Summer 2016

The Effect of High-Intensity Visible Light on the Bloom Niches of the Phototrophic Dinoflagellates Alexandrium fundyense and Heterocapsa rotundata

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THE EFFECT OF HIGH-INTENSITY VISIBLE LIGHT ON THE BLOOM NICHES OF THE PHOTOTROPHIC DINOFLAGELLATES ALEXANDRIUM FUNDYENSE AND HETEROCAPSA ROTUNDATA

By

Elizabeth Colleen Cooney
June, 2016

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

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MASTER'S THESIS

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A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

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ABSTRACT

Photosynthetic bloom-forming dinoflagellates heavily influence coastal ecosystems worldwide. Many of these protist algae bloom in surface waters during summer when light intensities are highest. Despite its likely role as a top-down regulator of bloom formation, the consequences of high-intensity sunlight exposure on cells are not well understood. This study sought to reveal the effect of high light exposure on cells, keeping in mind the potential consequences for bloom-formation. The suite of conditions under which a species is best adapted to bloom is referred to here as its "bloom niche". To investigate, I measured physiological changes deemed relevant to bloom health in the two distinct species, *Alexandrium fundyense* and *Heterocapsa rotundata* after exposure to high intensity visible light. Ultraviolet radiation (UVR) was eliminated from this study because these wavelengths do not penetrate far in coastal waters due to absorption by dissolved and particulate organic matter. Cells were exposed to high-intensity environmental sunlight (209-1607 µmol photons m^{-2} sec^{-1}) and compared to control cells which remained at growth culture light levels. After exposure, all cells were returned to growth light levels for recovery. Photosynthetic efficiency (F_v/F_m) was measured periodically throughout the experiment as a proxy for light stress; cells were considered to be stressed when F_v/F_m decreased significantly from levels associated with growth light conditions. Chlorophyll-*a* (chl-*a*), average cell volume, cell concentration, and both dissolved and particulate dimethylsulfoniopropionate (DMSP) were measured pre-exposure, post-exposure, and post-recovery. Both *A. fundyense* and *H. rotundata* exhibited stress in response to high

light exposure. Chl-*a* and DMSP did not change in response to high light in either species. Swelling took place in *H. rotundata* cells, resulting in cell lysis in the highest light treatment, but no change in volume or measurable damage occurred in *A. fundyense*. Grazing experiments with the tintinnid ciliate, *Schmidingerella* sp. were performed with each species to assess the effect of light stress on predator-prey interactions. No difference in grazing rates were observed, however variance increased with higher light exposure, indicating sunlight may have some effect on prey behavior. The differences in response by the two dinoflagellate species can be explained in large part by differences in cell size and structure. These factors, in concert with environmental stressors, likely shape the bloom niche of a species.

ACKNOWLEDGMENTS

First, I would like to thank my adviser, Dr. Suzanne Strom, whose mentorship, hard work, support, and spirit were the foundation upon which I was able to build this thesis. Thanks also to my other committee members, Dr. Brian Bingham and Dr. Robin Kodner, in addition to Dr. Sylvia Yang, for their help and guidance with statistics, manuscript revision, and thought process. Special thanks is due to Kelley Bright and Kerri Fredrickson, who invested a great deal of their time and energy to train and support me, and who lent their capable hands to my research, time and time again. Thanks as well to Tyler Spillane, Dr. Katerina Schoo, Melissa Ciesielski, Marguerite Cooney, Luis Valentin, Lyle Cook, and Olivia Barberi for assistance with experimental execution and methods development. Amy Fotheringham, Rose Kawczynski, Gene McKeen, and Nate Schwarck provided ordering, travel, and logistical assistance. This research was made possible through financial assistance from National Science Foundation grant no. 1434842 in addition to grants provided by Shannon Point Marine Center, Huxley College of the Environment, and Western Washington University.

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INTRODUCTION

Bloom-forming phytoplankton play an important seasonal role in coastal ecosystems. In high concentrations, unicellular algae can substantially affect the physical, chemical, and biological properties of the photic zone and associated habitats (Parsons et al. 1984). Algal biomass is recycled, exported, and reallocated as blooms decline, and the relative contribution of this biomass to all potential fates in the ecosystem is determined by the drivers of bloom regulation. Grazers, including heterotrophic protists and zooplankton, assimilate algal carbon and nutrients and pass them on to higher trophic levels (Sherr and Sherr 1984). Limiting resources cause cells to slow growth and change life stages or enter dormancy (Anderson et al. 1985, Kremp et al. 2009). Abiotic stressors and grazing lead to cell damage and death, releasing dissolved organic matter back into the water column (Strom et al. 1997). Compounds leaked or excreted from phytoplankton and grazers are taken up by bacteria, reentering the microbial food web, while cell debris may be remineralized or may flocculate and sink, sequestering organic matter at the sea floor (Sherr and Sherr 2002). To combat potential sources of mortality such as grazing and abiotic stressors, each bloom species possesses a suite of physiological adaptations. These traits help determine the conditions under which a species will bloom and decline (collectively referred to here as a "bloom niche"), and ultimately influence the fate of accumulated biomass.

Dinoflagellates are a ubiquitous group of protistan plankton common in most coastal environments. While some species form symbioses with corals or anemones and

many are parasites, over 1500 species of free-living dinoflagellates spanning 117 genera have been categorized to date (Coats 1999, Gómez 2005, de Vargas et al. 2015). Of these species, it is estimated that approximately half are autotrophic or mixotrophic while the rest are exclusively heterotrophic (Gaines and Elbrächter 1987). This diversity is bolstered by a broad range of cell sizes and life history strategies, allowing dinoflagellates to fill many ecological niches. Photosynthetic bloom-forming dinoflagellates such as *Alexandrium fundyense* and *Heterocapsa rotundata*, the two species observed in this study, are commonly researched because they provide a plentiful seasonal food source for heterotrophic protists and larger zooplankton. Many species, including *A. fundyense*, are also of particular interest because they produce toxins and can grow into harmful algal blooms (HABs) that threaten fisheries and human health (Shumway 1990). While both species exhibit phototrophy, *H. rotundata* and several *Alexandrium* taxa are also bacterivorous, and *A. tamarense* (closely related to *A. fundyense*) has even been observed consuming other small protists (Jeong et al. 2005a, Jeong et al. 2005b, Seong et al. 2006, Yoo et al. 2009). The complex ecological roles and economic impacts of these organisms make them a relevant topic of study in the plankton ecology field.

Despite the relevance of photosynthetic bloom-forming dinoflagellates, the elements that regulate the formation and decline of blooms are not wholly understood. Many factors contribute to dinoflagellate growth and death, and each bloom-forming species is regulated by a unique hierarchy of influences (Smayda 1997). Grazing by herbivorous protists and larger zooplankton is a known ‘top-down’ (biomass-removing)

bloom regulator, limiting or reversing population growth. Grazing is considered one of the primary drivers of bloom regulation and decline (Watras et al. 1985, Sellner et al. 1991). However, sub-optimal salinity and temperature or limiting levels of resources such as light and nutrients constitute 'bottom-up' (growth rate-limiting) drivers that also play a significant role in bloom regulation (Watras et al. 1982, Lewandowska and Sommer 2010). These factors limit population growth through limitation of individual cell growth.

Sunlight, investigated in the present study, is frequently discussed as a potential limiting resource and hence a bottom-up regulator - reduced light availability can restrict cell growth rates when solar irradiance is low, when mixing is deep (Sverdrup 1953), or when bloom densities increase and shading occurs (Raven et al. 2006). However, at the height of summer when waters stratify, cells at the surface can experience irradiances that far exceed the requirement for photosynthesis, with detrimental consequences (Neale et al. 1993, Rijstenbil 2002). The question motivating the present study is whether high-intensity sunlight can act also as a top-down, population-level regulator either directly, by killing or damaging cells, or indirectly, by rendering cells more susceptible to predation.

Instead of full-spectrum sunlight, this study specifically focused on stress elicited by visible spectrum sunlight. I chose to eliminate ultraviolet radiation (UVR) from light treatments because the harmful biological effects of UVR are already well-documented (Harm 1980, Karentz et al. 1991). Additionally, UVR wavelengths dissipate at a relatively shallow depth in turbid coastal ecosystems because they are readily absorbed and scattered by dissolved organic matter. In a review of water column UVR worldwide, Tedetti

and Sempéré (2006) showed that, in coastal environments, the depth at which light reaches 10% of surface irradiance (Z10%) for ultraviolet B (UVB) radiation ranged from 0.09 to 6.7 m, and for ultraviolet A (UVA) from 0.3 to 22 m in depth. The majority of coastal sites presented showed a Z10% of <5 m for both UVB and UVA. Since absorption by dissolved organic matter is higher for shorter wavelengths (Blough et al. 1993), much of the visible spectrum can penetrate past these depths, constituting the photic zone (Kirk 1994).

The two bloom-forming dinoflagellate species *A. fundyense* and *H. rotundata* were chosen for this study because of their individual relevance and contrasting cell morphologies. *A. fundyense*, a potentially toxic, chain-forming species, grows to 28-40 µm in cell diameter and forms large HABs that impact coastal ecosystems and fisheries (Anderson et al. 2005, Douchette et al. 2005). *H. rotundata* has much smaller (9-14 µm length), conically shaped cells with an outer cell structure so delicate, it was once falsely thought to be a 'naked' dinoflagellate lacking thecal plates (Dodge and Crawford 1970). Both species are prey for tintinnid ciliates, a prolific bloom-regulating predator group in some coastal habitats (Stoecker et al. 1981, Verity 1985).

I designed the present study to look for sunlight-induced changes in cell physiology that might translate to top-down regulation, either through destruction of cells or promotion of microzooplankton grazing via changes in behavior. Photosynthetic efficiency (F_v/F_m) was measured throughout all experiments as a real-time proxy for stress. Henceforth, the term "stress" will be used to describe treatment conditions that resulted in a continuous decline in F_v/F_m during exposure to light. While it does not reveal the

mechanism of light response, this fluorescence ratio acts as a simple tool for detecting changes in cellular photosynthetic capacity (Krause and Weis 1984). Although a decrease in F_v/F_m may not exclusively be associated with damage to the cell, it is a well-documented symptom of exposure to damaging conditions including excessive light (Parkhill et al. 2001).

To assess more specific physiological changes during light exposure, I measured cell volume, permeability, and dinoflagellate population density. Changes in these characteristics indicate compromised cell function that could affect the ability of cells to maintain homeostasis, resulting in release of dissolved compounds and/or lysis. Physical deterioration and lysis intuitively constitute the starkest indication of top-down regulation by sunlight. However, changes to cell shape or size may indirectly lead to top-down regulation by increasing susceptibility to predation. The concentrations of dimethylsulfoniopropionate (DMSP) in and outside cells were measured with predation in mind as well. As a group, dinoflagellates are one of the most significant producers of DMSP (Caruana and Malin 2013). This organosulfur compound and, in some cases, its enzymatic cleavage products, dimethylsulfide (DMS) and acrylate, are thought to function in osmotic regulation (Kirst 1996), reactive oxygen defense (Sunda et al. 2002), and chemical predator deterrence (Wolfe et al. 1997, Wolfe et al. 2000, Strom et al. 2003). If high sunlight stimulates changes in DMSP production in or outside the cells, this would have interesting implications for the role of sunlight in chemical signaling within the planktonic community and their impact on predator-prey interactions.

By characterizing the physiological response of *A. fundyense* and *H. rotundata* to light, I was able to explore the potential top-down role of high-intensity sunlight on bloom-forming cells, including indirect effects on grazing by *Schmidingerella* sp. (formerly *Favella* sp.), a tintinnid ciliate predator. I found that sunlight affected these dinoflagellate species very differently. My observations suggest the presence of high light helps define the bloom niches of dinoflagellates, but the way it does so is species dependent.

MATERIALS AND METHODS

Cultures

Experimental *Alexandrium fundyense* cultures were started from strain CCMP 1911, obtained from the National Center for Marine Algae and Microbiota (NCMA). The strain was originally isolated from Sequim Bay, WA. The origin of the *Heterocapsa rotundata* culture used in this experiment is unknown.

A. fundyense and *H. rotundata* cultures were maintained in f/2 medium at 15°C. *A. fundyense* was grown at a light level of 53 µmol photons m^{-2} s^{-1} and *H. rotundata* was grown at 12 µmol photons m^{-2} s^{-1}. Both cultures were grown under a 12L:12D light cycle and transferred every three weeks to new media. *A. fundyense* and *H. rotundata* cultures were each used in light exposure response and predation experiments.

The tintinnid ciliate *Schmidingerella* sp. was used for predation experiments. The original culture was isolated from East Sound on Orcas Island in the Salish Sea. *Schmidingerella* sp. cultures were maintained at 15°C in ciliate medium on a 12L:12D light cycle. Two times per week, *Schmidingerella* sp. cultures were transferred and inoculated with a combined diet of *Heterocapsa triquetra*, *Rhodomonas* sp., *Mantoniella squamata*, and *Isochrysis galbana*.

Sunlight Exposure Experiments

Sunlight Exposure Staging

A plexiglass tank with dimensions of 50.5 by 50.5 by 31 cm deep was used for all experiments. The tank was placed in an unshaded area and filled to 10-15 cm below the top edge with raw sea water. To maintain temperature, the tank was supplied with a constant flow of sea water drawn from just offshore at a depth of 9 meters. The ambient temperature of the incoming sea water was always between 12 and 15°C, which was cool enough to keep bottles in the tank at the growth incubator temperature of approximately 15 to 16°C for the duration of each experimental exposure period. The tank was fitted with an Onset HOBO H8 Pro Series temperature data logger to measure temperatures in the tank and a water-filled polycarbonate bottle containing a mercury thermometer to estimate temperatures inside experiment bottles. A Li-Cor LI-1400 data logger with a 2π photosynthetically active radiation (PAR) sensor was stationed adjacent to the tank, collecting incident irradiance data integrated over 5 min intervals.

Light Treatments

Separate light exposure response experiments (henceforth referred to as A1 and H1, respectively) were conducted for *A. fundyense* and *H. rotundata* to characterize changes in the cells when exposed to high-intensity visible-spectrum sunlight. For each species, the experimental design included two treatments, “highest light” and “control”, with four replicates each. Henceforth, the terms “high light” and “high light treatments”

will be used when referring to non-control conditions or treatments from more than one experiment, collectively. Layers of screen were used to adjust light levels. *A. fundyense* control treatment bottles were covered by four layers to approximate growth incubator light levels of 53 µmol photons m^{-2} s^{-1}, while highest light treatment bottles were left uncovered. Due to observations of rapid cell death in *H. rotundata* under full sunlight levels during pilot experiments, highest light treatment bottles for this species were wrapped in three layers of screen to extend the duration of the exposure period. *H. rotundata* control bottles were wrapped in eleven layers to approximate growth incubator light levels of 12 µmol photons m^{-2} s^{-1}.

Experiment Execution

Experimental *A. fundyense* was diluted to 2300 cells ml^{-1} for A1 and to 40 cells ml^{-1} for the later grazing experiment. *H. rotundata* was diluted to 33,000 cells ml^{-1} in H1 and 2000 cells ml^{-1} for grazing experiments. Experimental cell concentrations were determined simply by the maximum densities achievable through regular transfer methods from source dinoflagellate cultures, while grazing experiment cell concentrations were based on bloom densities observed in the field (Anderson et al. 2005, Michaud et al. 2007).

Dinoflagellate cultures were combined and divided into eight 250 mL polycarbonate bottles, which block penetration of ultraviolet radiation. These experiment bottles were returned to the growth incubator for 1 h to rest before the experiment began. After the 1h acclimation, “pre-exposure” samples were collected from each bottle for measuring

variable fluorescence (F_v/F_m), Chlorophyll *a* (Chl-*a*), cell permeability, particulate and dissolved DMSP, and cell concentration. Bottles were then covered in treatment-specific screening and placed in the outdoor tank to begin the light exposure period of the experiment. Beginning at this point, F_v/F_m was measured every 15 min over the course of the experiment. Based on pilot studies, I determined that the light exposure period for both species should approach but not exceed two hours to adequately stress the cells without killing them. However, due to inherent day-to-day variability in sunlight intensity, the exact length of time in the sunlight was ultimately determined using real time F_v/F_m measurements. Using this gauge, *A. fundyense* was placed in sunlight for 1.75 h and *H. rotundata* was placed in sunlight for 1.5 h. After the exposure period, all bottles were taken indoors for a second round of sampling (henceforth referred to as "post-exposure"). Bottles were rid of any screening and returned to their growth conditions for the recovery period. After 1.5 h of recovery in the growth incubator for *A. fundyense* and 1.75 h for *H. rotundata* (the latter species extended due to slow recovery of F_v/F_m), a third and final round of "post-recovery" samples were collected.

