Investigating the Impacts of Oil Exposure and Changing Snow Cover on Sea Ice Diatom Communities in the Alaskan Arctic, Appendix: Gene Expression Changes in *Fragilariopsis Cylindrus* Under Different Irradiances with Crude Oil Exposure

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METHODS

This supplemental report builds on the Coastal Marine Institute final report, reference number M20AC10007 CMI AK-19-02-11, and does not repeat methodology presented there. Please refer to the report for a detailed description of associated methodology and results.

18S metabarcoding from mixed community experiments

DNA sequences were demultiplexed using the Mr. Demuxy package (https://github.com/lefeverde/Mr_Demuxy). FastQC v0.11.9 (Andrews, 2010) was used for quality control of generated raw sequences and primers were trimmed using Cutadapt (Martin, 2011). Demultiplexed and trimmed sequences were quality filtered, denoised, potential chimeras were removed, and classified using DADA2 (Callahan et al., 2016) within the Qiime2 v2021.2 wrapper (Bolyen et al., 2019) on a high performance-computing cluster through UAF Research Computing Systems using the full length 18S rRNA genes in the PR2 v4.14.0 reference database (Guillou et al., 2012). Sequences belonging to metazoans, unassigned, plastids and bacteria were omitted before further analysis. The R package phyloseq v1.42.0 was used for analysis and visualization of relative read abundance (McMurdie & Holmes, 2013).

Differential gene expression analysis of *F. cylindrus*

FastQC was used to check the quality of the raw reads (Andrews, 2010). Trimmomatic was used to trim the adapter sequence bases with low quality using the following parameters: ILLUMINACLIP: TreSeq3-PE.fa:2:30:10 Leading:3 Trailing:3 SLIDINGWINDOW:4:15 MINLEN:50 (Bolger et al., 2014). Quality was again assessed using FastQC. The annotated *Fragilariopsis cylindrus* genome, isolate CCMP 1102, was indexed and sample reads were aligned to it using Bowtie2 (Langmead & Salzberg, 2012). Alignments were converted to .bam files and sorted using SamTools (Li et al., 2009) and count tables were generated using Featurecounts (Liao et al., 2014). Genes were annotated by Kyoto encyclopedia of genes and genomes (KEGG) with the online tool GhostKOALA (Kanehisa et al., 2016) and used for pathway level expression analysis. Additional assignments were utilized from multiple sources compiled in Joli et al. (2023). For instances where several gene accessions code for the same enzyme/function, count data were combined before further analyses. Differential expression analysis was conducted with DESeq2 v1.38.2 using the Wald test with a significance cutoff of $p < 0.05$ and correction for multiple testing (Love et al., 2014). Principal component analysis was conducted with the R package pcaExplorer v2.26.1 (Marini & Binder, 2019). Heatmaps were generated using the R package ComplexHeatmap v3.14 (Gu et al., 2016).

RESULTS

18S metabarcoding from mixed community experiments

The top 10 most abundant protistan eukaryotes, as represented by 18S sequence reads, was largely composed of diatoms (n = 6), with two dinoflagellates, a chlorophyte, and the diatom parasitoid Cryothecomonas (Fig. 1). The relative community composition shift between these 10 genera during the 9-day observation was similar within the unoiled and oiled treatments. In the unoiled treatments, Fragilariopsis and Navicula spp. increased in relative abundance while Navicula and Nitzschia increased in the oiled treatments. Fragilariopsis had the highest relative abundance at the highest irradiance level in

the unoiled treatments.

