

Update Report

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

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

STUDENTS SUPPORTED

Black, Megan, mmdblack@u.washington.edu, University of Washington, Biology, status cont, field of study Biology, advisor Cattolico, degree type PhD, degree date 2016-09-01, 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

Hovde, Blake, hovde@u.washington.edu, University of Washington, Genetics, status new, field of study Genetics, advisor R. Monnet, degree type PhD, degree date 2014-12-01, degree completed this period No

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 none

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 2014-10-01, degree completed this period No

Student Project Title Evolution of dual chlorophyll biosynthetic pathways in algae

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 Faculty or government research position

CONFERENCES / PRESENTATIONS

Presentation at Seattle Expanding Your Horizons science conference for middle school girls., public/profession presentation, 30 attendees, 2013-02-01

Chloroplast and mitochondrial genomes within the haptophyceae reveal early divergence in the Eukaryotic lineage

, public/profession presentation, 300 attendees, 2013-10-00

Mixotrophy in algal cultures, public/profession presentation, 30 attendees, 2014-03-00

Dual chlorophyll synthesis genes in the diatoms., public/profession presentation, 200 attendees, 2013-07-00

Growth and lipid production in the haptophyte *Chrysochromulina tobin* under salinity stress a potential role for stress response proteins, public/profession presentation, 900 attendees, 2013-11-00

Genome and transcript profiling of a new oil producing haptophyte, *Chrysochromulina tobin*, for targeted metabolic engineering., public/profession presentation, 900 attendees, 2013-11-00

Systems biology approach in understanding algal potential

, public/profession presentation, 30 attendees, 2014-09-00

ADDITIONAL METRICS

	Acres of degraded ecosystems restored as a result of Sea Grant activities	0
K-12 Students Reached	30	
Hands on workshop conducted by the graduate student and post-doctoral fellows from this laboratory contributed to the Seattle Expanding Your Horizons science conference for middle-school girls. By leading groups through a hands-on algae-focused workshop that we designed, new avenues of interest in science are opened to girls		
	Resource Managers who use Ecosystem-Based Approaches to Management	0
Curricula Developed	2	
Algal Cellular and Molecular Biology (biol. 446). Course designed to introduce biology majors to the use of algae as model test systems. Experiments include examination of		

algal toxicity on sea urchin egg development; examination algal microbial biomes and algal mixotrophy, and gaining proficiency in bioinformatic analyses by analyzing algal genome sequences.

Volunteer Hours 2

HACCP - Number of people with new certifications 0

These volunteers are looking at the bacterial biome, and considering questions of mixotrophy in algal systems. Presently, their work is focused on analyzing the bacterial biome associated with the water-polluting and toxic algal genus *Chrysochromulina*. This genus is being analyzed as a "model system" since it is associated with a minimum number of bacterial types (~ 10). However, given that this laboratory probably has the largest collection of *Heterosigma* strains in the world, and that this alga has now been shown to be mixotrophic, they will move to analyzing the bacterial biome of this alga as well.

Cumulative Clean Marina Program - certifications 0

PATENTS AND ECONOMIC BENEFITS

Description	Patents	Economic Benefit (\$)	Businesses Created	Businesses Retained	Jobs Created	Jobs Retained
Two patents for catatonic (2/1/2013 -	Actual 2	0	0	0	0	0

NLO chromophore for DNA and algal bioproduct standard. R/OCEH-7	1/31/2014) Anticipated (2/1/2014 - 1/31/2015)	0	0	0	0	0	0
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TOOLS, TECH, AND INFORMATION SERVICES

Description		Developed	Used	Names of Managers	Number of Managers
Dye for measuring DNA lipid levels in harmful algal species. R/OCEH-7	Actual (2/1/2013 - 1/31/2014) Anticipated (2/1/2014 - 1/31/2015)	0	1		0
Chysochromulin a as a model system for bacterial biome studies of harmful algal species. R/OCEH-7	Actual (2/1/2013 - 1/31/2014) Anticipated (2/1/2014 - 1/31/2015)	1	1		0
Genomics sequence, transcriptomic, and proteomic libraries for harmful algal species. R/OCEH-7	Actual (2/1/2013 - 1/31/2014) Anticipated (2/1/2014 - 1/31/2015)	1	1		0