Sampling

F_v/F_m was used as a proxy for cell stress (Krause and Weis 1984). This ratio represents photosynthetic efficiency via the maximum quantum yield of photosynthesis for stable charge separation of photosystem II (PSII), and is quantified according to the relationship, $F_v/F_m = \frac{Fm-F0}{Fm}$ in which F_m is the maximum Chl-*a* fluorescence in response to

photon saturation of PSII and F_0 is the dark-adapted minimum fluorescence (Krause and Weis 1984). To sample F_v/F_m, 1 mL samples were taken from each bottle, and dark-incubated at 15°C for 20 min. After dark acclimation of samples were measured using a Walz Water-PAM pulse amplitude-modulated fluorometer that measured F_0 from an initial weak light pulse followed by a high-intensity pulse to saturate PSII and measure F_m.

Chl-*a* concentrations were measured by filtering samples through 0.7 µm pore size 25 mm glass fiber filters. Pigments were extracted from these filters over 24 hours in a in 6 ml solution of 90% acetone in the dark at -20°C, and fluorescence was measured on a Turner 10-AU fluorometer before and after the addition of 2 drops of 1 N HCl. Chl-*a* was then calculated using the following equation (Lorenzen 1967):

$$Chl\ a\ (\mu g * L\ seawater^{-1}) = \frac{F_m k v (F_0 - F_a)(d)}{(F_m - 1)V_f}$$

where F_m is the maximum acid ratio, k is the calibration factor in µg Chl *a* * ml solvent^{-1} * instrument fluorescence unit^{-1}, v is the volume of acetone, F_0 is the fluorescence before acidification, F_a is the fluorescence after acidification, d is the sample dilution factor, and V_f is the volume of sample filtered.

Cell counts and cell volume data for *H. rotundata* were collected using live samples measured with a Beckman Coulter Z2 Particle Count and Size Analyzer with Z2 AccuComp software. For *A. fundyense*, cell count samples were preserved in a final concentration of approximately 2% acid Lugol's solution. Counts were done in a Sedgewick Rafter chamber and volume data were gathered using a microscope with Leica Application Suite X image

analysis software to measure length and width of cells. The formula for the volume of an oblate ellipsoid was used to calculate *A. fundyense* cell volume:

$$V = \frac{4}{3}\pi A^2 B$$

where A is the radius of the major axis and B is the radius of the minor axis of each cell.

DMSP samples were gravity-filtered through precombusted 0.7 µm effective pore size 25 mm glass fiber filters so as not to rupture the cells (Kiene and Slezak 2006). To measure DMSP in the extracellular (dissolved) phase, the first 4.5 mL of each sample's filtrate were caught in a 5 ml polystyrene culture tube, which was capped and stored at -80°C. Later, dissolved DMSP samples were thawed and sparged with N_2 gas for 1 min to remove any DMS present. Each sparged sample (4 ml) was then dispensed into a headspace vial containing 1 ml of 5 N NaOH, and sealed. Intracellular (particulate) DMSP was measured by placing filters into sealed 20-ml glass headspace vials containing 3 ml of 5 N NaOH. Upon being sealed, all prepared vial samples sat for at least 24 h to equilibrate before analysis.

Standards for particulate DMSP samples were prepared from pre-diluted DMSP solutions at the same time that samples were filtered and sealed into vials. Appropriate concentrations of pre-diluted DMSP solutions were pipetted into 20-ml glass headspace vials containing 3 ml of 5 N NaOH to bring each standard solution to its final concentration. Standard vials were then sealed, vortexed, and allowed to equilibrate for the same length

of time as the corresponding samples. Dissolved DMSP standards were made at the same time that samples were sparged and sealed into vials. Pre-diluted DMSP solutions were pipetted into headspace vials that contained 4 ml ultrapure water and 1 ml 5 N NaOH. As with particulate DMSP, standards for dissolved DMSP were allowed to equilibrate for the same amount of time as the corresponding samples. All samples were analyzed using a Shimadzu Gas Chromatograph 14-A equipped with a flame photometric detector and a Supelco packed Chromosil 330 column (Wolfe et al. 2000). The chromatograph was operated isothermally at 90°C with flow rates of hydrogen, air, and helium (carrier gas) at 50, 60, and 150 kPa, respectively. Particulate DMSP samples and standards were measured via direct injection while dissolved DMSP samples and standards were measured with a headspace sweep (flow rate of helium through headspace sampler was set at 40 kPa).

Cell permeability was measured using the high-affinity nucleic acid stain, SYTOX green (Thermo Fisher Scientific), which can only penetrate and stain cells with compromised plasma membranes (Lawrence et al. 2006). Prior to the experiment, samples of healthy cells from culture and heat-killed cells (prepared by placement in a water bath at 50°C for 10 min) of each species were prepared in the same fashion as samples in the following experiments. Aliquots (1 ml) were taken from each treatment and dispensed into 5 ml polystyrene culture tubes. Each sample was inoculated with 10µL of 50 µM SYTOX green in DMSO for a final concentration of 0.5 µM and then placed into dark incubation at 15°C for 10 minutes before being analyzed on a BD Facscalibur flow cytometer. For both species, flow rate was set on high (approximately 48.2 µl min^{-1}). The green fluorescence

detector (~530 nm) sensitivity was set at 269 volts for *A. fundyense* samples and 500 volts for *H. rotundata* samples. All samples were analyzed using CellQuest software. For the unstressed vs. heat-killed preliminary analysis, green (SYTOX green) and red (Chl-*a*) fluorescence signatures of these cells were used to define boundaries to differentiate intact culture cells from compromised heat-killed cells and debris (Figure 1).

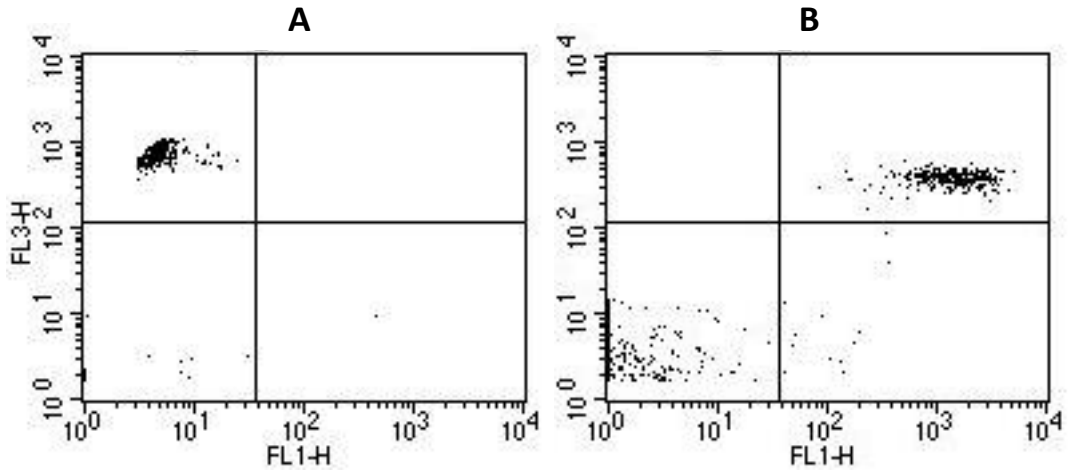

Figure 1. Examples of fluorescence scatter plots with a) live, non-light-exposed *Alexandrium fundyense* cells, and b) heat-killed *A. fundyense* cells. Y-axis shows red fluorescence (>650 nm) and x-axis shows green fluorescence (~530 nm), both on a logarithmic scale. The horizontal boundary line separates the detection events (signified by dots on the scatter plot) caused by particles of interest (upper) and debris (lower). The vertical boundary line was set to separate events caused by intact cells (left) from cells with compromised membranes that have been stained with SYTOX green (right). This way, a comparison could be made between upper left (UL) and upper right (UR) quadrants to determine the proportion of the population with compromised membranes.

In A1 and H1 experiments, a sample from each replicate within each treatment was prepared and run as described above at pre-exposure, post-exposure, and post-stress time points. The boundaries defined prior to experimentation remained fixed for these experiments. Heat-killed and non-exposed cells were run again as positive controls at the time of each experiment to be sure boundaries were still correctly placed.

Predation Experiments

Separate grazing experiments were performed for each species of dinoflagellate. Here, I will refer to the *A. fundyense* high light exposure experiment that included grazing as A2, and the *H. rotundata* exposure experiments with grazing as H2 and H3 (H3 was a re-do experiment performed because of changes in cell concentration in H2 that confounded the effect of light on grazing rates). Experimental design and sampling were nearly identical to the original light exposure experiments, except cell permeability was not measured. Additionally, instead of just one exposure treatment, two exposure treatments ("highest light" and "moderate light") accompanied the control for each species. Since these experiments took place in lower environmental light levels in late summer, the two exposure treatments for both A2 and H2 consisted of one screen layer (moderate light) and no screen (highest light)(irradiance data is presented in Results, Table 6). Exposure duration was 1.67 h for A2 and 1.58 h for H2, determined in part by real time F_v/F_m measurements. F_v/F_m was measured every 30 min and all other sampling took place before and after the exposure period and after a recovery period as previously described, the

latter lasting 2 h for both species. Due to low predator abundance and excessive stress resulting in cell loss in the H2 experiment, a simplified second grazing experiment was conducted for this species in which only F_v/F_m and cell concentration were measured in addition to grazing rates. The day of the experiment was in early autumn and had slight cloud cover. Since environmental light was considerably lower than in the earlier experiment, the same amount of screening used for the exposure treatment conditions in H2 were used in H3 to produce non-fatal irradiances. Bottles were exposed to light for 1.5 h before the grazing experiment, and recovery was not measured.

Prior to the grazing portion of each experiment, *Schmidingerella* cultures were sieved through 60 µm mesh, placed into fresh media, and held without food for 1 h so their food vacuoles would be empty. Roughly 1 h before the grazing experiment, unfed *Schmidingerella* were dispensed into 30 ml polycarbonate bottles containing enough ciliate media to bring the volume up to 25 ml, at 4 cells per ml. Bottles were then placed in the growth incubator until use. The grazing experiment began immediately after the dinoflagellate light exposure period, at which point the appropriate volume of dinoflagellate sample from each treatment was added to each prepared *Schmidingerella* bottle. Grazing took place in A2 for 40 min and in H2 and H3 for 20 min. A shorter grazing duration was allowed in *H. rotundata* due to the difficulty of counting high numbers of these smaller cells within the ciliates and for fear that chlorophyll would be digested more quickly, diminishing fluorescence necessary for analysis. At the end of the grazing period, samples were fixed in a final concentration of 0.5% glutaraldehyde and stained with 4',6-

diamidino-2-phenylindole (DAPI). Samples were filtered through 10 µm pore-size 25 mm polycarbonate filters after being allowed to sit overnight in darkness at 4°C. Filters were slide mounted with non-drying Type DF immersion oil (Cargille Laboratories Inc.) and frozen at -80°C until microscopic analysis. Within each sample, every *Schmidingerella* individual containing visible nuclei was counted and examined under blue light excitation to determine the number of dinoflagellates consumed. Dinoflagellates in ciliate food vacuoles were counted using the red fluorescence of their chlorophyll.

Statistics

Data for cell density, cell volume, cell permeability, Chl-*a*, particulate, dissolved, and total DMSP, and grazing were analyzed using SPSS 23.0 software. Only differences in post-exposure and post-recovery time points were compared statistically. Since samples were collected from the same bottles at each time point, alpha was adjusted to $\alpha=0.025$ using the Bonferroni correction to control for type I error associated with doing multiple tests. Independent samples T-tests were used with A1 and H1 data, while A2, H2, and H3 were analyzed using one-way ANOVA. Post-hoc Tukey tests were used for the latter to make pairwise comparisons. Even when transformed, some data sets (cell volume in H1 and A2, dissolved DMSP L cell vol^{-1}, total DMSP, and cells $gazer^{-1}$ in H2), did not meet the assumption of equal variance. In these instances, a Kruskal-Wallace one-way ANOVA analysis with multiple pairwise comparisons was employed.

Although Levene's test was employed to test for equal variance before each ANOVA, Hartley's F_{max} test was applied to grazing treatment data to compare variance between each pair of treatments in these experiments.

RESULTS

Overview

When exposed to high intensity visible-spectrum sunlight, both dinoflagellate species showed physiological responses, but *H. rotundata* showed a greater response than *A. fundyense*. While the effect on *A. fundyense* was limited to a drop in F_v/F_m, *H. rotundata* underwent a range of additional physiological changes, mostly associated with cell swelling (Tables 1-5 show measured result averages for all experiments). In the highest experimental light treatment, *H. rotundata* cells swelled and then lysed.

Table 1. *Alexandrium fundyense* experiment A1 result averages and standard deviations (SD). Bold, italicized values are significantly different from the control (no significant differences were observed). In treatments where SD is listed as "n/a", less than three replicate samples were taken.

Experiment A1		**Pre-exposure**		**Post-exposure**		**Post-recovery**	
	Treatment	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
cell concentration	Control	2290	174	2418	194	2193	114
(cells ml^{-1})	High Light	2340	145	2450	88.4	2250	63.1
cell volume	Control	22500	757	22800	996	22300	267
(μm^{-3})	High Light	22700	220	21000	1270	21600	989
cell permeability	Control	0.003	0.005	0.018	0.008	0.015	0.006
(%)	High Light	0.003	0.005	0.012	0.006	0.025	0.009
Chl-*a*	Control	38.3	2.98	36.5	3.53	37.8	3.36
(pg $cell^{-1}$)	High Light	37.9	2.91	36.9	2.59	37.7	0.36
Chl-*a*	Control	1710	190	1600	176	1690	143
(mg L cell vol^{-1})	High Light	1671.76	121	1750	96.7	1750	95.9
particulate DMSP	Control	129.37	n/a	148.51	17.19	166.02	12.56
(mmol L cell vol^{-1})	High Light	129.37	n/a	145.24	23.04	175.97	14.27
particulate DMSP	Control	2922	n/a	3377	233.2	3705	239.6
(fmol $cell^{-1}$)	High Light	2922	n/a	3039	379.1	3801	251.4
dissolved DMSP	Control	31.48	9.08	32.45	6.07	47.42	21.43
(nmol L $sample^{-1}$)	High Light	28.84	8.93	37.18	7.60	40.60	4.76
dissolved DMSP	Control	14.04	5.10	13.34	1.50	21.86	10.75
(fmol $cell^{-1}$)	High Light	11.92	3.46	15.20	3.08	18.04	1.89
total DMSP	Control	6.79	0.01	8.21	1.05	8.18	0.74
(μmol L $sample^{-1}$)	High Light	6.79	0.01	7.50	1.20	8.57	0.34

Table 2. *Alexandrium fundyense* experiment A2 result averages and standard deviations (SD). Bold, italicized values are significantly different from the control. Where dashes stand in the place of numbers, no measurements were taken. "Mod" is an abbreviation of "Moderate".