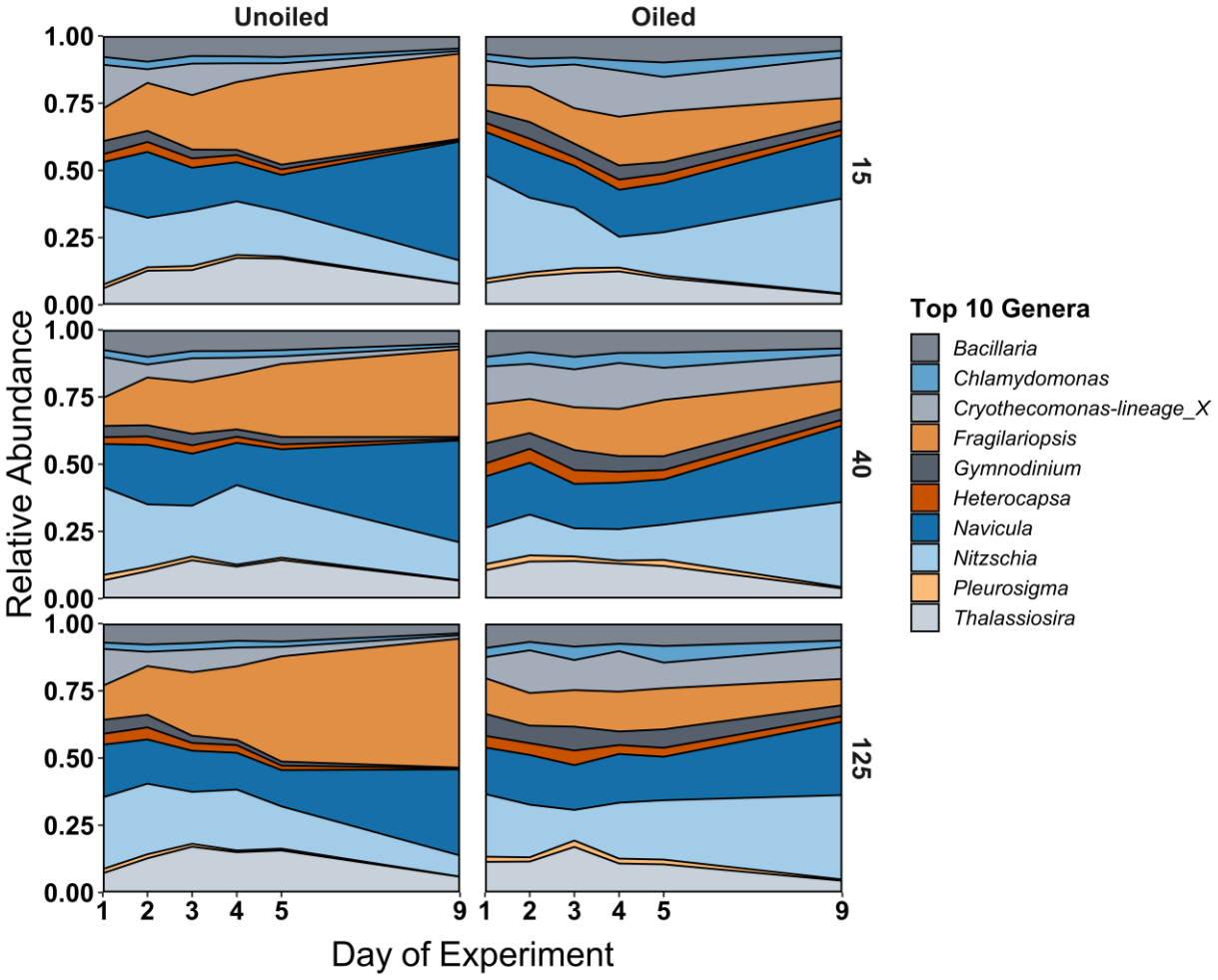

Figure 1. Relative abundance of the ten most abundant unicellular eukaryote genera determined by amplicon sequencing (18S rRNA) with and without oil at three different irradiances, 15 w/ snow in situ, 40 without snow in situ, and a representative melt pond 125 µmols photons m^2 s^{-1}.

Differential gene expression analysis of *F. cylindrus*

F. cylindrus treatments were distinct from one another based on the top 1,000 differentially expressed genes (Fig. 2). The unoiled low light treatment has the greatest distance between all other groups. 65 KEGG pathways were found to be significantly differentially expressed out of 101 total pathways. The heatmap in Figure 3 displays a subset of 14 significant pathways spanning core metabolic processes such as intracellular carbon cycling, cell growth and energy capture, as well as stress response pathways. Significant differences were observed between treatments relating to both irradiance and oil. In general, the unoiled treatments and the oiled treatments were more similar to one another while differences between light mediated expression was mostly confined to the unoiled treatments. Gene level differences related to photon capture and the electron transport chain, stress response, and carbon cycling were significantly different between treatments (Fig. 4). Antenna proteins associated with non-photochemical quenching (LHCx) were upregulated in the treatments with high light and containing oil. Basic light-harvesting complex proteins were significantly upregulated in the low light treatment without oil.