HAZARD RESILIENCE IN COASTAL COMMUNITIES

Name of coastal community	County		Number of resiliency trainings / technical assistance services provided	Was community hazard resiliency improved (e.g., via changes in zoning ordinances) ?
NONE		Actual (2/1/2013 - 1/31/2014)	0	Yes
		Anticipated (2/1/2014 - 1/31/2015)	0	

None	Actual (2/1/2013 - 0 1/31/2014)	Yes
	Anticipated (2/1/2014 0 - 1/31/2015)	

ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices

Actual (2/1/2013 - 1/31/2014)

Anticipated (2/1/2014 - 1/31/2015)

Number of fishers using new techniques

Actual (2/1/2013 - 1/31/2014)

Anticipated (2/1/2014 - 1/31/2015)

Sustainable Coastal Development

Actual (2/1/2013 - 1/31/2014)

Anticipated (2/1/2014 - 1/31/2015)

Coastal Ecosystems

Actual (2/1/2013 - 1/31/2014)

Anticipated (2/1/2014 - 1/31/2015)

PARTNERS

Partner Name Gordon and Betty Moore Foundation

Partner Name Los Alamos National Laboratory (US DOE)

Partner Name Northwest Fisheries Science Center (US DOC)

Partner Name Pacific Northwest National Laboratory (US DOE)

Partner Name University of California

Partner Name University of Washington

IMPACTS AND ACCOMPLISHMENTS

Title Washington Sea Grant research uncovers a key metabolic factor in toxic algae, which can be used to predict harmful blooms

Type impact

Relevance, Response, Results Relevance The single-celled alga *Heterosigma* forms massive toxic blooms, which have caused fish kills and destroyed millions of dollars' worth of farmed salmon in Puget Sound and neighboring waters. *Heterosigma* is elusive and resilient; it can go into a resting state for months in deep, cold waters, then change to a vigorous active state when growth conditions improve, swim back to the photic zone and initiate new blooms. How exactly *Heterosigma* makes this switch remains unknown, but it is important for understanding and anticipating toxic blooms. Response WSG researchers used a broad range of techniques to analyze *Heterosigma*'s change from an active to resting state, including 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. Results This effort has produced the only highly integrated study of both the behavioral and metabolic

responses of *Heterosigma*'s two life-history profiles. The data show a correlation between lipid production and swimming capacity. These findings can be used to build a testable model using cell-activation responses to predict bloom formation. They also suggest the possible presence of a sexual cycle and give new insight into the metabolic shifts that insulate harmful algal species from unfavorable conditions.

Recap Recap WSG researchers used a broad, integrated toolbox of techniques to uncover important information about *Heterosigma*'s behavior, life-history parameters, and metabolism. These data can be used to build a testable model for predicting harmful algal blooms.

Comments Primary Focus Area OCEH (HCE) Secondary Focus Areas COCC (HRCC), OCEH (SSSS) State 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 Los Alamos National Laboratory (US DOE), Gordon and Betty Moore Foundation, Pacific Northwest National Laboratory (US DOE), University of California

PUBLICATIONS

Title The complete mitochondrial and chloroplast genomes of a novel haptophyte, *Chrysochromulina tobin*, reveal unique repeat architectures, and evolutionary signatures

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents
Publication Year 2014 **Uploaded File** none **URL** none

