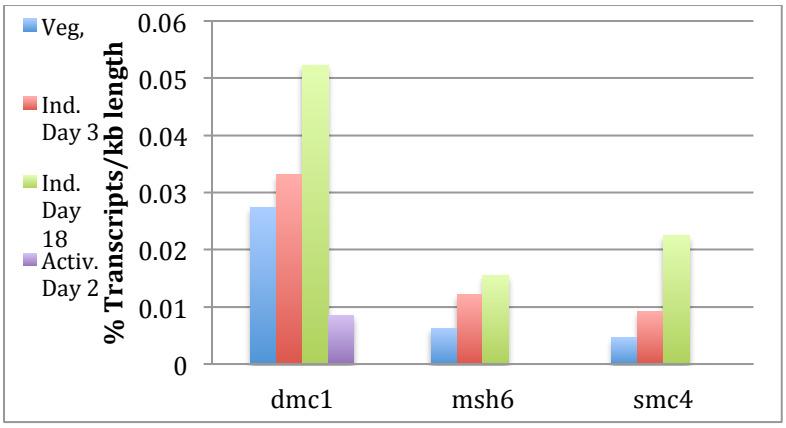


Fig. 7: Sampling of meiotic and recombination specific genes identified through preliminary bioinfomatic assessment of transcriptome data.

(b.) *Photosynthetic pigments*

For example, we have found that the gene encoding the enzyme that controls the penultimate step in chlorophyll biosynthesis is duplicated in *Heterosigma.* Our studies show that this gene set is very specifically regulated during resting cell formation and activation. As shown in Fig. 8, *por*2 is highly induced in resting cells while *por*1 expression is dominant in vegetative cells. Given that the POR protein is dependent on light to function, and given that *Heterosigma* can detect extremely low light levels, we hypothesize that the POR2 enzyme provides a mechanism for cells to maintain a chlorophyll complement, even under significantly diminished light availability. These data help us better understand what physiological cues support long term resting cell survival and/or successful resting cell induction at the metabolic level.

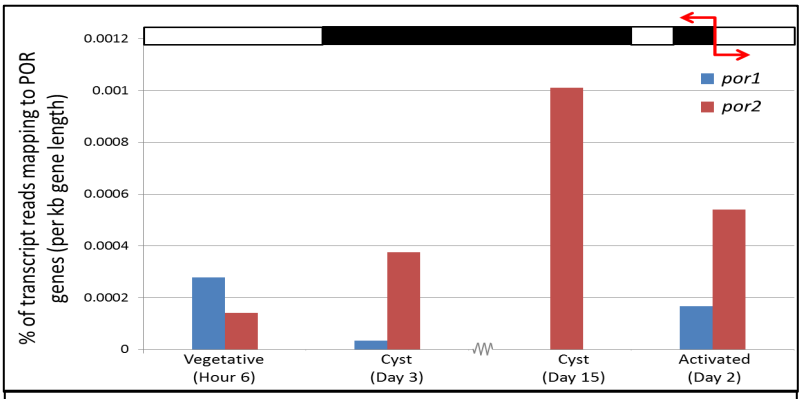


Fig. 8: *Por* mRNA abundance during *H. akashiwo* resting cell formation and activation. Arrow designates schematically abbreviated L/D photoperiod.

As anticipated, the fucoxanthin chlorophyll a-c binding gene transcripts were greatly (~ .9%) reduced in abundance when levels in cysts were compared to vegetative cells.

(c.) *CO_2 management*: We have discovered that *Heterosigma* has a duplicated RuBisCo activase gene – one copy is in the nucleus and a second in the chloroplast (Fig. 9). This observation opens an entirely new view of CO_2 processing, since RuBisCo activase has been shown to be indispensible to RuBisCo function. Our data show that the *Heterosigma* RuBisCo activase genes have a protobacterial origin, and are completely different from those found in terrestrial plants and green algae (chlorophyta). We have recently had a request from Los Alamos Laboratories for data concerning aspects of *Heterosigma* CO_2 processing, and are sending *Heterosigma* cultures, culture curation methodologies (including medium recipes developed in this laboratory), and

antibodies to RuBisCO to support their transformation efforts for optimizing carbon fixation in a biofuel producing algal strain.