Molecular chaperones, oxidative stress, beta oxidation and chrysolaminarin degradation genes were significantly upregulated in the presence of oil.

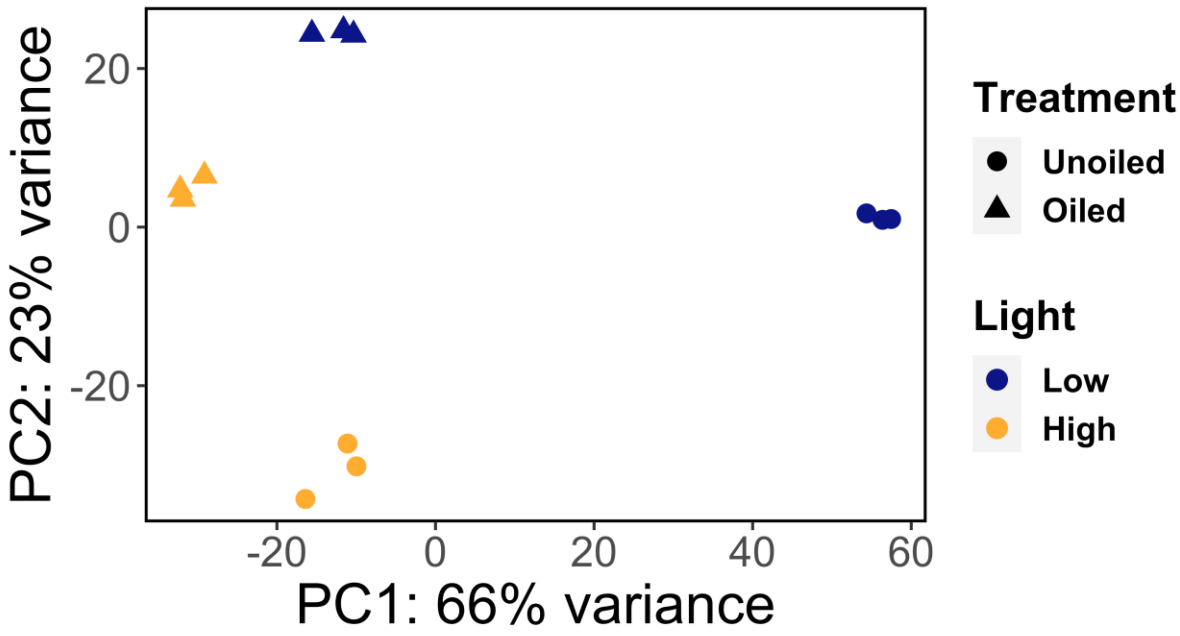

Figure 2. Principal component analysis grouping of *Fragilariopsis cylindrus* batch samples based on the top 1,000 differentially expressed genes.