Abstract **Abstract** **Background** Haptophytes are widely and abundantly distributed in both marine and freshwater ecosystems. Genomics of across this taxon remain understudied despite their early evolutionary divergence from other algal species, and their prominent role in global carbon fixation. **Results** The complete mitochondrial and chloroplast genome sequences of the haptophyte *Chrysochromulina tobin* (Prymnesiales) provide new insight into the architecture and gene content of these two haptophyte organellar genomes. The mitochondrial genome (~34 kb) encodes 21 genes and contains a 9 kb region consisting of tandem repeats. This chromosome has lost the *nad7*, *nad9*, *nad10* and *nad11* genes, a finding similar to that seen in other haptophytes and rhodophytic algae, but not in cryptophytes or stramenopiles— suggesting a possible polyphyletic haptophyte-cryptophyte host cell origin. The 112 chloroplast-encoded genes in the ~105 kb chloroplast genome included several potentially novel proteins an NmrA-like protein (*ycf39*) with strong structural homology to other nitrate transcriptional regulators; a divergent 'CheY-like' two-component response regulator (*ycf55*) protein; and Tic/Toc (*ycf60* and *ycf80*) membrane transporter proteins. Most notably, the laterally transferred ribosomal protein RPL36 (used previously to infer cryptophyte and haptophyte evolutionary relatedness) has a newly identified zinc finger allowing for additional functionality. The two *C. tobin* chloroplast ribosomal operons contain multiple single nucleotide polymorphisms (SNPs) and differ in tRNA content and identity. This pattern is observed in rhodophytes, but few stramenopiles. An analysis of small (~12-40 bp) chloroplast encoded tandem and inverted repeats in *C. tobin* and 78 other

algal chloroplast genomes, show that repeat type, size and location were correlated with gene identity and taxonomic clade. Conclusion The completely sequenced mitochondrial and chloroplast genomes of *C. tobin* provide new insight into additional understanding of organellar function and its evolutionary history. These are the first organellar genomes to be determined for the haptophyte taxon Prymnesiales, a dominant algal clade in both ocean and freshwater systems and a major primary photosynthetic producer and contributor to global ecosystem stability. Keywords haptophytes, chloroplast genome, mitochondrial genome, repeat structure, repeat function

Citation Hovde, B., Starkenburg, S., Hunsperger, H., Mercer, L., Deodato, C., Jha, R., Monnat, R., and R.A.Cattolico. The complete mitochondrial and chloroplast genomes of a novel haptophyte, *Chrysochromulina tobin*, reveals unique repeat architectures, and evolutionary signatures. BMC Genomics- to be submitted.

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Journal Title BMC Genomics -to be submitted

Title A pangenomic analysis of the *Nannochloropsis* organellar genomes reveals novel genetic variations in key metabolic genes

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents
Publication Year 2014 Uploaded File [nanpaper.pdf](#) URL none

Abstract Background Microalgae in the genus *Nannochloropsis* are photosynthetic marine Eustigmatophytes that are of significant interest to the bioenergy and aquaculture sectors due to their ability to efficiently accumulate biomass and lipids for utilization in renewable transportation fuels, aquaculture feed, and other useful bioproducts. To better understand the genetic complement that drives the metabolic processes of these organisms, we present the assembly and a comparative pangenomic analysis of the chloroplast and mitochondrial genomes from *Nannochloropsis salina* CCMP1776. Results The chloroplast and mitochondrial genomes of *N. salina* are 98.4% and 97% identical to their counterparts in *Nannochloropsis gaditana*. Comparison of the *Nannochloropsis* pangenome to other algae from within and outside of the same phyla revealed regions of significant genetic divergence in key genes that encode proteins needed for regulation of branched chain amino synthesis (acetohydroxyacid synthase), carbon fixation (RuBisCO activase), energy conservation (ATP synthase), protein synthesis and homeostasis (Clp protease, ribosome). Conclusions Many organellar gene modifications in *Nannochloropsis* are unique and deviate from conserved orthologs found across the tree of life. Implementation of secondary and tertiary structure prediction was crucial to functionally characterize many proteins and therefore should be implemented in automated annotation pipelines. The exceptional similarity of the *N. salina* and *N. gaditana* organellar genomes suggests that *N. gaditana* be reclassified as a strain of *N. salina*.