Experiment A2		**Pre-exposure**		**Post-exposure**		**Post-recovery**	
	Treatment	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
cell concentration	Control	1820	91.24	1880	113.21	1810	91.38
(cells ml^{-1})	Mod Light	1840	100.53	1770	109.62	1790	139.81
	High Light	1740	70.85	1810	52.07	1780	143.88
cell volume	Control	21700	1419.38	21700	312.63	20600	1061.72
(μm^{-3})	Mod Light	21400	929.45	***19900***	***911.79***	20600	1088.25
	High Light	20400	577.94	***19900***	***1071.80***	21000	1046.89
Chl-*a*	Control	38.0	1.72	36.1	1.39	37.1	1.52
(pg $cell^{-1}$)	Mod Light	36.8	1.37	37.0	5.46	35.3	4.68
	High Light	38.3	2.14	33.8	1.76	32.8	3.62
Chl-*a*	Control	1760	97.0	1670	70.4	1810	123
(mg L cell vol^{-1})	Mod Light	1720	132	1860	255	1720	257
	High Light	1880	136	1710	180	1560	192
particulate DMSP	Control	158.0	11.4	136.4	13.8	171.5	14.1
(mmol L cell vol^{-1})	Mod Light	151.7	7.5	***162.9***	***18.8***	170.9	19.1
	High Light	166.0	14.8	***162.7***	***11.1***	165.2	16.8
particulate DMSP	Control	3415.7	202.7	2932.8	279.2	3519.1	224.6
(fmol $cell^{-1}$)	Mod Light	3249.1	222.7	3467.8	400.2	3509.0	346.6
	High Light	3390.8	280.2	3465.2	236.2	3462.7	274.8
dissolved DMSP	Control	54.33	24.69	132.90	100.87	30.73	5.67
(nmol L $sample^{-1}$)	Mod Light	309.50	115.84	64.60	8.35	44.79	14.92
	High Light	662.99	423.65	41.98	11.36	47.81	13.30
dissolved DMSP	Control	30.08	14.40	73.33	61.54	17.10	4.09
(fmol $cell^{-1}$)	Mod Light	169.22	66.59	36.73	7.01	24.94	8.18
	High Light	377.92	236.40	23.09	5.55	26.83	6.70
total DMSP	Control	6.26	0.31	5.32	1.00	6.40	0.32
(µmol L $sample^{-1}$)	Mod Light	6.29	0.71	6.19	0.50	6.31	0.33
	High Light	6.56	0.28	6.31	0.39	6.17	0.10
grazing	Control	---	---	1.28	0.03	---	---
(cells $grazer^{-1}$)	Mod Light	---	---	1.44	0.16	---	---
	High Light	---	---	1.40	0.17	---	---
grazing (fraction grazers fed)	Control	---	---	0.71	0.03	---	---
	Mod Light	---	---	0.75	0.06	---	---
	High Light	---	---	0.76	0.07	---	---

Table 3. *Heterocapsa rotundata* experiment H1 result averages and standard deviations (SD). Bold, italicized values are significantly different from the control.

Experiment H1		**Pre-exposure**		**Post-exposure**		**Post-recovery**	
	Treatment	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
cell concentration	Control	32800	417	33000	699	32600	1020
(cells ml^{-1})	High Light	32500	499	32700	451	32900	485
cell volume	Control	173.33	13.28	179.38	8.07	184.20	4.05
(μm^{-3})	High Light	178.34	4.56	***209.68***	***0.39***	***211.93***	***5.72***
cell permeability	Control	0.083	0.017	0.077	0.018	0.146	0.099
(%)	High Light	0.083	0.017	0.086	0.015	0.065	0.043
Chl-*a*	Control	1.21	0.03	1.18	0.08	1.22	0.07
(pg $cell^{-1}$)	High Light	1.23	0.05	1.19	0.02	1.21	0.07
Chl-*a*	Control	6990	244	6570	343	6630	98.8
(mg L cell vol^{-1})	High Light	6870	256	***5660***	***125***	***5740***	***349***
particulate DMSP	Control	123.93	12.49	114.70	13.37	112.81	9.53
(mmol L cell vol^{-1})	High Light	111.11	9.75	99.04	6.43	99.70	4.87
particulate DMSP	Control	21.36	0.69	20.52	1.91	20.77	1.66
(fmol $cell^{-1}$)	High Light	20.07	1.79	20.77	1.37	21.13	1.13
dissolved DMSP	Control	17.36	3.85	14.57	1.06	12.39	3.58
(nmol L $sample^{-1}$)	High Light	17.16	2.95	15.38	1.95	12.16	1.90
dissolved DMSP	Control	0.53	0.12	0.44	0.03	0.38	0.12
(fmol $cell^{-1}$)	High Light	0.53	0.10	0.47	0.05	0.37	0.06
total DMSP	Control	0.73	0.032	0.69	0.064	0.69	0.043
(µmol L $sample^{-1}$)	High Light	0.67	0.068	0.69	0.051	0.71	0.031

Table 4. *Heterocapsa rotundata* experiment H2 result averages and standard deviations (SD). Bold, italicized values are significantly different from the control. Treatments in which no signal was detected are marked "ND". Where dashes stand in the place of numbers, no measurements were taken. "Mod" is an abbreviation of "Moderate".

Experiment H2		**Pre-exposure**		**Post-exposure**		**Post-recovery**	
	Treatment	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
cell concentration	Control	11800	1140	11900	166	12100	465
(cells ml^{-1})	Mod Light	11700	841	12000	1660	10500	835
	High Light	12100	519	***8400***	***1460***	***7340***	***1040***
cell volume	Control	181.17	4.56	185.02	5.85	204.46	5.74
(μm^{-3})	Mod Light	182.46	4.82	211.39	2.23	217.53	12.09
	High Light	179.94	9.88	200.70	46.63	***138.11***	***15.12***
Chl-*a*	Control	1.40	0.11	1.41	0.03	1.36	0.07
(pg $cell^{-1}$)	Mod Light	1.47	0.10	1.30	0.11	1.32	0.09
	High Light	1.42	0.04	***0.44***	***0.14***	***0.33***	***0.08***
Chl-*a*	Control	7740	532	7640	348	6680	493
(mg L cell vol^{-1})	Mod Light	8050	670	***6150***	***561***	6050	275
	High Light	7920	623	***2190***	***715***	***2400***	***479***
particulate DMSP	Control	150.5	14.3	138.2	10.8	126.5	5.5
(mmol L cell vol^{-1})	Mod Light	154.3	12.7	125.2	11.8	138.1	10.6
	High Light	152.3	18.0	95.0	28.4	***ND***	***---***
particulate DMSP	Control	27.3	2.5	25.7	1.4	25.8	0.9
(fmol $cell^{-1}$)	Mod Light	28.1	1.9	26.4	2.3	***30.0***	***1.2***
	High Light	27.3	2.0	***18.3***	***2.9***	***ND***	***---***
dissolved DMSP	Control	13.28	1.55	7.92	0.73	13.43	1.62
(nmol L $sample^{-1}$)	Mod Light	9.94	3.38	10.74	1.81	12.32	1.55
	High Light	9.85	0.98	***66.70***	***10.47***	***20.84***	***2.91***
dissolved DMSP	Control	1.15	0.18	0.68	0.06	1.13	0.17
(fmol $cell^{-1}$)	Mod Light	0.88	0.34	0.93	0.25	1.20	0.23
	High Light	0.83	0.06	***8.29***	***2.80***	***2.91***	***0.63***
total DMSP	Control	0.33	0.006	0.32	0.013	0.32	0.005
(µmol L $sample^{-1}$)	Mod Light	0.34	0.016	0.33	0.013	0.33	0.018
	High Light	0.34	0.023	***0.22***	***0.013***	***0.11***	***0.016***
grazing	Control	---	---	1.86	0.19	---	---
(cells $grazer^{-1}$)	Mod Light	---	---	1.39	0.36	---	---
	High Light	---	---	***0.33***	***0.25***	---	---
grazing (fraction grazers fed)	Control	---	---	0.69	0.16	---	---
	Mod Light	---	---	0.60	0.09	---	---
	High Light	---	---	***0.21***	***0.13***	---	---

Table 5. *Heterocapsa rotundata* experiment H3 result averages and standard deviations (SD). Bold, italicized values are significantly different from the control. Treatments in which no signal was detected are marked, "ND". Where dashes stand in the place of numbers, no measurements were taken. "Mod" is an abbreviation of "Moderate". In treatments where SD is listed as "n/a", fewer than three replicate samples were taken; samples were not replicated because the only purpose of this experiment was to measure grazing and these measurements were not being taken for interpretation as they were in the H2 experiment.

Experiment H3		**Pre-exposure**		**Post-exposure**	
	Treatment	**Average**	**SD**	**Average**	**SD**
cell concentration	Control	20600	n/a	20900	n/a
(cells ml^{-1})	Mod Light	21000	n/a	21600	n/a
	High Light	21300	n/a	20700	n/a
cell volume	Control	161.43	n/a	166.70	n/a
(μm^{-3})	Mod Light	160.60	n/a	192.15	n/a
	High Light	149.48	n/a	180.23	n/a
grazing	Control	---	---	2.37	0.16
(cells $grazer^{-1}$)	Mod Light	---	---	2.39	0.30
	High Light	---	---	2.38	0.63
grazing	Control	---	---	0.66	0.05
(fraction grazers fed)	Mod Light	---	---	0.64	0.03
	High Light	---	---	0.70	0.04

Alexandrium fundyense

A. fundyense underwent mild physiological change when exposed to high light, mainly in the form of decreased F_v/F_m. In exposure treatments, F_v/F_m decreased during the exposure period and increased again during recovery (Figure 2). However, while the magnitude of F_v/F_m decrease tended to correlate with cumulative exposure during the exposure period, the F_v/F_m in the A1 highest light treatment (3.70 mol photons m^{-2}) decreased more than the lowest F_v/F_m measured in the A2 moderate light treatment (4.95 mol photons m^{-2}). While the cumulative exposure of A2 moderate light surpassed that of A1 highest light, the highest maximum instantaneous irradiance occurred in A1 (Table 6). The variability of instantaneous irradiance and the way the light changed over time were also notably different between the two experiments and may have played an important role in dictating the F_v/F_m response (Figure 3).

Aside from F_v/F_m, no physiological changes were observed in the highest light treatment of A1, but in post-exposure highest light and moderate light treatments in A2, particulate DMSP increased and average cell volume decreased (Tables 1 and 2). Since this difference ($p=0.024$, Table 7 contains all experimental p-values in the present study) did not carry over into post-recovery and because it was not observed in A1, it is likely the result of error in cell volume estimates. Because the particulate DMSP concentration was calculated using cell volume, and decreased DMSP was only observed in treatments with increased volume, the change in DMSP ($p=0.016$) is likely due to the cell volume change and not a physiological stress response. For comparison, particulate DMSP concentrations

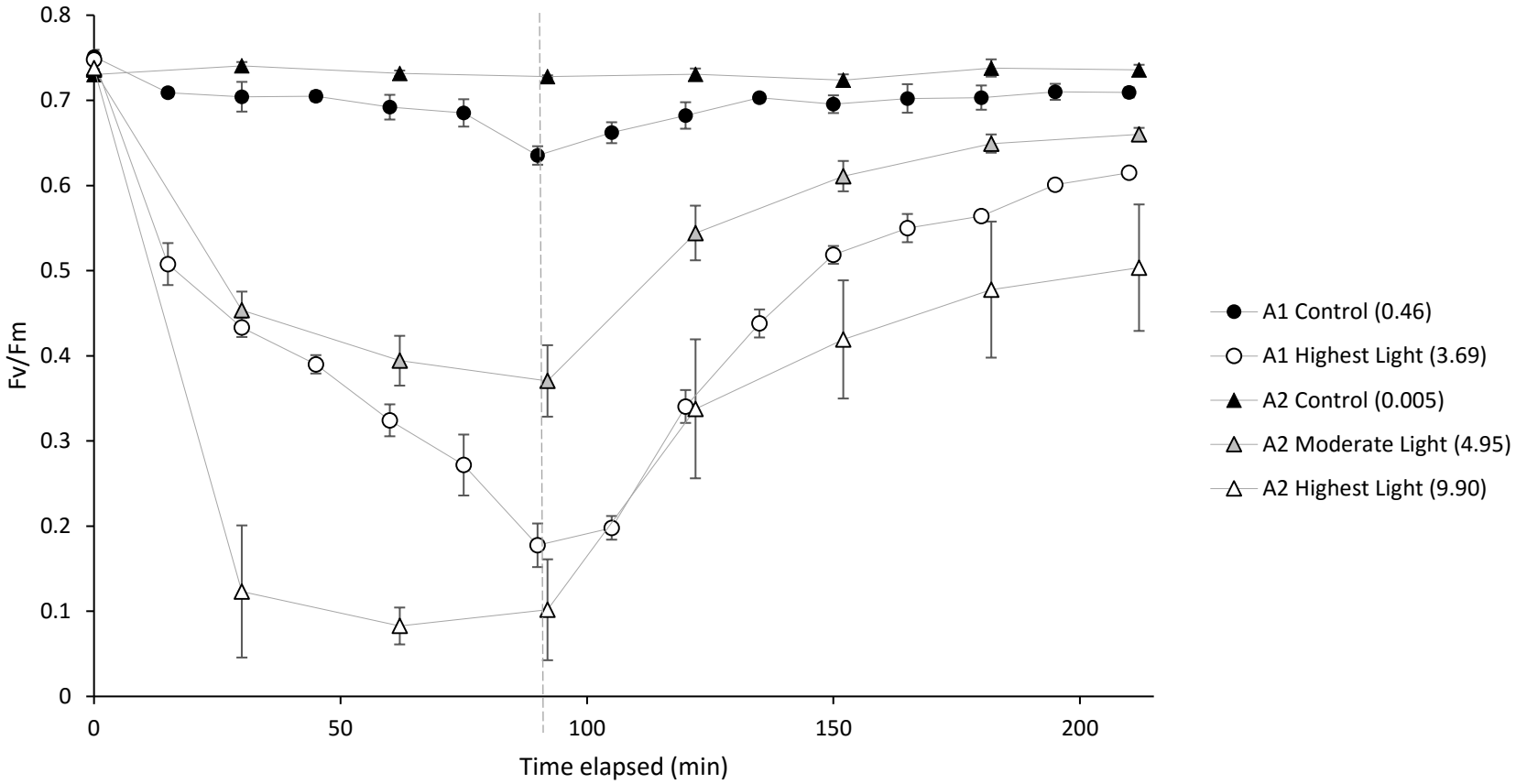

Figure 2. Average F_v/F_m of each *Alexandrium fundyense* treatment. The vertical dashed line marks the time at which samples were moved from light exposure back into growth conditions for recovery. The legend presents cumulative light exposure (mol photons m^{-2}) during the exposure period for each treatment in parentheses. Error bars represent ± 1 SD.

Table 6. Irradiance during the exposure period in each experiment, including average instantaneous, maximum instantaneous, and cumulative exposure for each treatment of *Alexandrium fundyense* and *Heterocapsa rotundata* experiments. Values do not include light exposure during recovery.

Species	Experiment	Treatment	Average Instantaneous (μmol photons m^{-2} s^{-1})	Maximum Instantaneous (μmol photons m^{-2} s^{-1})	Cumulative Exposure (mol photons m^{-2})
A. fundyense	A1	Control	69.97	121.40	0.46
		Highest Light	559.79	971.17	3.69
	A2	Control	0.70	0.78	0.005
		Moderate Light	717.17	803.33	4.95
		Highest Light	1434.34	1606.67	9.90
H. rotundata	H1	Control	0.82	0.85	0.00
		Highest Light	208.69	217.37	1.25
	H2	Control	0.70	0.71	0.004
		Moderate Light	719.42	727.33	4.32
		Highest Light	1438.83	1454.67	8.63
	H3	Control	0.36	0.68	0.002
		Moderate Light	364.75	694.00	2.25
		Highest Light	729.50	1388.00	4.50

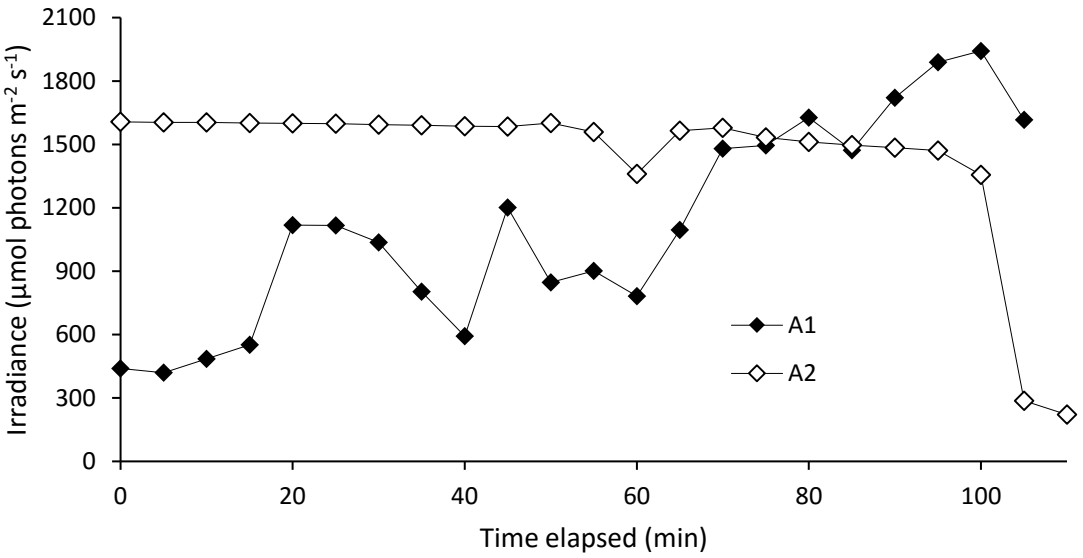

Figure 3. Instantaneous irradiance (average during 5 min measurement intervals, µmol photons m^{-2} s^{-1}) over the course of the exposure periods in *Alexandrium fundyense* experiments, A1 and A2. These data represent environmental irradiance at the surface of the water with no screen. For irradiance within sample bottles, see Table 6.