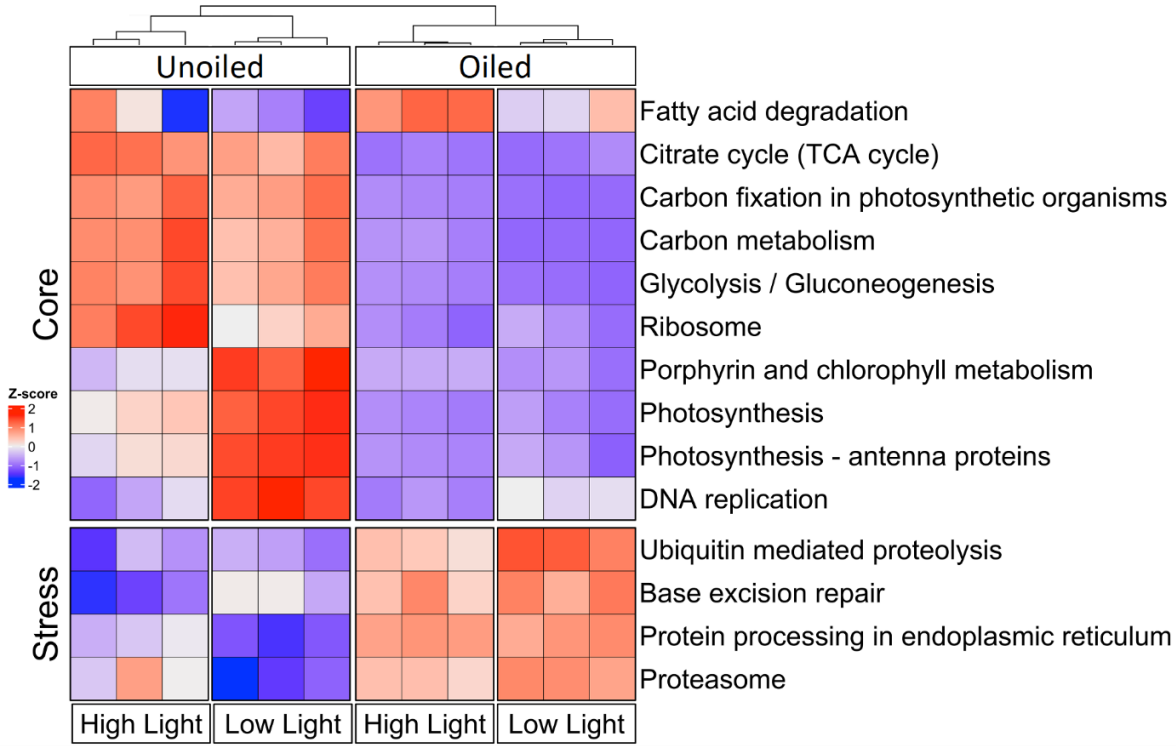

Figure 3. Subset of significant (p<0.05) differentially expressed KEGG pathways of *Fragilariopsis cylindrus* batch samples categorized as core or stress related metabolic pathways for all sample replicates. Red=upregulation and blue=downregulation.