Citation Starkenburg, S.R., Kwon, J., Chertkov, O., Jha, R., Twary, S., Rocap, G. and R.A. Cattolico (2013). A Pangenomic analysis of the *Nannochloropsis* organellar genomes reveals novel genetic variation in key metabolic genes. BMC Genomics. 2014, 15 212 doi

10.1186/1471-2164-15-212

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Journal Title BMC Genomics

Title Chrysochromulina sp. A proposed lipid standard for the algal biofuel industry and its application to diverse taxa for screening lipid content

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents
Publication Year 2013 Uploaded File [standardsfinal.pdf](#) URL none

Abstract Background The production of algal-derived oil has been recognized as an expanding new industry. Algal oil recovery and quality are impacted by both biological (algal cell type, growth physiology) and technical (recovery and extraction methodologies) constraints. Unfortunately, and unlike other well-established food and oil commodities, presently no universal reference standard exists for use in the algal oil industry. Results A laboratory-optimized strain of Chrysochromulina sp. is proposed as a natural matrix reference standard for algal fatty acid analysis. The alga is amenable to this purpose because (a) as a soft-bodied organism, it is susceptible to many disruption and fatty acid extraction techniques; (b) it has a high fatty acid content (~40% dry weight); (c) the growth response and lipid profiles of this organism are highly reproducible; (d) unlike many algae that have limited fatty acid distributions, Chrysochromulina sp. cells contain a broad representation of both saturated and unsaturated fatty acids ranging from C 14 to C 22. As a proof of concept, Chrysochromulina sp. was used as a reference standard for comparing 20 taxonomically diverse algal cultures, grown under identical physiological conditions and analyzed for fatty acid content using a micro-GC/MS analytical technique. Conclusions Expanding efforts in both commercial and research facilities will require the screening and monitoring of candidate algal strains for lipid synthesis. Universal adoption of a reference standard will provide a common platform to compare the fatty acid compositions of different algal strains grown under diverse environmental conditions and subjected to different oil recovery methods. A reproducibly generated natural matrix standard will have two distinct advantages (a) as a reproducibly generated standard, it can supplant reference products that vary markedly among suppliers; and (b) the use of a natural matrix standard will help in the identification and elimination of errors in lipid extraction, derivatization and analysis

Citation Cattolico (2013) Chrysochromulina sp. A proposed lipid standard for the algal biofuel industry and its application to diverse taxa for screening lipid content. Algal Research doi.org/10.1016/j.algal.2013.07.001

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Journal Title Algal Research

Title Behavioral and Physiological Changes during Benthic- Pelagic Transition in the Harmful Alga, Heterosigma akashiwo Potential for Rapid Bloom Formation

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents

Publication Year 2013 Uploaded File [liz_tobin.pdf](#) URL <http://www.ncbi.nlm.nih.gov/pubmed/24124586>

Abstract Many species of harmful algae transition between a motile, vegetative stage in the water column and a non-motile, resting stage in the sediments. Physiological and behavioral traits expressed during benthic-pelagic transition potentially regulate the timing, location and persistence of blooms. The roles of key physiological and behavioral traits involved in resting cell emergence and bloom formation were examined in two geographically distinct strains of the harmful alga, *Heterosigma akashiwo*. Physiological measures of cell viability, division and population growth, and cell fatty acid content were made using flow cytometry and gas chromatography – mass spectrometry techniques as cells transitioned between the benthic resting stage and the vegetative pelagic stage. Video-based tracking was used to quantify cell-level swimming behaviors. Data show increased temperature and light triggered rapid emergence from the resting stage and initiated cell swimming. Algal strains varied in important physiological and behavioral traits, including survivorship during life-stage transitions, population growth rates and swimming velocities. Collectively, these traits function as “population growth strategies” that can influence bloom formation. Many resting cells regained the up-swimming capacity necessary to cross an environmentally relevant halocline and the ability to aggregate in near-surface waters within hours after vegetative growth supporting conditions were restored. Using a heuristic model, we illustrate how strain-specific population growth strategies can govern the timescales over which *H. akashiwo* blooms form. Our findings highlight the need for identification and quantification of strain-specific physiological and behavioral traits to improve mechanistic understanding of bloom formation and successful bloom prediction.