Table 7. P-values for data collected in each experiment of *Alexandrium fundyense* and *Heterocapsa rotundata*. Values for A1 and H1 were calculated using independent samples T-tests, while a one-way ANOVA was used for A2, H2, and H3. The two p-values in each experiment represent post-exposure and post-recovery. Bold values are significant (α=0.025). Italicized numbers represent values obtained using a Kruskal-Wallace analysis. Superscripts of M (moderate light) and H (highest light) indicate which exposure treatments differed from the control. Superscripts separated by a comma indicate that non-control light treatments differed from one another, as well. Superscripts with no comma separation were not significantly different from one another. Values in parentheses are p-values recalculated using control treatment (i.e. treatments without swelling) volumes.

	Alexandrium fundyense				***Heterocapsa rotundata***				
	A1		**A2**		**H1**		**H2**		**H3**
	Post-exposure	**Post-recovery**	**Post-exposure**	**Post-recovery**	**Post-exposure**	**Post-recovery**	**Post-exposure**	**Post-recovery**	**Post-exposure**
cell concentration	0.795	0.428	0.299	0.915	0.383	0.607	**0.005^{H}**	**<0.001^{H}**	----
cell volume	0.069	0.221	***0.024^{MH}***	*0.799*	***0.005***	***0.021***	0.292	**<0.001^{H}**	----
cell permeability	0.354	0.110	----	----	0.469	0.182	----	----	----
Chl-*a* (pg cell^{-1})	0.891	0.971	0.418	0.267	0.859	0.851	**<0.001^{H}**	**<0.001^{H}**	----
Chl-*a* (mg L cell vol^{-1})	0.189	0.543	0.354(0.439)	0.258	**0.002**	**0.003**	**<0.001^{M,H}**	**<0.001^{H}**	----
particulate DMSP (mmol L cell vol^{-1})	0.823	0.334	**0.016^{MH}**(0.038)	0.793	0.079	0.050	**0.001^{H}**	**<0.001^{H}**	----
particulate DMSP (fmol cell^{-1})	0.182	0.599	0.042	0.921	0.840	0.733	**<0.001^{H}**	**<0.001^{M,H}**	----
dissolved DMSP (nmol L sample vol^{-1})	0.795	0.428	----	0.155	0.501	0.912	**<0.001^{H}**	**0.001^{H}**	----
dissolved DMSP (fmol L cell vol^{-1})	0.334	0.512	----	0.138	0.351	0.852	***0.018^{H}***	***0.024^{H}***	----
total DMSP	0.405	0.367	0.137	0.501	0.976	0.513	**<0.001^{H}**	***0.022^{H}***	----
grazing (cells grazer^{-1})	----	----	0.519	----	----	----	***0.018^{H}***	----	0.686
grazing (fraction grazers fed)	----	----	0.824	----	----	----	**0.001^{H}**	----	0.196

for these treatments were recalculated with the average of pre-exposure, post-exposure, and post-recovery control treatment volumes. Corrected particulate DMSP values were no different from the post-exposure control and all other treatments in A2. Volumetric Chl-*a* (mg L cell vol^{-1}), which was not different from the control using experimentally measured volumes, was also recalculated using corrected volumes, but remained the same as the control.

The highest light treatment in A1 showed a slight increase in membrane compromised cells (by proxy of mean SYTOX green fluorescence) after exposure (Figure 4). However, this increase can likely be attributed to shear stress caused by handling since both control and highest light treatments exhibited this increase (Table 1). Of the parameters measured, no other changes in *A. fundyense* physiology were observed in response to high light exposure.

Ingestion (cells $grazer^{-1}$) by *Schmidingerella* was not different among treatments of *A. fundyense* (Figure 5). However, the variance of the control was smaller than in the moderate light and highest light treatments.

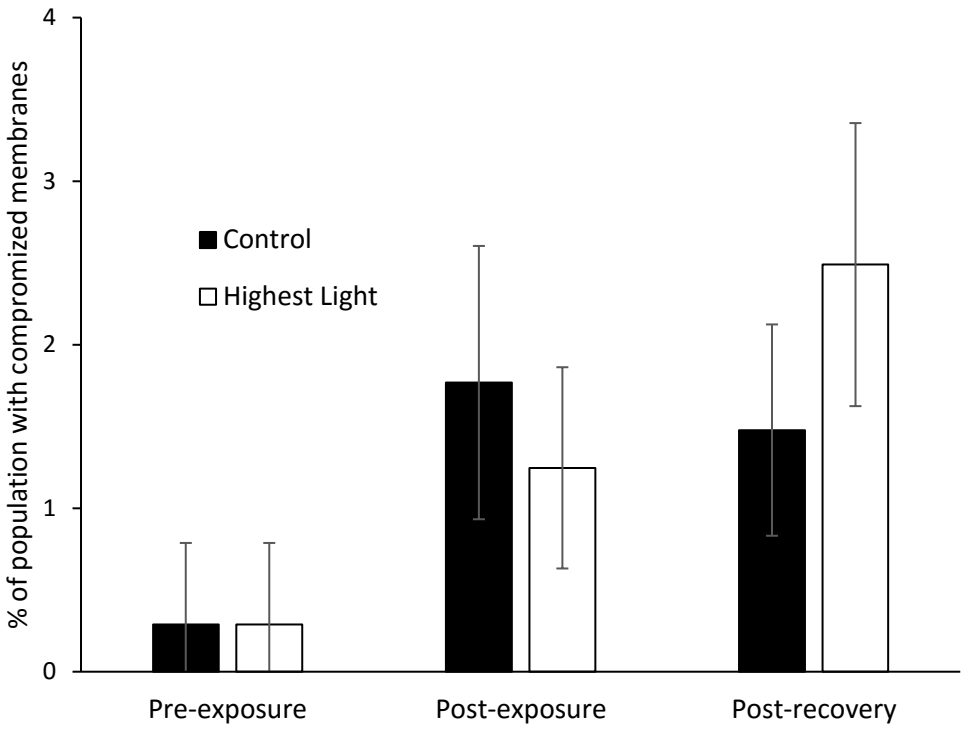

Figure 4. Percent of the measured cell population with compromised membranes. See Figure 1 for explanation of how compromised and intact cell detection events were differentiated from one another. Error bars represent ± 1 SD.

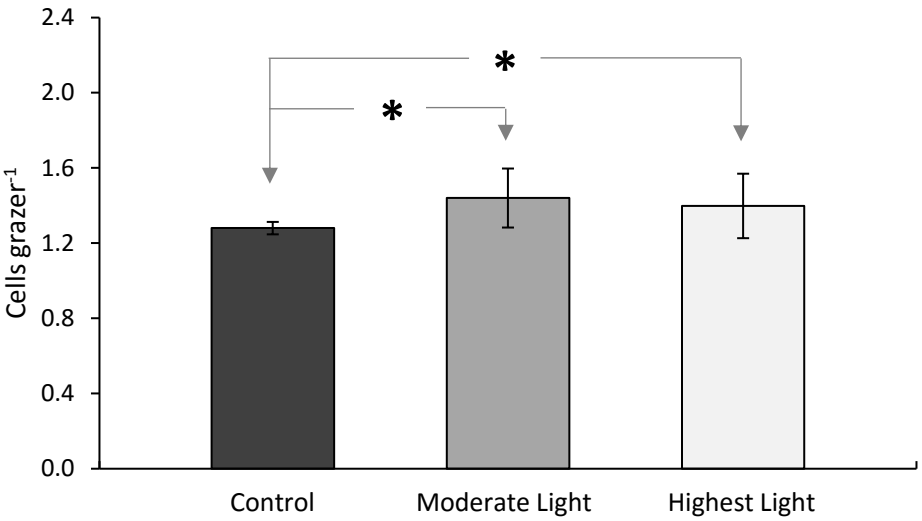

Figure 5. Average ingestion of *Alexandrium fundyense* (cells grazer^{-1}) in control, moderate light, and highest light treatments in the A2 grazing experiment. *Schmidingerella* sp. was allowed to feed for 40 min. Asterisks indicate significant differences between variance ($p<0.05$). Error bars represent ± 1 SD.

Heterocapsa rotundata

Compared to *A. fundyense*, more dramatic physiological changes were observed in *H. rotundata* after exposure to high light conditions. Irradiance levels, and thus the magnitude of the physiological responses exhibited by *H. rotundata*, were highly variable (Table 6). In H1, I erred on the side of excess screen for fear of killing cells. In an effort to elicit a more substantial stress response in H2, exposure levels were inadvertently set too high (less screen), causing cell death. In H3, the same amount of screening was applied as in H2; however, with waning light conditions and increased cloud cover, substantial but non-fatal irradiance conditions were achieved (Figure 6). Ultimately, this variability in light exposure between experiments provided a gradient of stress across which I could compare responses.

In stressed *H. rotundata* that did not undergo significant cell death (H1 highest light, H2 moderate light), an increase in average cell volume and a decrease in volumetric Chl-*a* (mg L cell vol^{-1}) were the only changes observed (Tables 3, 4). In these samples as well as in H3, the F_v/F_m of higher light treatments declined during the exposure period and increased again during recovery (Figure 7). In all experimental exposure treatments, cell size distribution showed a shift toward larger average cell size directly after exposure to sunlight (Figures 8, 9). Volumetric Chl-*a* (mg L cell vol^{-1}) in highest and moderate light treatments decreased after exposure when compared to the control, while cellular Chl-*a* (pg $cell^{-1}$) in all high light treatments except H2 highest light stayed the same (Figure 10).

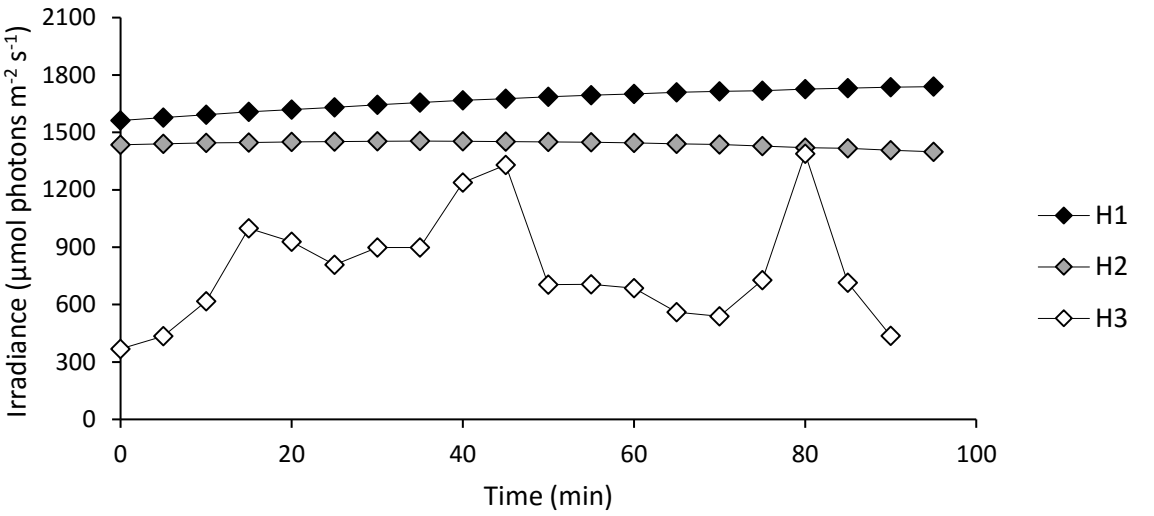

Figure 6. Instantaneous irradiance (average during 5 min measurement intervals, µmol photons m^{-2} s^{-1}) over the course of the exposure period in *Heterocapsa rotundata* experiments, H1, H2, and H3. These data represent environmental irradiance at the surface of the water with no screen. For irradiance within sample bottles, see Table 6.

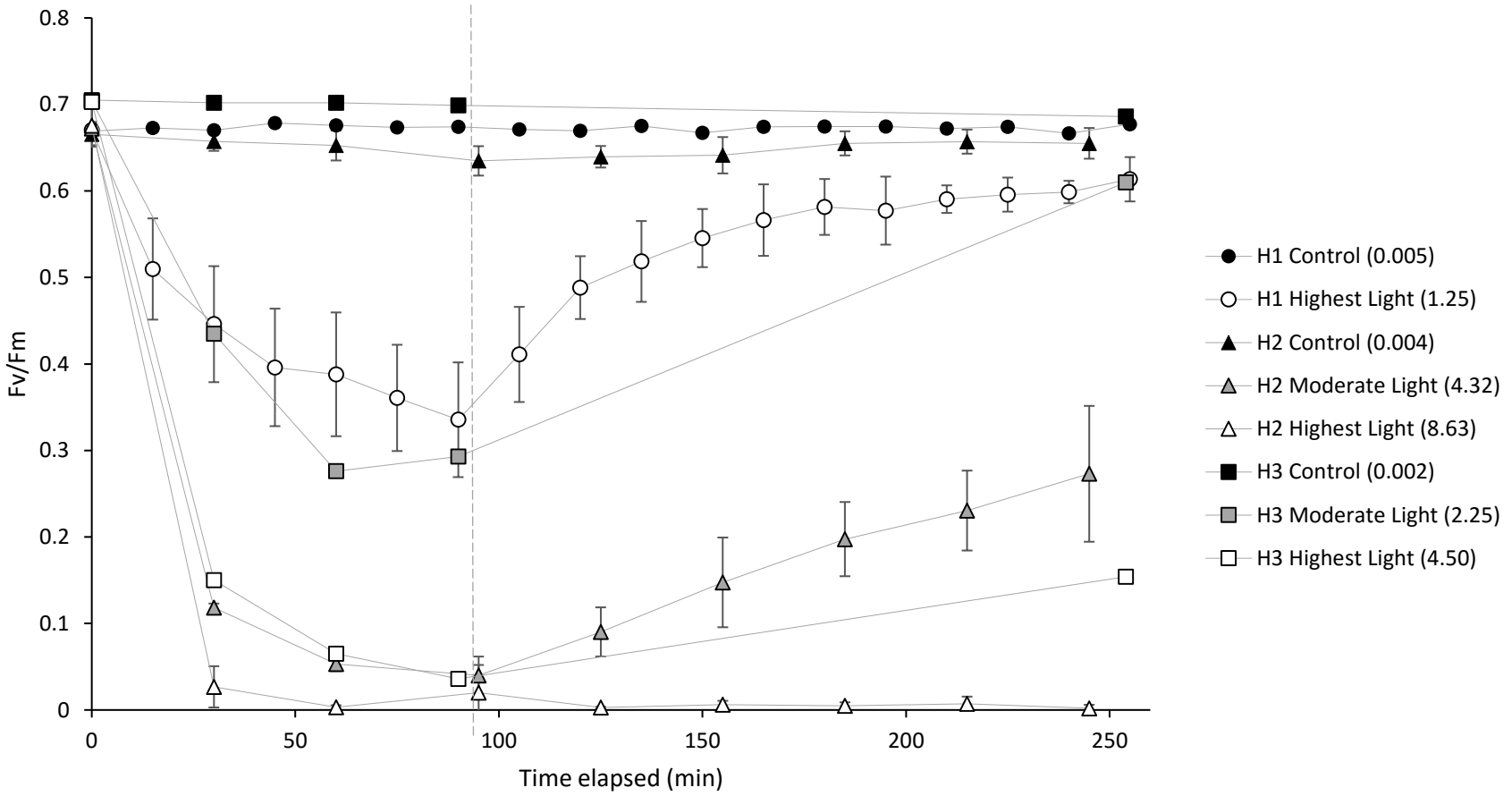

Figure 7. Average F_v/F_m of each *Heterocapsa rotundata* treatment. The vertical dashed line marks the time at which samples were moved from light exposure back into growth conditions for recovery. The legend presents cumulative light exposure (mol photons m^{-2}) during the exposure period for each treatment in parentheses. Error bars are not shown for H3 because samples were not replicated in this experiment. Error bars represent ± 1 SD.