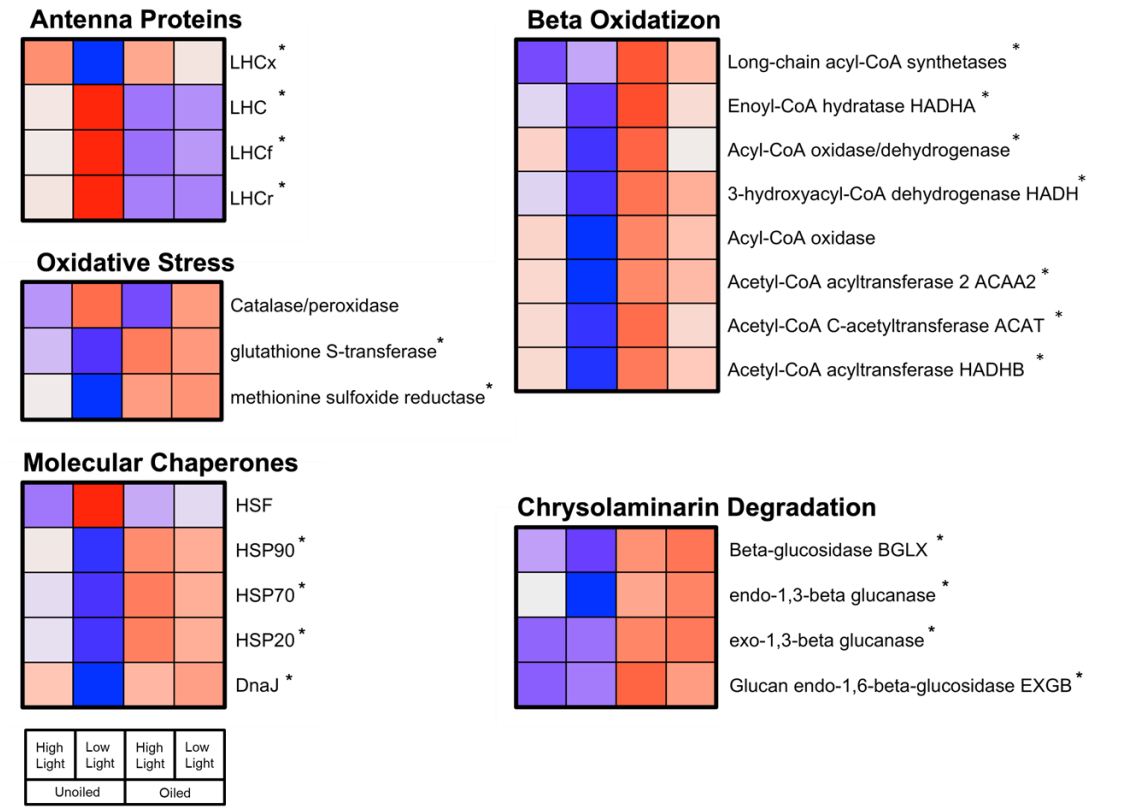

Figure 4. Subset of averaged genes and sample replicates of *Fragilariopsis cylindrus* batch samples from select gene groups of interest. * indicates significance ($p < 0.05$).

DISCUSSION

18S metabarcoding from mixed community experiments

Our sea ice inoculum, collected from Utqiaġvik landfast sea ice, was similar to previous years (pers. obs.). Initial observations of the sea-ice algal samples do not indicate a prevalence of dinoflagellates, as the sequencing data implies. Dinoflagellates are known to have a large number of 18S gene copies that can inflate their relative abundance based on sequencing techniques that utilize polymerase chain reaction (Gong and Marchetti, 2019). Cell count data will provide additional information on the community composition of these samples. Cryothecomonas is a genus of heterotrophic parasitoid that has long been known to occur in Arctic sea ice (Thomsen et al., 1991), but little is known about its distribution, species diversity, life cycle, or role in Arctic systems. These organisms were observed as parasitoids within diatom cells and consuming them from the inside out, a first observation from the Arctic and sea ice (Fig. 5).

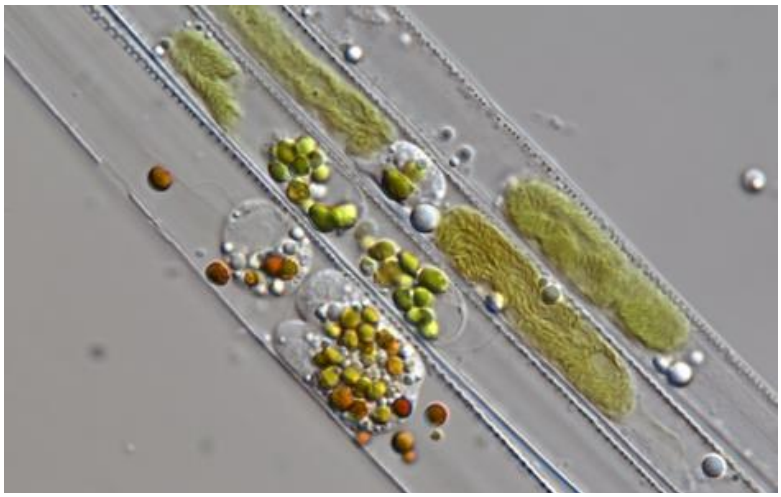

Figure 5. The parasitoid, *Cryothecomonas*, consuming diatom cellular contents from within the cell.

As sea ice and snow thickness declines, the amount of light reaching the bottom of the ice and the upper water column increases. Determining the response of Arctic sea-ice algae to increases in irradiance is an ongoing topic of research. We investigated how a sudden increase in irradiance influences community composition of sea-ice algae by monitoring their relative abundance using 18S metabarcoding (Fig. 1) during a 9-day batch experiment. The natural sea-ice algal community was exposed to irradiance measured in-situ with (15 µmols photons m-2 s-1) and without (40 µmols photons m-2 s-1) snow, and representative of a melt pond (125 µmols photons m-2 s-1). The lowest irradiance functioned as the control (no irradiance shift) while the other light treatments represented a 2.7 and 8.3-fold increase in irradiance, respectively. Crude oil contaminated medium was also used to investigate the combined effects of these two potential stressors. The community composition shifts in the unoiled treatments were similar despite a shift in irradiance. The increase in relative abundance of Navicula was most pronounced in the control (15 µmols photons m-2 s-1) treatment, while Fragilariopsis dominated at the highest irradiance. Navicula spp. are common in bottom-ice communities and well adapted to the light levels present in this environment. Fragilariopsis spp. are found in both sea ice and water, with F. cylindrus growing well in both. The increase in abundance of Fragilariopsis in the unoiled treatment follows the general succession of ice to water column blooms in the Arctic (Croteau et al., 2022). Our ice core samples had to be melted and maintained as liquid cultures so this shift was expected. Fragilariopsis decreased in relative abundance in the oiled treatments. This confirms our previous work using an isolate of F. cylindrus which exhibited the greatest decrease in growth rate in the presence of crude oil out of all isolates tested. The two genera, Nitzschia and Navicula, increased in relative abundance in the presence of crude oil by the end of the experiment. This behavior likely indicates a tolerance to oil exposure for these genera, with the possibility of limited growth occurring over the course of the experiment. Cell counts are still required to determine if growth occurred or whether cell death and senescence of more sensitive taxa lead to the change in community composition.