Citation Tobin#, L., Grunbaum, D., Patterson, J., and R.A. Cattolico (2013). Behavioral and physiological changes during benthic-pelagic transition in the harmful alga, *Heterosigma akashiwo* Potential for rapid bloom formation. *PLoS 1* doi 10.1371/journal.pone.0076663

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Journal Title PLOS1

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

No Leveraged Funds Reported This Period

UPDATE NARRATIVE

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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. It is known that *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, these are summarized below. In these studies, vegetative cells harvested at the 6th hour of a 12 hour light:12 hour dark photoperiod (20 °C), are placed in total darkness (10 °C) for 18 days, then returned to a 12 hour light:12 hour dark photoperiod (20 °C).

Lipid production: 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.) Dependent on induction conditions and strain identity, *Heterosigma* cells lose lipids as they transition from vegetative to resting cell life history phase (Fig.1).

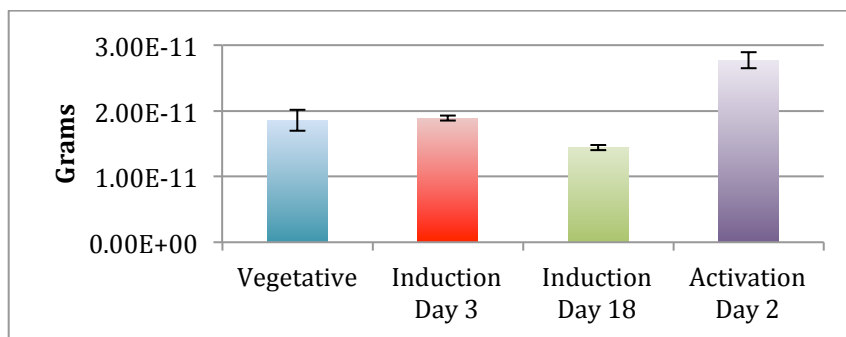


Fig.1: Differences in the amount of total lipids per cell as *Heterosigma* transitions through phases of its life history.

(b.) The types of lipids found in vegetative and resting cells differ. 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). On resting cell activation, lipid amount per cell increases significantly (Fig. 2);