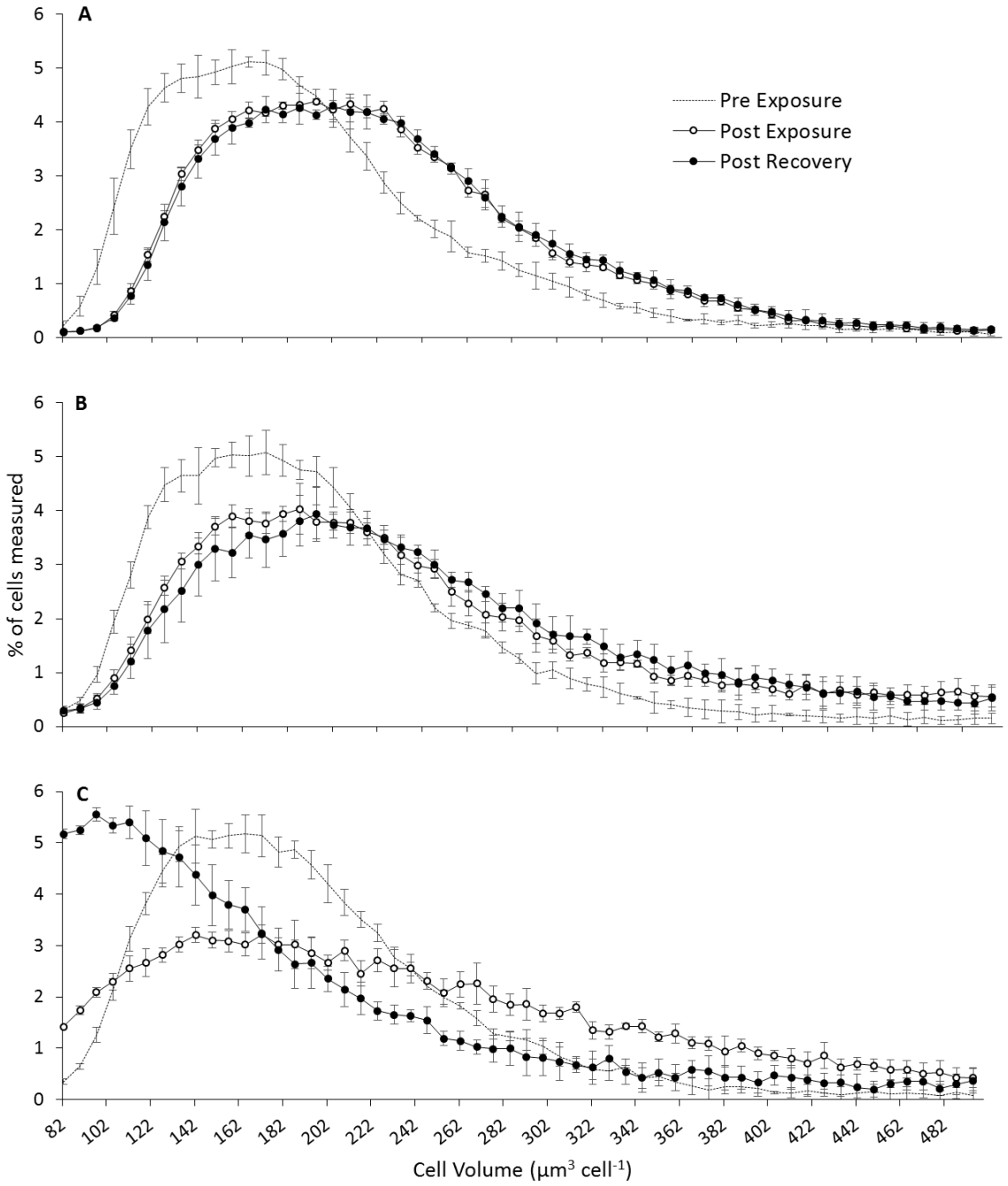

Figure 8. Cell volume distribution of cells measured using a Coulter Counter for each treatment at each stage of *Heterocapsa rotundata* experiments A) H1 (cumulative exposure: 1.25 mol photons m^{-2}) and B) H2 (cumulative exposure: 4.32 mol photons m^{-2}) and C) 8.63 mol photons m^{-2}. Error bars represent ± 1 SD.

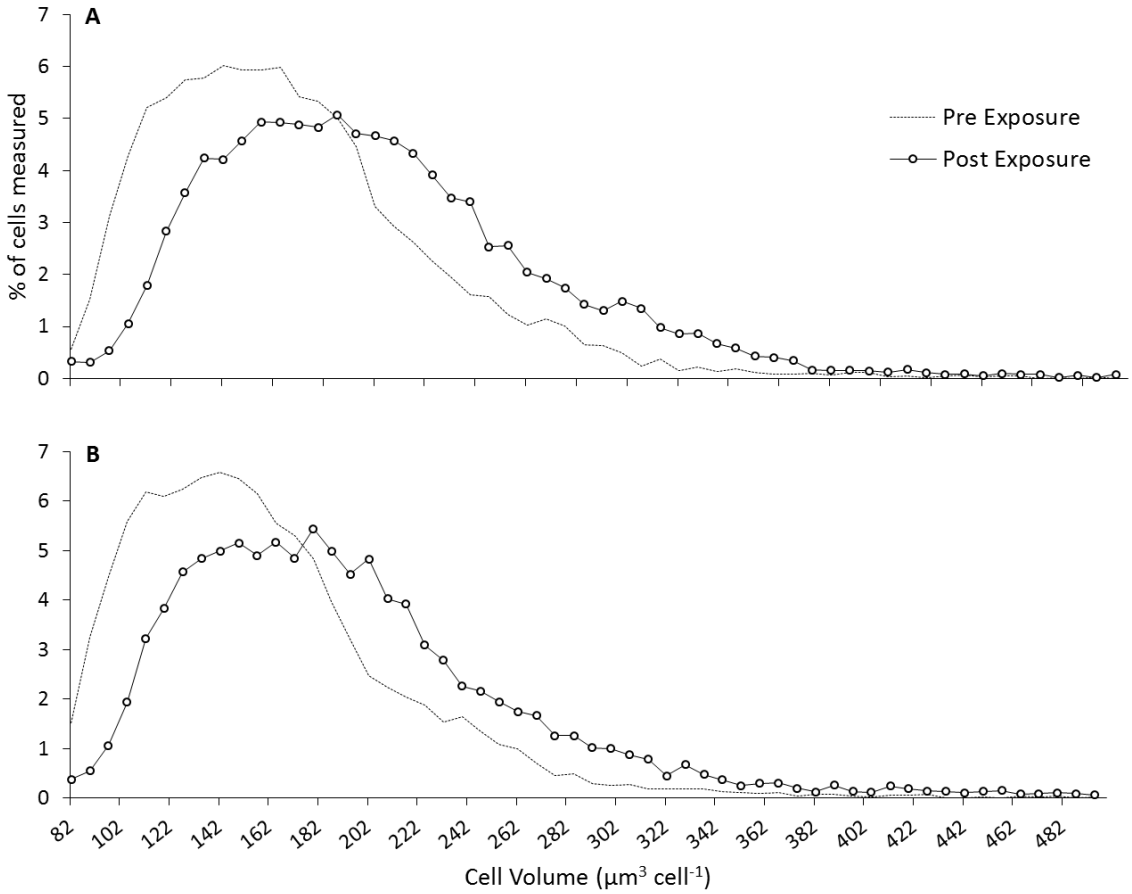

Figure 9. Cell volume distribution of *Heterocapsa rotundata* cells measured using a Coulter after exposure to A) moderate light (cumulative exposure: 2.25 mol photons m^{-2}) and B) highest light (cumulative exposure: 4.50 mol photons m^{-2}) treatments before and after exposure in H3. Error bars are not shown because samples were not replicated in this experiment.

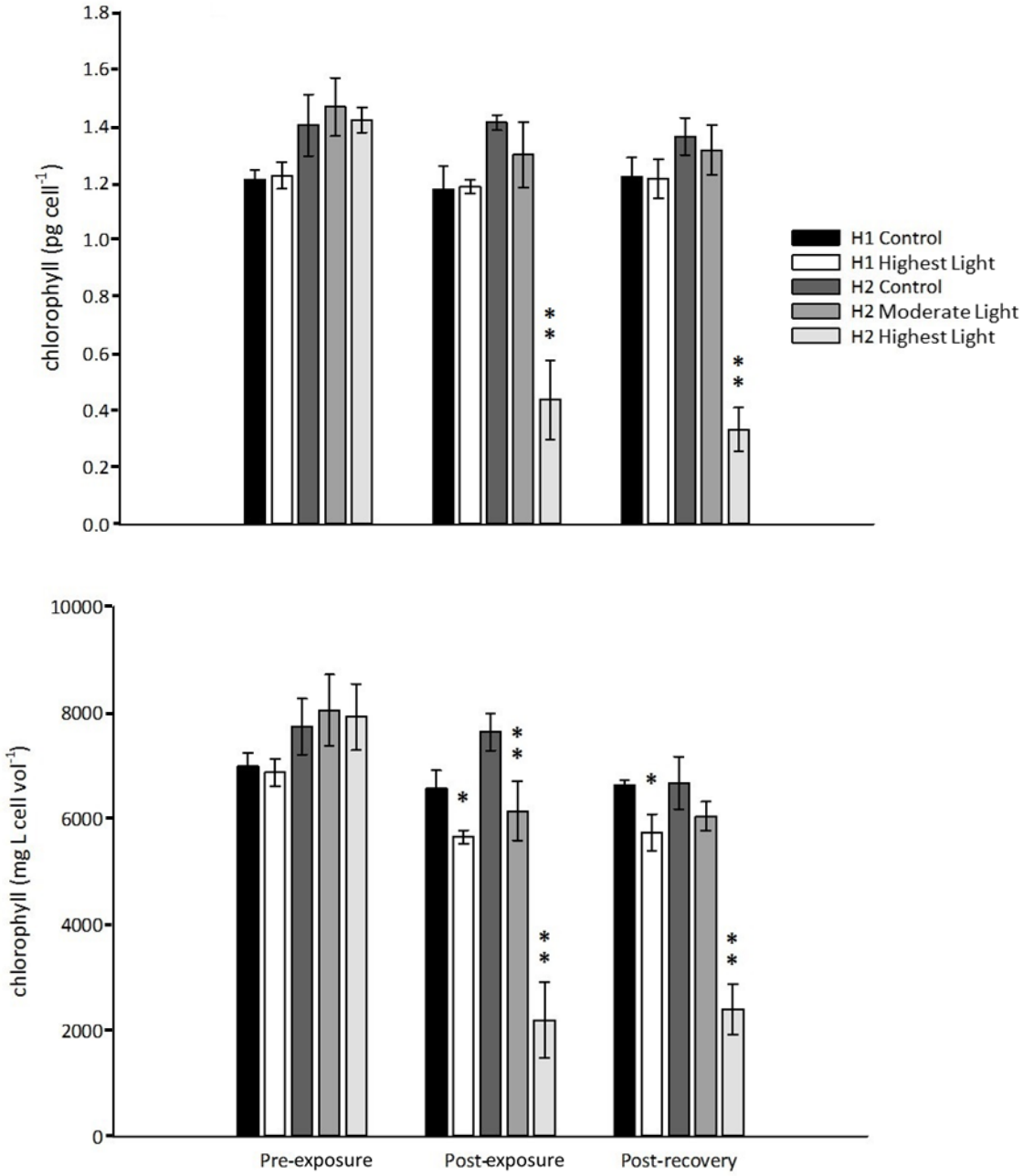

Figure 10. *Heterocapsa rotundata* chlorophyll content (pg cell^{-1} and mg L cell vol^{-1}) in H1 and H2 at three time points. Asterisks denote high light treatments that are significantly different from their respective control (*, 0.025>p≥0.001; **, p<0.001). Error bars represent ± 1 SD.

No change in the proportion of membrane compromised cells was observed in *H. rotundata*.

The trend in increased cell volume after exposure is consistent with pre-experimental observations that *H. rotundata* cells gradually swelled when viewed under a microscope, presumably due to light and heat-induced stress from the instrument light source. The H1 highest light treatment showed an increase in average cell volume ($p=0.005$) after light exposure, but no change in cell concentration, suggesting the shift in cell size was the result of swelling as opposed to a disproportionate loss of smaller cells (Figure 11). Decreased volumetric Chl-*a* (mg L cell vol^{-1}) in highest light H1 is also consistent with the swelling hypothesis, since cellular Chl-*a* (pg $cell^{-1}$) remained unchanged (Figure 10). Pilot observations of cells also revealed that after some time, swelling can result in lysing and rapid disintegration of cells. This fragmentation is presumed to have been the fate of cells in the highest light treatment in H2 since this population shifted toward a smaller average particle size after recovery, corresponding with a decrease in cell concentration (Figure 8).

Cell lysis in H2 was responsible for other changes observed in the highest light treatment in this experiment. Particulate DMSP was disproportionately low in the high light treatment post-exposure ($p=0.001^{H}$), becoming undetectable in post-recovery sampling (Figure 12a). In the same treatment, dissolved DMSP clearly increased post-exposure ($p=<0.001^{H}$) (Figure 12c,d). Due to the destructive level of light stress, these results indicate weakening and rupturing of cells, causing release of intracellular materials. Total DMSP

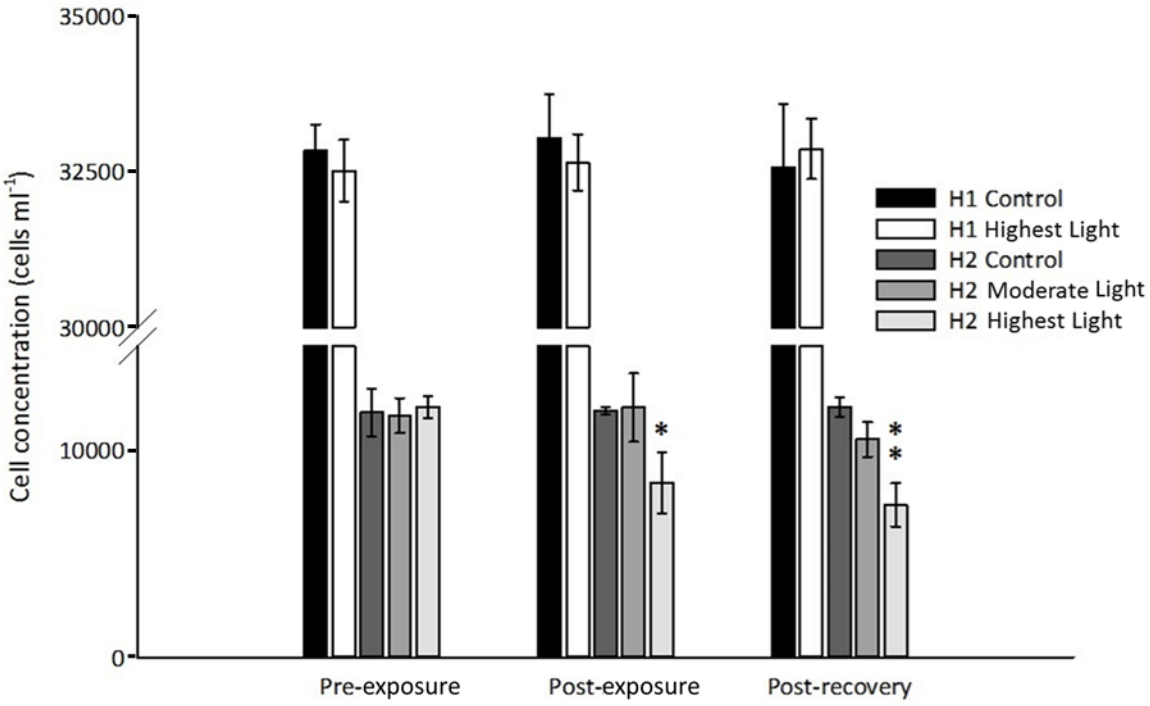

Figure 11. *Heterocapsa rotundata* cell concentrations (cells ml^{-1}) of all treatments in H1 and H2 at three time points in the experiment. Asterisks denote high light treatments that are significantly different from their respective control (*, 0.025>p≥0.001; **, p<0.001). Error bars represent ± 1 SD.