Differential gene expression analysis of *F. cylindrus*

A separate experiment using batch cultures of the F. cylindrus isolate investigated the gene expression response to oil exposure under ideal conditions, i.e., previously light acclimated and nutrient replete

condition, using RNA-seq. F. cylindrus was selected for its relatively high sensitivity to oil exposure as determined by the previously reported growth experiments, abundance in Arctic sea ice and water, and previously sequenced and curated genome. Data from the oil tolerant S. hyperborea has not yet been processed. F. cylindrus induced a major cellular stress response in the presence of crude oil, with indications of damage to multiple macromolecular groups (Figs. 3 & 4). Gene expression analysis indicates that there is a general shunt of energy from growth to repair and damage processing. This can be seen in Figure 3 as a reduction of ribosome and DNA replication and an increase in stress response pathways. Ubiquitin mediated proteolysis is a process where damaged proteins are ubiquinated, which acts as a signal for the proteasome to hydrolyze damaged proteins (Hofmann and Falquet, 2001). The pathway "protein processing in the endoplasmic reticulum" suggests repair and generation of new proteins, common when extensive cellular damage occurs. These pathways were upregulated in the presence of oil. The base excision repair pathway indicates that damage to DNA has occurred from oxidation and/or alkylation, the most common route of DNA base damage (Hang, 2007). The two oxidative stress proteins, glutathione S-transferase and methionine sulfoxide reductase, are involved with the destruction of reactive oxygen species that may have been generated during oil exposure resulting in DNA and protein damages (Fig. 4). In addition to these indications of a robust cellular stress response, DNA replication was downregulated in the oiled treatments, cell growth and division ceases during cellular stress and is further backed by the chlorophyll growth previously reported (Kültz, 2003; Kültz, 2020). Perhaps the most utilized genes for monitoring cellular stress response include the molecular chaperones known as Heat Shock Proteins (HSPs) that help to refold denatured proteins. Oil exposure resulted in the significant upregulation of the molecular chaperones HSP90, HSP70, HSP20 and DNAJ (Fig. 4). These data provide clear evidence that F. cylindrus was damaged in the presence of oil regardless of irradiance level.

A shift in metabolism can also be seen as a downregulation in photosynthesis (Fig. 3). Work by Kamalanathan et al. (2021) showed that the photosynthetic pigments of Thalassiosira pseudonana remain intact in the presence of oil, but light harvesting complex (LHC) proteins are damaged. Our chlorophyll a and phaeophytin pigment ratios support this observation that antenna pigments are not degraded, but liquid chromatography would be required to confirm this. A clear shift in LHC proteins can be seen as a shift from LHC, LHCf, and LHCr to the non-photochemical quenching (NPQ) LHCx. NPQ is the dominant mechanism for modulating diatom photosynthesis when irradiances are excessive by dissipating excess energy as heat through the coordinated action of diatoxanthin and LHCx (Buck et al., 2019). LHCx expression was similar for all treatments except for the low-light unoiled treatment which was the lowest. These data show that NPQ was likely occurring at the high irradiance light level in the absence of oil and is normal for F. cylindrus at these irradiances (Kropuenske et al., 2009). The induction of LHCx upregulation by the low-light oiled treatment suggests that NPQ is operating to prevent electron transport at photosystem II which would likely result in reactive oxygen species generation and oxidative damage. Kamalanathan et al. (2021) also showed that carbon fixation was is impaired, and that the addition of a carbon substrate helped to alleviate growth inhibition. The upregulation of beta oxidation and chrysolaminarin degradation genes show that alternative carbon substrates, i.e., lipids and chrysolaminarin, are being utilized for energy production in the citrate cycle (Fig. 4). Chrysolaminarin is the main storage carbohydrate of diatoms, and is visible as large clear vacuoles; we were able to observe the perceived degradation of chrysolaminarin during the experiment and will quantitatively confirm these observations using the stain Aniline Blue (Fig. 6).