Fig. 9: Phylogenetic assessment of CbbX proteins. From Hovde et al, 2015.

III. Ecotype variation: To investigate the extent of ecotype variability among populations worldwide, we sequenced five genes (*cox*1, *nad*2, *nad* 4, *nad* 5 and *nad* 7) from 50 strains of *H akashiwo* that are housed in the U. of W. culture collection. Each non-synonymous amino acid change in our sequence data set that resulted in an altered protein was assigned a unique number. Thus each of the 50 strains was assigned to one of twelve ecotypes types at the functional protein level (Black and Cattolico, unpub.). These haplotypes show some geographic affinity, although some regions, such as the Puget Sound, clearly have mixed populations. Although, most of the nucleotide changes encode synonymous amino acids, some changes are more drastic and code for a different amino acid in the protein. Some substitutions (polar/non-polar) have the potential to alter the shape and efficiency of a protein. These mitochondrial gene haplotypes could potentially cause functional changes in the cells.

IV. Genome mining: We have successfully sequenced the genome of the haptophyte *Chrysochomulina tobin* (Fig. 10). This alga is a member of a clade that has been found to be

dominant in many aquatic ecosystems world-wide. In spite of the huge contribution of haptophytes to both global CO_2 management, and their impact as HAB organisms, our work represents only the second organism to be sequenced in this huge phylogenetic assembly. Though haptophytes are toxic, and present in Pacific Northwest waters – to our knowledge, no focused study has targeted this taxon in our local. Both chloroplast (Fig. 11) and mitochondrial genomes of this alga have now been published (Hovde et al, 2014). The 59 MB *Chrysochromulina* genome has been a gold-mine of gene discovery (a new rhodopsin; chloroplast encoded gene for nitrogen repression; ribosomal genes lacking a zinc finger domain; several peptides that serve as anti-bacterials; a new polyketide synthase arrangement; previously unreported duplication genes critical to chlorophyll biosynthesis, etc.) as well as new insight to laterally transferred genes originating from green algae(Chlorophyta) and bacteria (Hunsperger et al, 2015; Hovde et al, 2015). Because *Heterosigma* has such a huge genome (the size of that found in humans), it is presently cost prohibitive to sequence the genome of this raphidophyte. However, using our newly defined *Chrysochromulina* database, we are successful probing the transcriptome of *Heterosigma*. The "closer" phylogentic relatedness of these two algae is giving new opportunity for gene identification. Important to our studies is the fact that several new genes, never previously identified in algae, have already been observed.