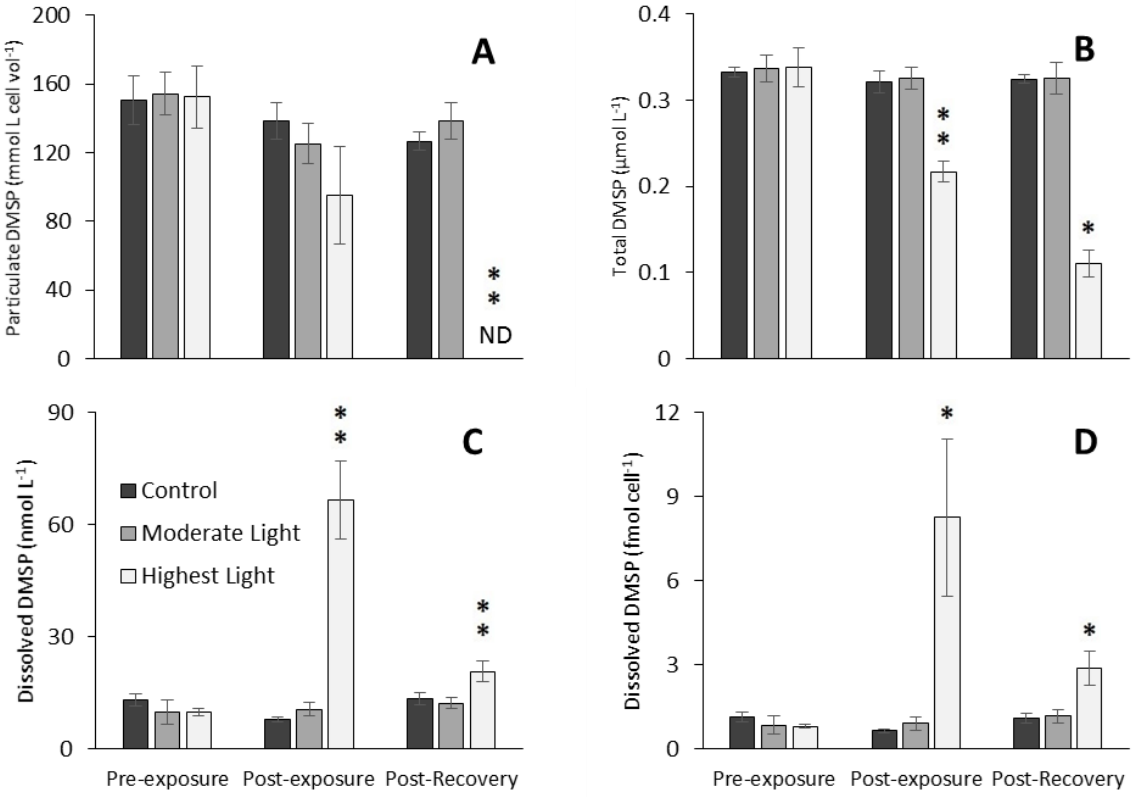

Figure 12. DMSP concentrations for control, moderate light, and highest light treatments of *H. rotundata* in H2 at three time points in the experiment. Concentrations of a) particulate DMSP (mmol L cell vol^{-1}), b) total DMSP (µmol L^{-1}), and c,d) dissolved DMSP (nmol L sample^{-1}, fmol cell^{-1}) are presented. ND signifies no signal detected. Asterisks denote high light treatments that are significantly different from their respective control (*, 0.025>p≥0.001; **, p<0.001). Error bars represent ± 1 SD.

decreased in the H2 highest light treatment ($p=<0.001^{H}$), likely because it was consumed by reactive oxygen and DMSP lyase also released from lysed cells (Sunda et al. 2002, Li et al. 2016)(Figure 12b). In the H2 moderate light treatment, volumetric Chl-*a* decreased post-exposure ($p=<0.001$) while per cell Chl-*a* remained similar to the control, staying consistent with observations from the highest light treatment in H1 (Figure 10). However, in the H2 highest light treatment, Chl-*a* decreased both volumetrically and per cell. The difference in pattern between the H2 highest light treatment and all other high light treatments in the *H. rotundata* experiment series suggests that at some level between 4.32 and 8.63 mol photons m^{-2} there lies a light intensity threshold at which low light-acclimated *H. rotundata* cells began to lyse. This is the only treatment in which F_v/F_m did not begin to increase during the recovery period, effectively signifying collapse (Figure 7).

Non-fatal light stress and physiological response in *H. rotundata* had no effect on *Schmidingerella* sp. grazing rates. However, grazing on *H. rotundata* decreased compared to the control ($p=0.018^{H}$) on *H. rotundata* exposed to the highest light treatment in experiment H2 (Figure 13). This was undoubtedly due, in part, to the decrease in *H. rotundata* concentration in this treatment. In H3, where prey cell concentrations were equivalent among treatments, grazing was not different among treatments. However, as observed for grazing on *A. fundyense*, the variation increased significantly with light exposure (Figure 14). In both exposure treatments of H3, a slight redistribution was observed in ingestion (cells $grazer^{-1}$), wherein *Schmidingerella* containing no *H. rotundata*

cells after grazing comprised a smaller percentage of the population than in the control (Figure 15).

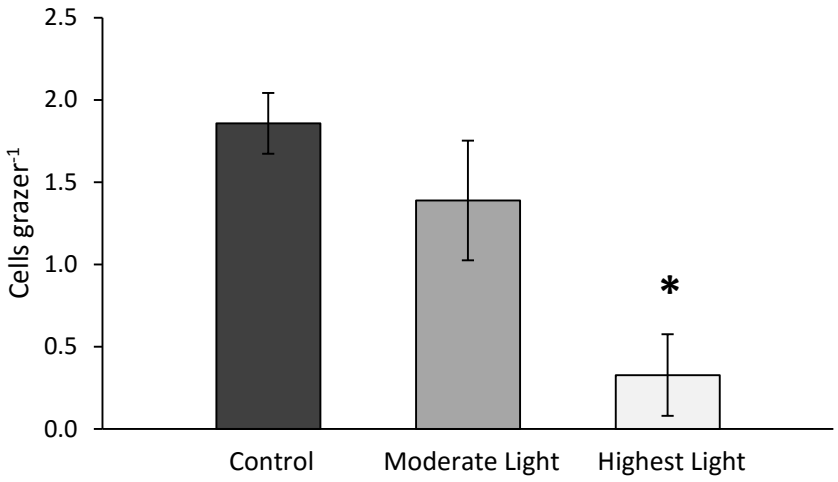

Figure 13. Average ingestion of *Heterocapsa rotundata* (cells grazer^{-1}) in control, moderate light, and highest light treatments in the H2 grazing experiment. *Schmidingerella* sp. was allowed to feed for 20 min. The asterisk denotes significant difference from the control ($p<0.025$). Differences in variance were not analyzed for these data since cell loss confounded the role of light exposure in the grazing results. Error bars represent ± 1 SD.

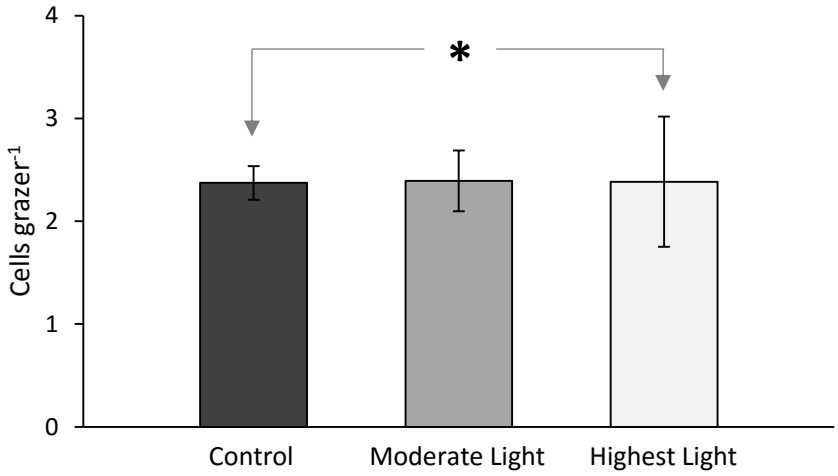

Figure 14. Average ingestion of *Heterocapsa rotundata* (cells grazer^{-1}) in control, moderate light, and highest light treatments in the H3 grazing experiment. *Schmidingerella* sp. was allowed to feed for 20 min. Asterisks indicate significant differences between variance ($p<0.05$). Error bars represent ± 1 SD.

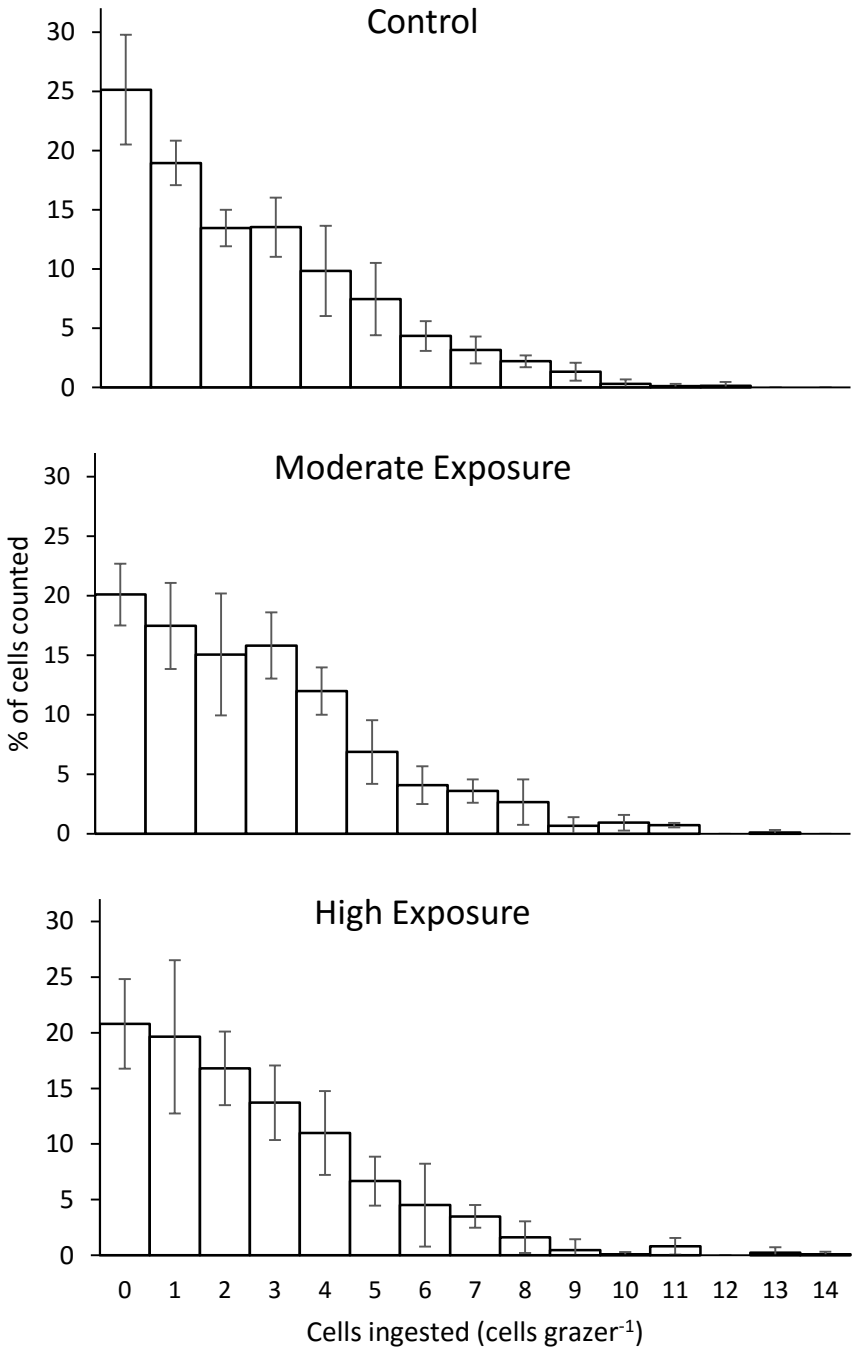

Figure 15. *Heterocapsa rotundata* ingestion distribution of measured *Schmidingerella* over the 20 min grazing period in H3. Error bars represent standard deviation. Error bars represent ± 1 SD.

DISCUSSION

Overview

High-intensity sunlight exposure has been documented as a source of stress in many photosynthetic organisms (Powles et al. 1984, Long et al. 1994). Bloom-forming dinoflagellates encounter high irradiance in the field because they accumulate near the water's surface under stratified conditions (Margalef 1978). Therefore, understanding the impact of light on cell physiology and its role as a potential stressor may be important for predicting bloom formation and decline. In this study, I characterized physiological responses of *A. fundyense* and *H. rotundata* to high-intensity visible spectrum light exposure and found that the latter underwent more dramatic physiological change. To explore whether the effects of high light exposure affect predation on stressed cells, I compared grazing rates by *Schmidingerella* sp. on high light-exposed versus growth light-exposed cells. High light exposure did not affect average grazing rates, however, variability of grazing rates on high light-exposed cells notably increased. The results presented here suggest that *A. fundyense* and *H. rotundata* inhabit different bloom niches, with resistance to high light defining the bloom niche of the former species more so than the latter.

Physiological Response

Stress-Inducing Light Levels

Physiological responses to visible light exposure by *A. fundyense* and *H. rotundata* were markedly different. While *A. fundyense* showed no physiological changes other than a drop in

F_v/F_m after high light exposure, *H. rotundata* also underwent changes in chlorophyll, DMSP in and outside the cells, cell volume, and even began to lyse in the highest light treatment. Qualitative observations in pilot experiments revealed that, when exposed to high light, *H. rotundata* cells visibly swelled from a slim, conical football shape to a more distended egg shape. In the non-fatal high light treatment of *H. rotundata*, the increase in cell volume had not diminished by the end of the recovery period (H1: 1.75, H2: 2 h), suggesting that once imposed, this alteration either is sustained for multiple hours, or is permanent.

Photosynthetic efficiency (F_v/F_m) was used as a proxy for stress in the current study because it is a symptom of damage caused by intense light exposure (Parkhill et al. 2001). Damage resulting from excess reactive oxygen (hydrogen peroxide - H_2O_2; hydroxyl radical - $HO^{\bullet}$; superoxide anion - O_2^-; singlet state oxygen - $^1O_2$) production in the chloroplasts has been cited as the mechanism through which light stress occurs (Lesser 2006; Pospíšil 2009). These reactive molecules are thought to cause damage directly to PS II (Nishiyama et al. 2001) or to decrease chlorophyll and Rubisco activity (Lesser 1996). Whatever the mechanism, damage induced by excess light results in the decrease of photosynthetic efficiency (F_v/F_m). This simple fluorescence ratio reflects the photon-utilizing capacity of PS II, which relates directly to how efficiently cells can harvest light energy for carbon fixation, ultimately translating to growth capacity (Krause and Weis 1984). It is important to note that decreased F_v/F_m is not unequivocal evidence of damage, since evidence has been found in symbiotic dinoflagellate species that downregulation of F_v/F_m may be associated with a photoprotective strategy (Hoegh-Guldberg and Jones 1999). While the mechanistic causes of stress must be sought through other

measurements, the rise and fall of F_v/F_m (the latter considered indicative of stress for the purposes of this study) effectively reflects the fitness of the cells regarding capacity for growth, which is ecologically meaningful in the context of bloom-formation.

Stress-inducing light levels in the field promote water column stratification through warming of the surface layer. A long-standing paradigm is that the association of planktonic dinoflagellate blooms with stratified water during summer can be explained by a lack of shear stress from the relative lack of mixing (Margalef 1978). However, more recent observations suggest that, while the lack of stress-causing turbulence in stratified water may have some effect, this association has more to do with other conditions that tend to co-occur with stratification (Smayda 1997; Smayda 2002). Rainfall-runoff, which often precedes dinoflagellate blooms, has been classically assumed to promote bloom formation via the strengthening of stratification. However Smayda (1997) hypothesized that the nutrient contribution of runoff may be the bloom-determining factor, while maintained stratification (which is typically already present in these instances) simply keeps these nutrients concentrated in the surface layer where phytoplankton can easily access them.

Whether due to the lack of mixing or the retention of nutrient pulses from runoff, stratification promotes the accumulation of dinoflagellates near the surface of the water column where sunlight is most intense, and it is here that blooms often form. Since exposure to high and variable irradiance is a common feature of their niche, bloom-forming dinoflagellates must have adaptations for coping with light stress. Evidence of specific adaptations to the dynamic light conditions of coastal environments can be seen when comparing oceanic and

coastal diatoms, which possess notably different photosynthetic architecture. Strzepek and Harrison (2004) showed that oceanic species have much lower photosystem I and cytochrome b_6f complex concentrations, an adaptation likely selected for in these oligotrophic environments because it reduces need for iron. Cytochrome b_6f is associated with the regulatory mechanism on the thylakoid membrane that switches the light harvesting apparatus into a state of photoprotective thermal dissipation (Munekage et al. 2001). The fact that coastal diatoms have retained higher concentrations of these complexes suggests photo-related mechanisms serve a comparatively important purpose in coastal environments. The authors reasoned that this retention can be explained by the requirement for coping with dynamic light conditions characteristic of coastal ecosystems. Some studies have observed greater photoprotective fluorescence quenching and pigment adjustment (Demers et al. 1991) as well as comparatively higher photoprotective pigment content (Jeffery et al. 1999) in bloom-forming dinoflagellates than in other taxa. Depth regulation via motility (Heany and Talling 1980) in addition to strong circadian rhythms (Prézelin 1992) have also been cited as possible adaptations for avoiding light stress.