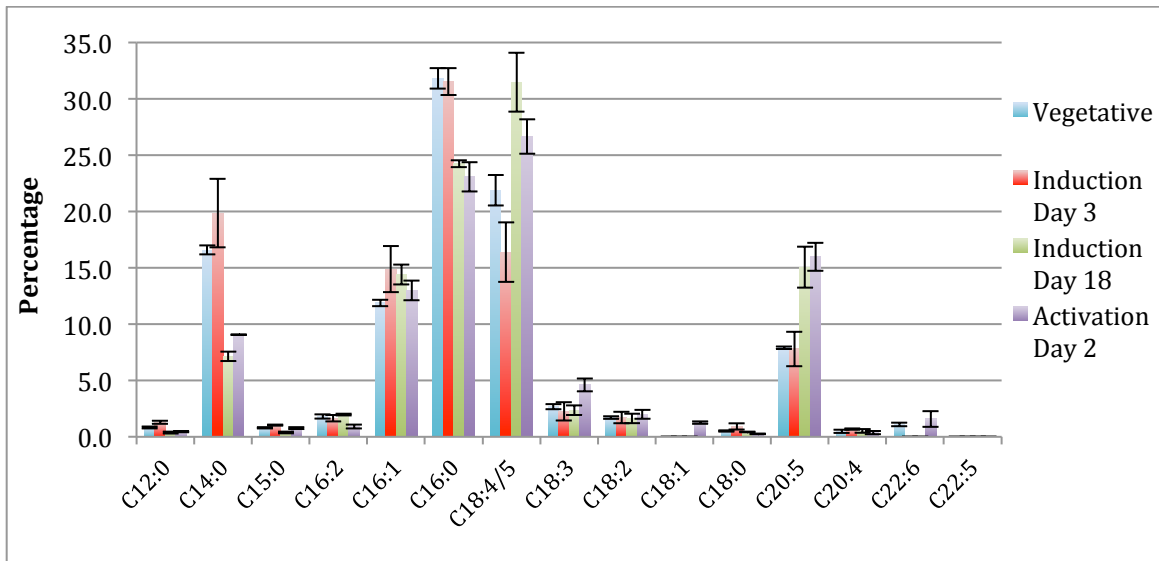


Fig. 2: GC/MS analysis of lipid content in cells as cultures progress through different life history phases. Each data point is a replicate of 4 samples.

(c.) Transcriptome studies 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.3).

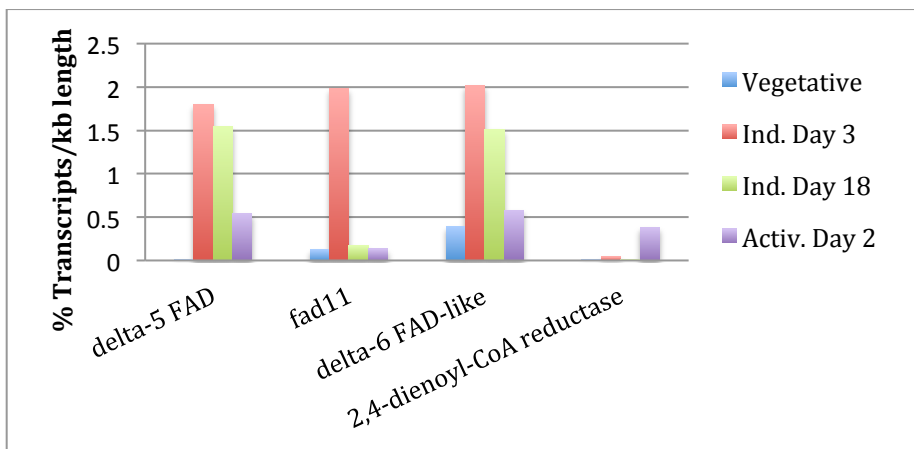


Fig.3: Differential regulation of fatty acid biosynthesis genes in response to *Heterosigma* life cycle progression.

(d.) Cell swimming behavior is directly correlated with amount of lipid per cell. That is, as an increasing number of cells enter the activated (i.e., swimming) state, lipid content per cell increases (Fig. 4). 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.

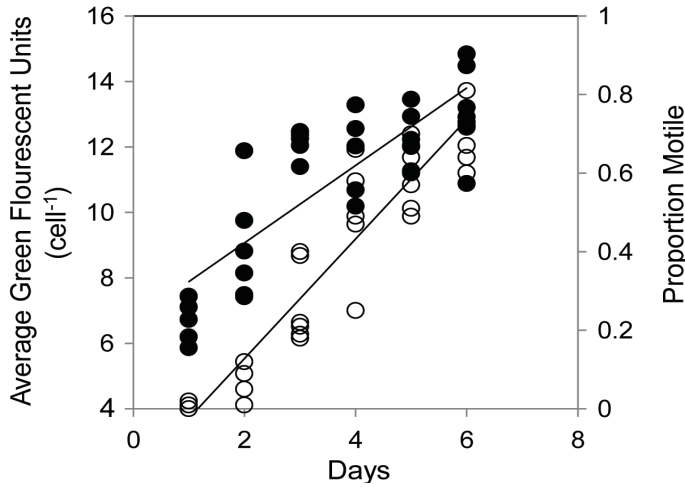


Fig. 4. Changes in neutral lipid content cell-1 and cell motility following exposure to activation conditions. The scatter plot shows the association of average neutral lipid content cell-1 (BODIPY 505/515 signal; black circles) and the proportion of motile cells (open circles) with time under activation 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 ($N = 65, p < 0.001$).

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. 5). Tubulin synthesis would be needed to support mitotic (and possibly meiotic – see below) events, flagellar function and intracellular transport activity.