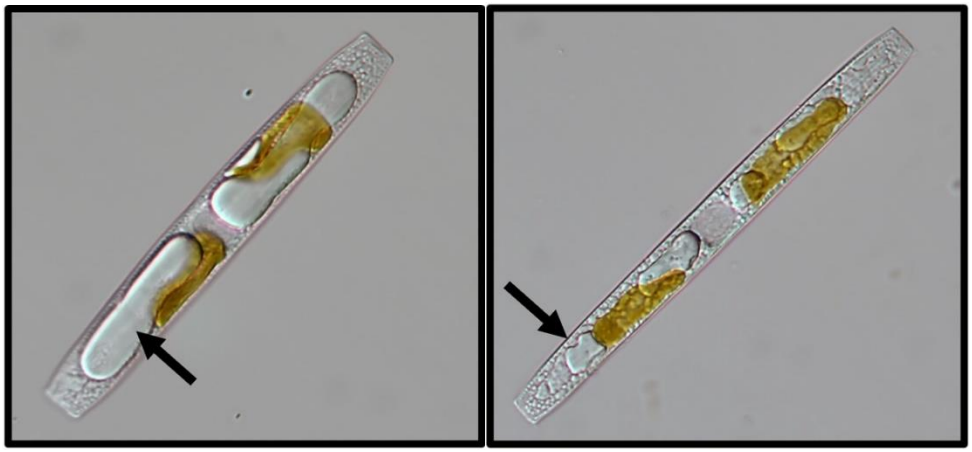

Figure 6. A representative diatom cell, *Nitzschia sp.*, from unoiled (left) and oiled (right) treatments at the end of the experiment. Arrows point to chrysolaminarin storage vacuoles.

CONCLUSIONS

Differences in community composition shifts in the presence of oil suggests differences in sensitivity. Previous work investigating sublethal growth rate inhibition helps explain these observations, including the decline of F. cylindrus in the presence of oil. Metabolic pathways measured from F. cylindrus batch experiments were differentially expressed between light and oil treatments. In response to oil, core metabolic processes were downregulated, including photosynthesis, while chrysolaminarin degradation genes were upregulated. A robust chrysolaminarin reserve may allow diatoms to persist through transient exposure events. Oil exposure induced a strong cellular stress response in F. cylindrus that will be compared to the oil tolerant S. hyperborea to identify molecular underpinnings that may be responsible for oil sensitivity, allowing predictions from untested taxa.

ACKNOWLEDGEMENTS

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STUDY PRODUCTS

Outreach

Eben Hopson 8th Grade Field Trip (2023). “Sea-Ice Biology.” Two days of sea ice science, one day in the field and one day in the classroom. Dilliplaine, K.

Presentations

Dilliplaine, K. and Hennon, G. M. M. "Crude oil concentration and light level determines the sublethal impact on sea-ice diatom growth" (2023) Alaska Marine Science Symposium

Dilliplaine, K. and Hennon, G. M. M. "Sensitivity of sympagic algae to crude oil in a brighter Arctic" (2023) Gordon Research Seminar

Dilliplaine, K. and Hennon, G. M. M. "Sensitivity of sympagic algae to crude oil in a brighter Arctic" (2023) Gordon Research Conference

Publications

Dilliplaine, K. and Hennon, G. M. M. "Impacts of crude oil on Arctic sea-ice diatoms modified by irradiance" (in review) Elementa Science of the Anthropocene

Dilliplaine, K. and Hennon, G. M. M. "Gene expression signatures of sea ice diatoms exposed to crude oil" (in prep) Ecotoxicology or similar journal

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