The depth to which light penetrates before and during coastal blooms must be important for determining where cells like *H. rotundata* (which exhibited lysis under high light exposure in the present study) proliferate. Penetration depth of UVR is more variable in coastal ecosystems than other photosynthetically-active wavelengths because it is disproportionately absorbed and scattered by dissolved organic matter (Kirk 1994). Excessive photosynthetically active radiation (PAR) in the visible spectrum can also be detrimental to photosynthetic cells

(Walker 1992, Foyer et al. 1994, Long et al. 1994). While it attenuates only marginally slower than UVR with depth in clear seawater, visible spectrum sunlight, otherwise known as photosynthetically active radiation (PAR) penetrates deeper than UVR when dissolved organic matter and particles are at high concentrations, as they are during blooms (Belzile et al. 2002). The present study focused only on PAR for this reason.

To estimate the depth at which *H. rotundata* cells would exhibit cell swelling in the field according to observations made in the present study, I constructed depth profiles of irradiance. PAR attenuation coefficients ($k_{no\ bloom}$=0.3, k_{bloom}=0.9) were borrowed from Paul (2010) and corresponded with days of lowest (July 9, no bloom) and highest (August 6, bloom) environmental chlorophyll in East Sound, Orcas Island during the summer of 2007 (Figure 16). The highest average instantaneous surface irradiance from the present study (1439 µmol photons m^{-2} s^{-1}) was used to calculate irradiance at depth, although the highest observed light reading during the four experiments performed in summer 2014 was in mid-July, at 1942 µmol photons m^{-2} s^{-1}.

Coefficients were entered into the following equation to determine irradiance at depth:

$$I_z = I_0 e^{-kz}$$

I_z is the irradiance at a given depth (µmol photons m^{-2} s^{-1}), I_0 is the average instantaneous surface irradiance (1439 µmol photons m^{-2} s^{-1}) recorded during the H2 experiment (chosen over the H1 experiment surface irradiance because of the greater range of light response by cells and higher overall light levels in H2), k is the attenuation coefficient (m^{-1}) and z is depth (m).

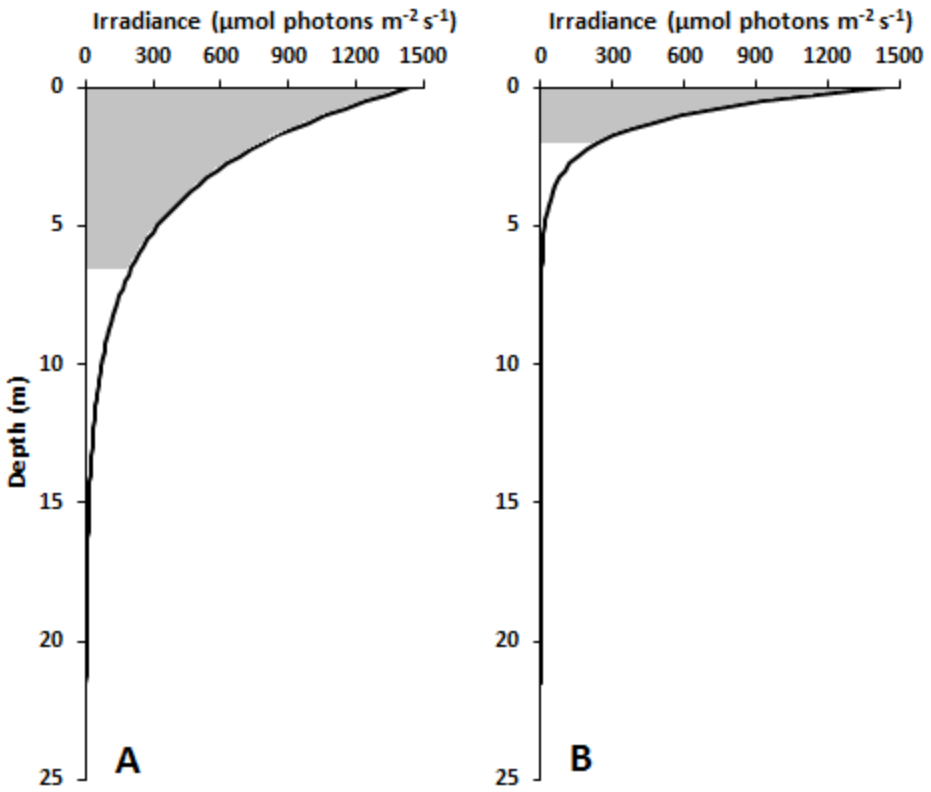

Figure 16. Irradiance depth profiles created using A) no bloom (k=0.3) and B) bloom (k=0.9) attenuation coefficients from a summer 2007 bloom in East Sound, Orcas Island (Paul 2010). Grey shading represents the depth at which irradiance is sufficient to cause cell swelling in low light-acclimated *Heterocapsa rotundata*; i.e. the depth at which irradiance was 209 µmol photons m^{-2} s^{-1}, the lowest irradiance at which swelling was observed in *H. rotundata* in the present study.

Since the highest light treatment in H1 was the lowest average instantaneous irradiance at which cell swelling was observed in all of the *H. rotundata* experiments, this irradiance was set as the lower limit of stress in the depth profiles (Figure 16a,b). It is possible that the swelling response would occur at even lower irradiances, but further studies are required to know for sure.

Based on the theoretical PAR irradiance depth profiles (Figure 16a,b), the "stress-layer" for *H. rotundata* does not penetrate especially deep during blooms; however, mild mixing could easily inject cells into the top 2 m, exposing them to stress-inducing light conditions. Observations by Helbling et al. (2008) revealed that mixing conditions exacerbated UVR-induced photoinhibition in a *Heterocapsa* species (*triquetra*). The authors consider the light fluctuation imposed by mixing as the likely driver of this stress response, which is consistent with the hypothesis that *Heterocapsa* species are not well-equipped to cope with rapid changes in light. The association of *Heterocapsa* species with habitats of comparatively low mixing is also consistent with this idea (Smayda and Reynolds 2001). While lack of shear stress is likely one of the qualities that make stratified waters conducive to dinoflagellate blooms, the zonation effect of stratification may also provide spatial stasis, allowing C-strategy bloomers like *H. rotundata* to remain wherever conditions are optimal for cell growth and division.

Ultraviolet radiation, while omitted from this study due to its rapid attenuation in coastal environments, is present in the environment near the water's surface. These shorter wavelengths are well-documented to cause stress in unicellular algae, and undoubtedly affect

summer plankton communities. The photoinhibitory effect of UVR can substantially slow growth in some dinoflagellate species (Ekelund 1991) and is exacerbated by nutrient limitation (Litchman et al. 2002), which is a common condition of the stratified surface waters where dinoflagellates often bloom. UV wavelengths have also been shown to inhibit the motility in dinoflagellates (Ekelund 1991, Hessen et al. 1997), affecting the ability of cells to control their placement in the water column. Despite the potential negative effects of UVR, these wavelengths have been shown to drive changes in plankton communities that may promote the formation of dinoflagellate blooms. In a microcosm study by Mostajir et al. (1999), UVR exposure resulted in a decrease of ciliates and diatoms within planktonic community. This change led to an eventual increase in autotrophic dinoflagellates, likely due to a decrease in ciliate grazing and competition from diatoms. This community shift is evidence that, in addition to PAR, UVR is likely an important factor in bloom formation and regulation where it is present.

In *H. rotundata*, both maximum instantaneous irradiance and cumulative exposure resulted in a greater decrease in F_v/F_m than in *A. fundyense* (Figure 17a,b). In *A. fundyense* however, maximum irradiance may have had a greater impact than cumulative exposure. In the A1 highest light treatment, there were higher total photons over the course of exposure compared to the A2 moderate light treatment, but F_v/F_m decreased more in the latter treatment. This can likely be explained by the fact that the maximum instantaneous irradiance in A2 was higher than in the A1 exposure treatment, even though the latter had higher cumulative photons m^{-2}.

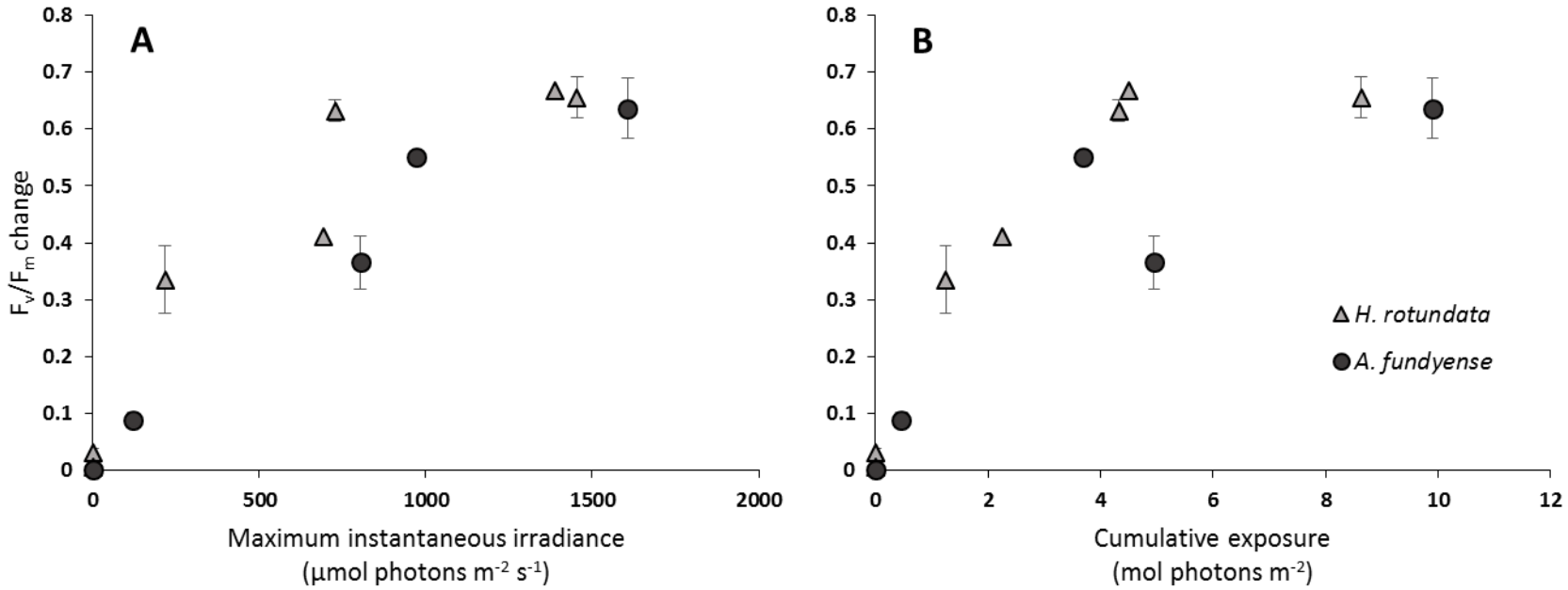

Figure 17. Change in F_v/F_m (calculated by subtracting the last F_v/F_m value in the exposure period from the respective initial F_v/F_m value in each experiment) in relation to A) maximum instantaneous irradiance (µmol photons m^{-2} s^{-1}) and B) cumulative exposure (mol photons m^{-2}) in *Alexandrium fundyense* and *Heterocapsa rotundata*. Error bars represent ± 1 SD.

Photosynthetic unicellular algae are known to possess a variety of adaptations to deal with daily fluctuations in irradiance (Falkowski and LaRoche 1991) across a range of time scales (Long et al. 1994). In dinoflagellates, resistance to extreme oscillations in light exposure are particularly well-documented in species that live symbiotically within corals and anemones (Hoegh-Guldberg and Jones 1999). The impact of light variability has been less thoroughly explored in free-living dinoflagellate species, however an increase in water column mixing was shown to increase UVR-induced photoinhibition in *Heterocapsa triquetra*, presumably due to the resulting light fluctuation (Helbling et al. 2008). Variable light is most certainly encountered by coastal dinoflagellates because of mixing, which is itself variable due to the influence of tidal cycles (Allen et al. 1980). Other factors, like cloud cover and smoke from forest fires (both of which impacted light levels in the present study), alter the frequency and magnitude of light change on both hourly and daily time scales.

Morphology and Photophysiology

The discrepancy in cell size and structure between *A. fundyense* and *H. rotundata* is likely important for interpreting the light response differences observed between the two species. The comparatively delicate amphiesma (the layered cell covering that includes the cortex and the pellicle) of *H. rotundata*, as well as its smaller size, may have rendered these cells more susceptible to damage from light stress. Raven (1998) discussed the advantages and disadvantages of small cell size, noting that smallness is favored for nutrient acquisition when environmental nutrient concentrations are low. More efficient nutrient transport, however, is

accompanied by a higher energy cost for maintaining homeostasis due to unwanted leakiness. Additionally, the greater ease with which light can penetrate smaller cells exposes structures within the cell, including perhaps those responsible for maintaining homeostasis, to damaging light levels (Raven 1998).

The amphiesmal structure of *H. rotundata* seems ill-equipped to combat excess leakage when damage occurs. Within dinoflagellates there is a continuous spectrum of amphiesmal complexity ranging from "naked" (lacking thecal plates) to "armored" (Pfiester 1989). *H. rotundata* (formerly *Katodinium rotundata* or *Katodinium rotundatum*) was considered a naked dinoflagellate until "delicate" thecal plates and scales were discovered in the amphiesmal vesicles near the surface of the cell (Dodge and Crawford 1970). Compared to the rigid amphiesma of *A. fundyense*, the flimsier external structure of *H. rotundata* is less suited for keeping the cell intact if homeostasis is disrupted and turgor pressure increases as water enters the cell. Consistent observations of empty cortices from dead cells persisting intact in *A. fundyense* cultures but never in *H. rotundata* provide further evidence that the amphiesma of the latter species is structurally weaker. Additionally, the larger cell size of *A. fundyense* gives it a comparative photoprotective advantage over *H. rotundata*. While small size is favored for light absorption when light is limited, low volume and a high surface-area-to volume ratio allow for less shading of internal structures by pigments and shorten the path length for light attenuation in the cell (Raven 1984a, Raven 1984b, Karentz et al. 1991, Garcia-Pichel 1994). These features make smaller cells more susceptible to light-induced damage and could have

promoted the swelling and lysis observed in this species by compromising intracellular homeostatic mechanisms.

Further evidence of the importance of cell morphology in bloom-forming species was presented in a study by Smayda and Reynolds (2001), in which they found certain planktonic "morphotypes" (defined by general cell size and shape) were associated with specific habitat types. *H. rotundata* was categorized as predominating in relatively shallow zones with reduced offshore water-mass exchanges. *A. fundyense* was considered well-adapted for entrainment and dispersal in coastal currents (i.e. a more advective environment). Both ecosystems are characterized by periodic inorganic nutrient limitation. It is thought that most photosynthetic dinoflagellates, including *H. rotundata* and *Alexandrium* species, are able to persist in nutrient-deficient conditions by feeding on other organisms (Jeong et al. 2005a, Jeong et al. 2005b, Seong et al. 2006, Yoo et al. 2009). As a result, even when nutrient injection from runoff is sparse, bloom-forming dinoflagellate cells can persist high in the water column during times of limited mixing. According to the morphotype-habitat associations made by Smayda and Reynolds, *A. fundyense* predominates in comparatively higher shear-stress environments than *H. rotundata*. This is intuitively consistent with the hypothesis made in the present study that amphiesmal structure is stronger in *A. fundyense* than in *H. rotundata*.