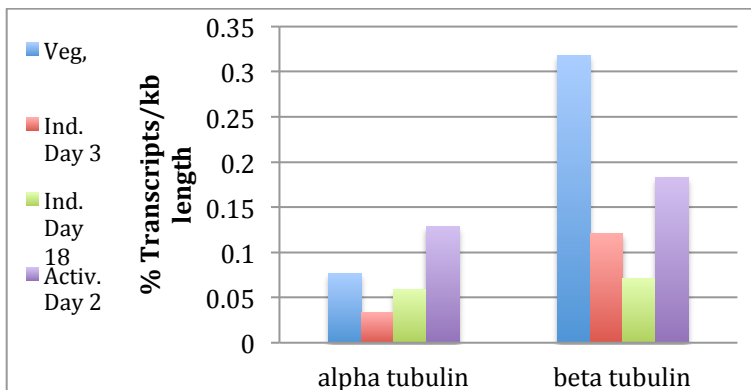


Fig 5: Differential regulation of tubulin genes as *Heterosigma* cells progress through life history phases.

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 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. 6). These genes include: *dmc1*, *mer3*, *spo1*, *msh4* as well as several DNA recombination and repair genes (e.g., *msh2*, *msh6*, *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.

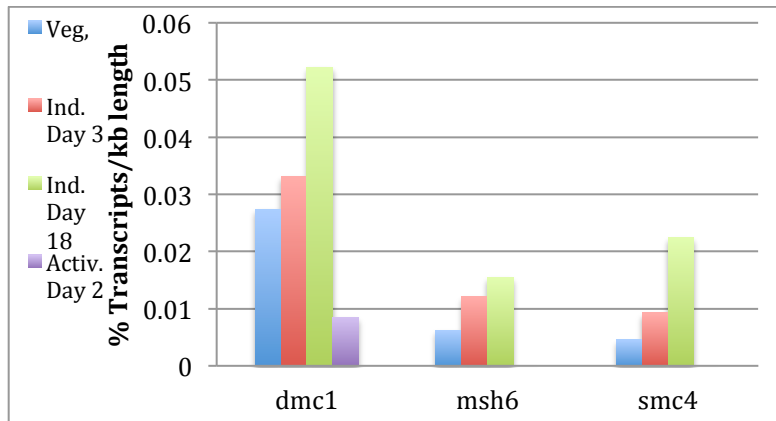


Fig. 6: Sampling of meiotic and recombination specific genes identified through preliminary bioinformatic assessment of transcriptome data.

Metabolic control: 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. Interesting data has already been observed. 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

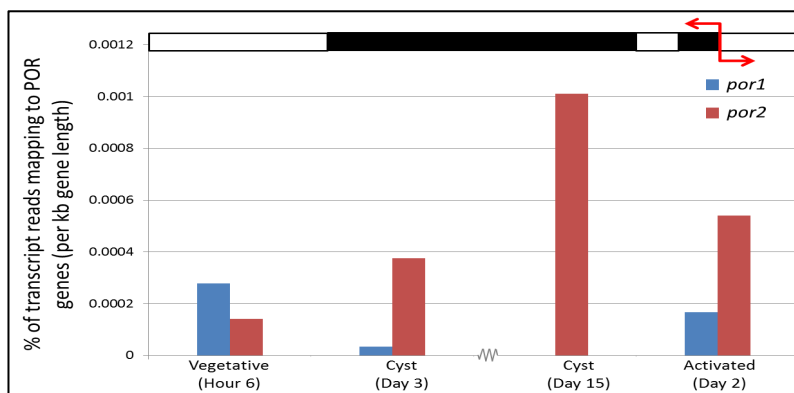


Fig. 7: *Por* mRNA abundance during *H. akashiwo* resting cell formation and activation. Arrow designates schematically abbreviated L/D photoperiod.

gene set is very specifically regulated during resting cell formation and activation. As shown in Fig. 7, *por2* is highly induced in resting cells while *por1* 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.