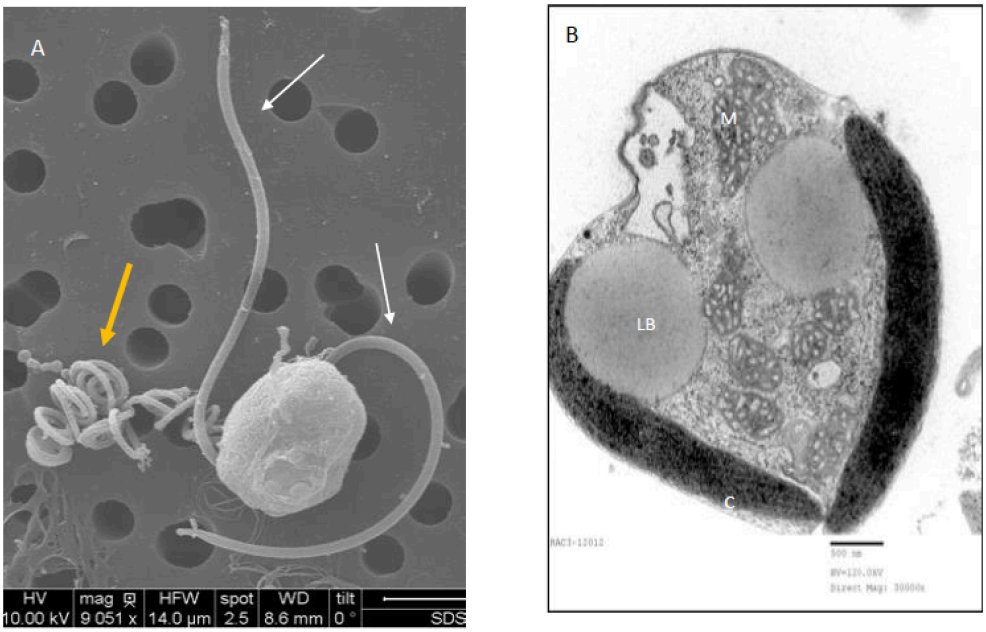


Fig. 10:*Chrysochromulina* cell structure. (A) Scanning electron micrograph of *C.tobin*. Two flagella are visible (white arrows) along with the coiled haptonema (orange arrow). (b.) Electron micrograph of whole cell: Lipid body (LB); chloroplast (C); Mitochondrian (M). Scale bar represents 500 nannometers. From Hovde et al, 2015.