In addition to cell size and structure, pigment composition is an important determinant of resistance to light stress. The hypothesis stated earlier, of superior photoprotective capacity in dinoflagellates relative to other phytoplankton taxa, is perhaps dependent upon the time scale, as evidenced by studies involving xanthophyll cycle pigments and mycosporine-like amino

acids (MAAs). These compounds are thought to be mechanisms used by phytoplankton, including dinoflagellates, to combat light stress (Hager 1975, Hager 1980, Shick and Dunlap 2002). Few comparisons of short-term response to high light fluctuation between dinoflagellates and other phytoplankton exist. However, in one study, the dinoflagellate *Alexandrium excavatum* was shown to exhibit quicker and higher-magnitude quenching and pigment alteration over the course of an hour than the diatom *Thalassiosira pseudonana* (Demers et al. 1991). Demers et al. (1991) ambiguously interpreted the greater response by *Alexandrium* as either a superior stress response or, since *Thalassiosira* appeared comparatively unaffected by the light change, evidence that the dinoflagellate was more stressed. Another study by Jeffery et al. (1999) observed that among 152 species from 12 classes of marine phytoplankton, bloom-forming dinoflagellates contained the highest UV-absorbing pigment-to-carbon ratios, intuiting that this indicates greater photoprotective advantage in dinoflagellates. However ample evidence exists that dinoflagellates tend to grow and photosynthesize best at lower irradiances than diatoms (Richardson et al. 1983). This suggests that other phytoplankton possess alternate photoprotective adaptations that dinoflagellates lack, causing dinoflagellates to be comparatively less resilient under high light conditions in the long term. Conclusively determining the placement of dinoflagellates among the hierarchy of planktonic photoprotection will require further comparative studies.

The benefit of photoprotective pigment production weighed against cost in phytoplankton is likely dependent upon cell size. Like *Alexandrium*, *Heterocapsa* species have been shown to possess photoprotective pigments (Laurion and Roy 2009; Korbee et al. 2010).

In the Baltic Sea, xanthophyll pigments in *Heterocapsa triquetra* have been shown to increase with rising light levels in the morning, and decrease as light wanes in the evening (Łotocka 2015). These same cells showed variability in concentrations of the carotenoid peridinin (characteristic of dinoflagellates) in an opposite relationship to changing light, suggesting this pigment is either degraded by high sunlight or plays a role in photoprotection. *H. triquetra* grown in enhanced UVB light conditions increased xanthophyll cycle pigments and slowed growth, a pattern observed in an identically treated strain of *Alexandrium tamarense* (Laurion & Roy 2009). While similar traits are bound to exist in *Alexandrium* and *Heterocapsa* species, in the context of photoprotection cost efficiency, the size difference between the species chosen for the present study is not trivial. According to the bio-optical model presented by Garcia-Pichel (1994), species like *H. rotundata* in the nanoplankton size category (cell radii, 1-<10 µm) may reap survival-determining benefits from producing self-shading compounds, unlike even smaller cells for which the benefits are negligible. However, the effectiveness of photoprotective pigments relative to the energetic cost of production for cells in the nano size category is much lower than for larger (*A. fundyense*) cells. As a result, selective pressure for this adaptation in *H. rotundata* may be comparatively weak.

The concentration of photoprotective pigments within phytoplankton cells is also heavily dependent upon acclimation light levels, which may present a confounding variable, as *A. fundyense* and *H. rotundata* were grown and effectively acclimated under different light intensities. Acclimation growth irradiance has been shown in some species to affect short-term responses to light exposure, even between strains of the same species (Laurion and Roy 2009;

Archer et al. 2010). In the present study, attempts were made to culture *H. rotundata* at the same light level as *A. fundyense*; however, under the higher light regimen, *H. rotundata* would not grow to concentrations necessary for experimentation. Appropriate cell concentrations were only achieved when *H. rotundata* cultures were grown at a reduced light level. The discrepancy in growth irradiance during pre-experiment acclimation may have partially accounted for the difference in light response between species. Low acclimation irradiance in *H. rotundata* may have made them more susceptible to light damage (Demers et al. 1991). However, this susceptibility could also be attributed to niche-defining physiological differences between species that we know to exist, the consequences of which are substantiated by the findings in the present study.

The difficulty with which *H. rotundata* grew at an elevated growth irradiance (53 µmol photons m^{-2} s^{-1}) may be a clue into the bloom niche of this species. In the literature, *Heterocapsa* species are consistently reported to be less resilient when exposed to high light and light fluctuations compared to other taxa (Laurion and Roy 2009, Lewandowska and Sommer 2010, Enberg et al. 2015), even when acclimated to relatively high light levels (Helbling et al. 2008). *H. rotundata* cells grown in different light levels prior to the present study not only differed in growth rate, but were also visibly different in coloration, likely due to pigment alteration. Slow growth and pigment change in the higher irradiance *H. rotundata* cultures persisted for months, suggesting that no substantial acclimation to the higher light condition was occurring in these cells.

It is possible that many of the species within the *Heterocapsa* genus, including *H. rotundata*, rely primarily on high rates of cell division for bloom formation and less on adaptations that protect individual cells. In the classic C-S-R model by Reynolds (1988), *H. rotundata* fits the description of the C-strategist, characterized by rapid growth, small cell size, and high surface area to volume ratio. The small cell size and weak amphiesma (Dodge and Crawford 1970) of *H. rotundata* suggest that production of new cells is relatively cheap for this species. In theory, if a species with low-cost cells can divide very rapidly (which may be the case due to the energy saved by sacrificing individual cell resilience), the population could fill in the portions of the water column that exhibited optimal conditions for net growth. In this scenario, many cells would still be lost in areas with less optimal conditions, like near the surface where light and temperature are high; however as long as cell division elsewhere in the water column exceeds this loss, a bloom should still form. Additionally, by lysing when they die, *H. rotundata* cells may effectively recycle some nutrients back into their own population, promoting further growth of more optimally-located cells.

DMSP

The organosulfur compound, DMSP, is thought to be yet another tool phytoplankton cells use to combat light stress, although no evidence of this was observed in *A. fundyense* or *H. rotundata*. The enzymatic cleavage product of DMSP, DMS, is potentially a climate-regulating gas, making it a compound of great interest on many ecological scales. On a plankton ecology scale, DMSP is thought to have a variety of biological functions in dinoflagellates and other

phytoplankton including osmotic regulation (Kirst 1996), predation defense (Strom et al. 2003), and deactivation of reactive oxygen (Sunda et al. 2002). Archer et al. (2010) observed an increase in DMSP accumulation within the coccolithophore *E. huxleyi* after just 1 hr of visible plus UV light exposure. In the same study, cells acclimated to lower light before exposure accumulated more DMSP over the exposure period than cells acclimated to ten-fold higher irradiance. Archer et al. (2009) showed that seasonally, environmental DMSP-to-chlorophyll *a* ratios varied by 40-fold, with high ratios coinciding with elevated PAR and UVB irradiance in the temperate shelf seas of the western English Channel. This fluctuation was coupled with changes in concentrations of the dinoflagellate-associated pigment peridinin, indicating that an increase in dinoflagellate abundance contributed substantially to the DMSP measured. While the two species of dinoflagellates in the present study did contain measurable amounts of DMSP, no change in intracellular DMSP was observed in direct response to high light. If intracellular DMSP did respond to changes in irradiance, the concentration might increase (via upregulation if DMSP itself is the compound responsible for combating the products of stress) or decrease (if ROS quenching by DMSP results in loss of the compound, or if cells actively cleave DMSP, following the hypothesis that the enzymatic cleavage products of DMSP are the more effective ROS quenchers). Overall, my results suggest that, unlike its role in *E. huxleyi*, neither DMSP nor its enzymatic cleavage products serve as mechanisms for immediate protection against light stress in *A. fundyense* or *H. rotundata*.

The most ecologically consequential observation gained from DMSP measurements in this study occurred in the highest *H. rotundata* light treatment, in which cells lysed. This was

the only treatment in which dissolved DMSP increased, presumably due to release from rupturing cells. The decline in total DMSP observed in this treatment supports a hypothesis by Wolfe and Steinke (1996) who proposed that, unless damage occurs, DMSP is always segregated from the cleavage enzyme DMSP lyase within the cell, and the two only intermix as a result of lysis. The results of experiment H2 are consistent with this hypothesis since DMSP only decreased in the treatment in which cells lysed. To confirm that DMSP in this treatment was lost to an enzymatic cleavage reaction with DMSP lyase, this experiment should be replicated with the addition of DMS measurements. Although the compartmentalization of DMSP and its lyase in microalgae is still unknown, a DMSP synthesis pathway ending in the chloroplast has been described in the macroalgae, *Enteromorpha intestinalis*, and involves intermediate compounds known to occur in some species of microalgae (Gage et al. 1997). However, Uchida et al. (1996) proposed a theoretical DMSP synthesis pathway for the heterotrophic dinoflagellate *Crypthecodinium cohnii* that does not involve these intermediates. When the synthesis pathway of DMSP can be definitively described in dinoflagellates, it will further elucidate the situational interactions, if any, of DMSP and its lyase within the cell.

Predation Response

In the present study, grazing by the ciliate *Schmidingerella* sp. was not affected by prey light exposure. However, changes in algal morphology brought on by abiotic stress have been shown to affect grazing on some species. A large body of work exists on the grazing behavior of *Daphnia,* a freshwater microcrustacean, on stress-treated algae. Van Donk and Hessen (1993)

demonstrated that phosphorus starvation in *Scenedesmus subspicatus* and *Selenastrum capricornutum* inhibited the assimilation of these prey cells in the *Daphnia* gut, allowing them to pass through the predator nearly intact. A subsequent study by van Donk et al. (1997) suggested that an alteration in cell wall morphology caused by nutrient deficiency in prey cells is responsible for hindering assimilation. UVB exposure during growth in algal prey cells has also been shown to inhibit prey assimilation, and to increase or decrease grazing rates depending on the predator (van Donk and Hessen 1995, De Lange and Lürling 2003). Unlike these studies, any changes in cortical structure that did occur in my experiments were induced on an instantaneous time scale rather than over multiple growth cycles. Therefore, structural changes in the present study were likely more damage-driven than defensive.

Although they yielded no differences in feeding among treatments, predation experiments in the present study were motivated by the hypothesis that stress would alter chemical signal production by dinoflagellate prey cells. In addition to its potential role in reactive oxygen defense, DMSP and its enzymatic cleavage products are thought to act as a predation-deterring infochemical in some algal species (Wolfe et al. 1997, Wolfe et al. 2000, Strom et al. 2003). Alternatively, Breckels et al. (2011) hypothesized that the cleavage product, DMS, released by photosynthetic algae may attract predatory *O. marina* which exploits these compounds as a defense against its own copepod predators. Although cell wall distention was observed in *H. rotundata*, this did not appear to result in greater DMSP release or a change in predation. Observations in the present study are not consistent with the hypothesis that high

light affects DMSP production in cells. Therefore, no conclusions could be drawn from the data collected here about the role of DMSP as a chemical signal, or its effects on grazing.

While average grazing rates remained the same among treatments, the variability in ingestion increased with intensity of prey light exposure. In the H3 *H. rotundata* experiment (which is considered the primary grazing experiment for this species since cell loss confounded grazing rate comparisons in H2), the increase in variance for ingestion rate was exponential with increasing light exposure. This pattern suggests that light exposure does affect the predator-prey interaction in some way, at least on an individual cell basis, even if it does not alter the average ingestion rate on a population level.

Alteration of swimming behavior could explain the increased variability of feeding on stressed algal cells. Solar radiation has been shown to alter motility in flagellate phytoplankton by causing loss of orientation, flagellar inactivation, and even flagellar loss (Häder 1985, van Donk and Hessen 1996). Qualitative microscopic observations in pilot experiments with *H. rotundata* cells revealed an increase in swimming speed followed closely by sluggishness over the course of continued high light exposure. Variability in prey cells, either from mutations in clonal cells or through genetic recombination via sexual reproduction, likely results in a range of timing in the onset of behavioral changes during stress and recovery. Likewise, inherent variability in predator cells results in a range of grazing aptitude on changing and increasingly behaviorally-diverse prey cells. The nuances of this hypothesized gradual change in population dynamics would be best explored through more qualitative observations and behavioral experimentation. An additional observation of *H. rotundata* swimming behavior took place

within minutes of cells being exposed to sunlight. It was clear to see with the naked eye that *H. rotundata* cells were actively swimming downward and collecting at the bottom of their bottle, presumably in a light avoidance response. Similar avoidance behavior was observed by Latasa and Berdalet (1994) in *Heterocapsa* sp. after 3 h of 150 µmol m^{-2} s^{-1} PAR exposure, suggesting light avoidance is a trait recurring within this genus. In contrast, no obvious change in behavior by *A. fundyense* was observed during light exposure.

If *A. fundyense* is more resistant to light stress, this species and others like it may benefit from this trait by avoiding predation and outcompeting less resistant phytoplankton. The tintinnid ciliate *Favella ehrenbergii* preferentially feeds on dinoflagellates, showing a strong correlation in distribution with this prey group in the water column (Stoecker et al. 1981, Stoecker et al. 1984). Unfortunately, mention of *Schmidingerella* sp. in the literature is scant, since the genus was very recently established (Agatha and Strüder-Kypke 2012). It is likely however, that species within the newly-described genus have been studied in depth as *Favella*. For example, a strain within *Favella ehrenbergii*, the species used in studies by Stoecker et al. (1981, 1984), was recently reassigned to the genus, *Schmidingerella*. In order to interpret grazing data collected in the present study, I am assuming *Schmidingerella* sp. to be functionally similar to the *Favella* species described in the literature. *Favella* are known to help suppress bloom formation and can be responsible for significant mortality of dinoflagellate cells during blooms (Watras et al. 1985). Given the choice, *Favella* exhibits preference toward larger photosynthetic dinoflagellates, and is well-documented as a predator on *Alexandrium* (previously *Gonyaulax*) species (Stoecker et al. 1981, Watras et al. 1985). Stoecker et al. (1981)

even demonstrated a specific preference for *Alexandrium tamarense* (previously *Gonyaulax tamarensis*) over *Heterocapsa* sp. However, ciliate grazers such as *Favella* are sensitive to light stress themselves (Häder et al. 2011). In vitro experiments by Mostajir et al. (1999) showed that UVB radiation actually hindered feeding by heterotrophic ciliates to the point of promoting flagellate prey abundance. If *Alexandrium* are generally resistant to light exposure, as demonstrated in the present study, high-irradiance surface waters may provide refuge from predation, allowing blooms to form. Additionally, growth of coexisting bloom-forming dinoflagellates of smaller cell size that compete for nutrients may be suppressed by the high light.

Between *A. fundyense* and *H. rotundata*, the latter species may bloom more readily when light stress is not present due to higher division rates and more efficient nutrient acquisition of the smaller cells (Raven 1998). However, in high light conditions, exploiting the suppression of competition and predation may be a defining characteristic of the *A. fundyense* bloom niche. In a parallel example, refuge-seeking in low salinity water has been observed as a possible predator avoidance strategy in *Heterosigma akashiwo*, a toxic bloom-forming raphidophyte (Strom et al. 2013). Extremophilic refuge-seeking may be an effective strategy for bloom-forming species, and should be explored further in this group of organisms.

Conclusion

My results indicate that, at high intensities, sunlight may contribute to top-down bloom regulation in *H. rotundata*. The destruction of *H. rotundata* cells at high light intensities has

clear implications for changing the ecological pathways through which primary production cycles. While intact cells may be consumed by grazers and assimilated into higher trophic levels (Sherr and Sherr 1984), materials from lysed *H. rotundata* cells may become a substrate for bacteria or, as particulate debris, may flocculate and sink (Cole et al. 1988, Sherr and Sherr 2002). Additionally, the dramatic rupture of cells under light-induced stress may release DMSP and its enzymatic cleavage products into the surrounding environment more rapidly and on a larger spatial scale than grazing. Since these compounds likely affect grazing behavior in heterotrophic grazers, this could influence plankton community dynamics on a multidimensional scale (Wolfe et al. 1997, Wolfe et al. 2000, Strom et al. 2003). As far as direct species-specific consequences go, however, based on the findings of this study, environmental light stress is likely a more important factor for bloom suppression in *H. rotundata* than for *A. fundyense*. In contrast, high light intensity conditions may define the bloom niche for the latter species through its high light tolerance combined with inhibition of both competitors and predators.

To further elucidate the implications of light stress on these dinoflagellate species, a series of follow-up studies should be conducted. First, *H. rotundata* and *A. fundyense* should be cultured under identical light conditions to remove differences in acclimation as a confounding variable. Once this is achieved, both species should be exposed simultaneously to light stress in order to compare responses under identical light stress conditions. Samples for qualitative observations of each species should be collected at close time intervals during exposure and examined microscopically for changes in behavior. The ciliate predator *Schmidingerella* sp. must

also be exposed to the same light levels as dinoflagellate prey stress treatments in order to observe any changes in behavior that might compromise grazing in the field. These additional investigations will provide a more informed assessment of the role of high light in defining dinoflagellate bloom niches. This will, in turn, help direct further explorations into the role high-irradiance sunlight plays in the formation and suppression of algal blooms.

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