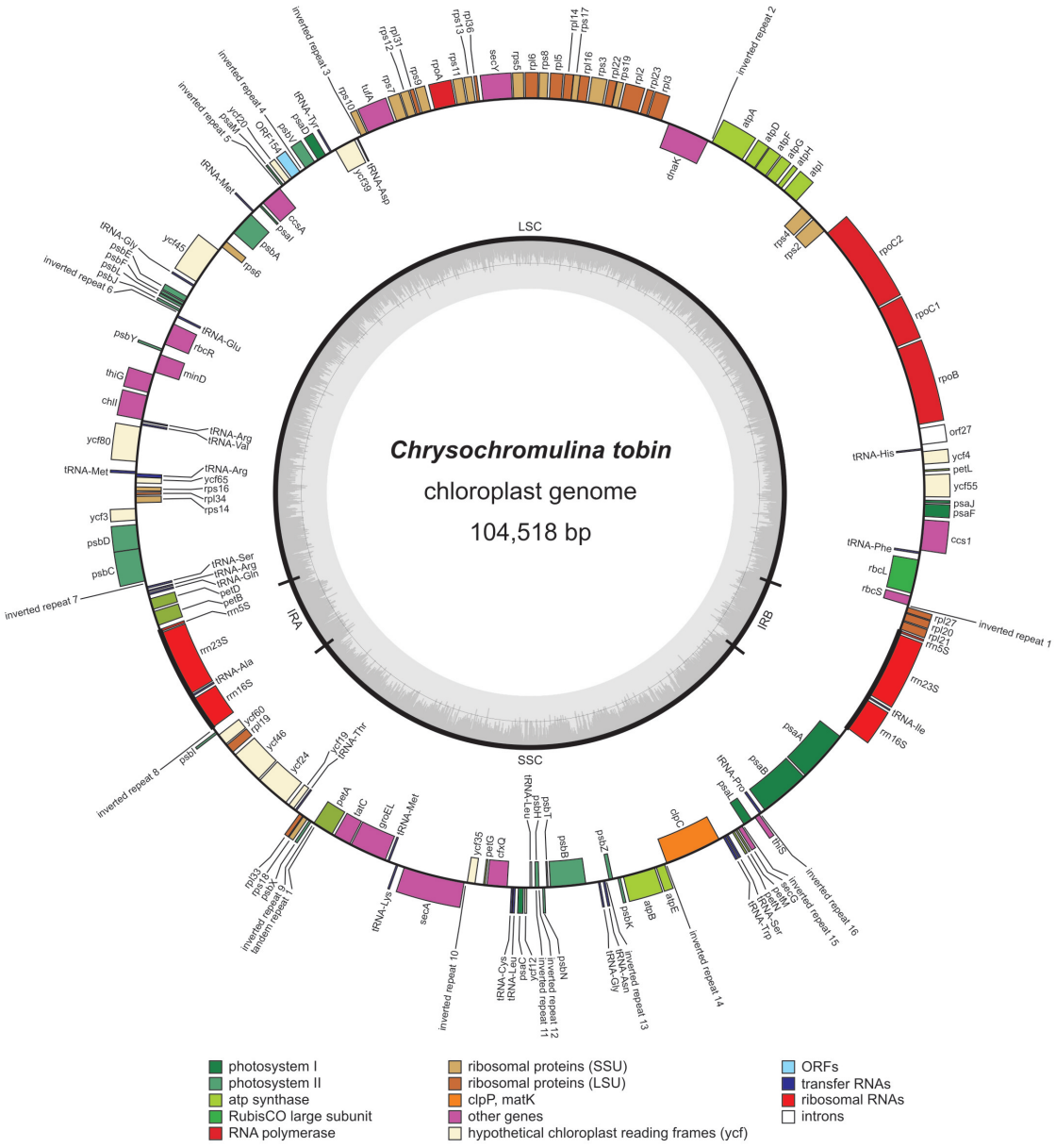


Fig. 11:. *Chrysochromulina tobin* chloroplast genome map. Genes facing outside are transcribed in the counter-clockwise direction and genes facing inside are transcribed in a clockwise direction. Two copies of the ribosomal operon are inverted and the repeat region contains no other genes beyond the ribosomal subunits. The small single copy (SSC) and large single copy (LSC) regions are labeled. Inverted and tandem repeats are also designated. From Hovde et al, 2014

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Students supported through this proposal

Graduate students

Megan Black
Blake Hovde
Heather Hunsperger
Elizabeth Tobin

Undergraduates (Represents the year that students entered the laboratory: many continued for at least 1 to 2 years to do research in our team)

2011

Nishant Sharma
Daniel Hunter
Jessica Farrow-Johnson Presently volunteering in the laboratory
Jessica Chess
Michelle Chang
Rebecca Bolla

2012

Suven Nair
Rahul Farwaha
Zachary Watanabe Visiting student – Pomona College
Boris Rozenberg
Jordan Prosch
Kelsey Lewis
Philippe Enos Awarded NASA Fellowship
Max Coulter
Phyll Eier
Chris Ford
Hannah Roberts

2013

James Miller
Connor Cummerlowe Visiting student – Pomona College
Tejinder Randhawa Awarded Mary Gates Fellowship
Ryan Sinit

2014

Sahutchai (Gla) Inwongwan Exchange student fro Thailand
Zach Smith
Jenni Bradstreet
Andrew Torok
Benjamin Pelle
Garrett Pickard

Partnerships

Gordon and Betty Moore Foundation
Los Alamos National Laboratory (US DOE)
Northwest Fisheries Science Center (US DOC)
Pacific Northwest National Laboratory (US DOE)
University of California

Outreach

Lecturing to community groups; providing supplies and mentoring for science projects; in-laboratory job mentorship supporting job shadowing; working with local teachers to generate new high school curricula; and planning no-cost oceanography-based field trips for under-represented science students. Participation in the Seattle Expanding Your Horizons science conference for middle-school girls, by leading groups through a hands-on algae-focused workshop that we designed. Graduate students and post-doctoral fellows continue to act as mentors to undergraduate students, helping to foster the next generation of scientifically informed Americans. These combined experiences should serve to produce young professionals highly desirable to both industry and academic institutions. Successful participation in competition for Fry-Hotson-Rigg and May Garrett and Mary Gates awards for undergraduatesYearly undergraduate presentations at theU of W Mary Gates Undergraduate Symposium

Technology transfer

University of Washington Commercial Endeavors
GC/MS micro-technique for assessing lipids
Technology for fingerprinting harmful algal species