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Effects of Episodic Turbulence on Diatoms: with Comments on the use of Evans Blue Stain for Live-Dead Determinations

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**Effects of Episodic Turbulence on Diatoms,
with Comments on the Use of Evans Blue Stain for Live-Dead Determinations**

A Thesis

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

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

by

Haley S. Garrison

2013

APPROVAL SHEET

This thesis submitted in partial fulfillment of

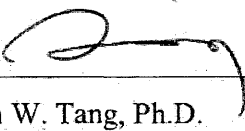
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Master of Science



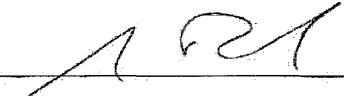
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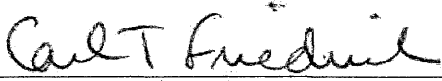


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DEDICATION

This thesis is dedicated to my parents, Mary Beth Garrison and Lehman Haley Garrison III. It is only by their unfailing support, belief, and love that I am where I am today.

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ABSTRACT

Episodic turbulence is a short-lived, high-intensity phenomenon in marine environments produced by both anthropogenic and natural causes, such as boat propellers, strong winds, and breaking waves. Episodic turbulence has been shown to cause mortality in zooplankton, but its effects on marine phytoplankton have rarely been investigated. This study focused on two diatoms: *Thalassiosira weissflogii* and *Skeletonema costatum*. I found that exposure for 45 s to turbulence intensities above $2.5 \text{ cm}^2 \text{ s}^{-3}$ caused 24-32% reduction in diatom abundance and increased the amount of intact dead cells to 22%. Turbulence also caused extracellular release of optically reactive DOM. At a turbulence level of $4.0 \text{ cm}^2 \text{ s}^{-3}$, photosynthetic efficiency (F_v/F_m) decreased from 0.51 to 0.38 and 0.55 to 0.50 in *T. weissflogii* and *S. costatum* respectively. These turbulence levels are comparable to those under breaking surface waves and are much smaller than those generated by boat propellers.

Despite its relatively short duration, episodic turbulence has the potential to affect phytoplankton via lethal and sublethal effects. An improved technique using the Evans Blue stain was developed to enable visual live/dead plankton cell determinations. When used in conjunction with preservation and flow cytometry, this staining method allows the study of phytoplankton mortality due to turbulence and other environmental stresses.

Effects of Episodic Turbulence on Diatoms,
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Chapter 1: General Background

General Background

The effects of turbulence on plankton have been widely studied. Turbulence in the marine environment spans a variety of spatio-temporal scales, from mesoscale turbulence on the order of tens of kilometers that influences biological production (Lévy et al 2009) to micro-scale turbulence on the order of microns. Turbulence intensity also spans several orders of magnitude. Measured by turbulent energy dissipation rate (ϵ), or the rate at which turbulent energy is converted to thermal energy per unit time, turbulence ranges from 7.4×10^{-4} to $1.5 \times 10^4 \text{ cm}^2 \text{ s}^{-3}$ (Peters and Marrasé 2000).

Observations of small-scale turbulent flow in aquatic systems indicate that ϵ is inversely related with depth and correlated with wind speed (Dillon 1981). Wind stress from storm events causes a more extreme relationship of depth with turbulence (z^{-4}) compared to calm conditions (z^{-1}) (Gargett 1989). Observations by Kitaigorodskii et al. (1983) suggest that breaking surface waves 1-2 m high produce high-magnitude energy that penetrates to depths 10 times the wave amplitude.

As a result of techniques and equipment recently developed, our knowledge of ocean turbulence has increased. Acoustic Doppler Velocimeters (ADVs) and Acoustic Doppler Current Profilers (ADCPs) are able to measure turbulent eddies by tracking particle movement in the x, y, and z directions. Real-time profiling allows characterization of low-frequency turbulence such as Langmuir events, the magnitude of

which may have been previously underestimated (Weller et al. 1985). ADVs may be deployed for months, and arrays of ADCPs can accurately observe large-scale turbulent convection (Gargett et al. 2008). Microstructure temperature profiles made with fast-response thermistors can also be used to compute turbulent energy dissipation rate; temperature measurements may be even more accurate than velocity measurements at detecting turbulent fluctuations with high wavenumbers (Lorke and Wüest 2005). Despite increased knowledge of the properties and magnitude of ocean turbulence, relatively few studies of the effects of intense turbulence on microscopic organisms such as phytoplankton have been completed.

Turbulence affects phytoplankton physiology and community structure. Even weak turbulence may alter phytoplankton composition and physiology: chain-forming diatoms produce thicker tests and more flexible mucous filaments when exposed to turbulence with an energy dissipation rate of $10^{-1} \text{ cm}^2 \text{ s}^{-3}$ (Clarson et al. 2009). At higher levels of turbulence, diatoms become dominant over dinoflagellates, while the latter thrive in quiescent environments (Estrada and Berdalet 1997, Kiørboe 1993, Margalef 1978). Dinoflagellates use their flagella to maintain rotational motion, increasing nutrient flux relative to non-motile diatoms in calm water, but under turbulent conditions they lose this advantage due to velocity fluctuations in the shear field (Karp-Boss et al. 1996). Small-scale turbulence also alters food web interactions. The likelihood of encounter between two objects in a given amount of time and space, for example, increases contact between grazers and phytoplankton by 50% (Yamazaki et al. 1991), or increase bacterial abundance due to organic material released by increased sloppy feeding as consumers come in more frequently come in contact with large phytoplankton (Peters et al. 1998).

Micro-scale turbulence is often overlooked but has the potential to be beneficial to phytoplankton at an intermediate level (Thomas and Gibson 1990), in part due to the fact that turbulence has the potential to overcome the limits of diffusive transport. For example, NO₃ uptake increased 10% in *Ditylum brightwellii* cultures exposed to intermediate shear levels in a Couette cylinder, but uptake of ¹⁴C-bicarbonate decreased (Pasciack and Gavis 1975). Turbulent motion is one of the mechanisms that cause cell aggregation. If turbulence induces aggregate formation, phytoplankton may be unable to remain buoyant and will sink from the euphotic zone; large cells are more likely to form flocs and sink than small cells (Jackson 1990). On the other hand, if intense turbulence tends to destroy large cells, a trend toward smaller cells under conditions of intense turbulence could lead to slower sinking rate, increasing potential for remineralization of organic matter higher in the water column (Anderson and Sarmiento 1994).

Despite the myriad of potential effects of turbulence on phytoplankton, there are few studies addressing the effects of episodic turbulence. Episodic turbulence here is defined as turbulence occurring over a short period (seconds to minutes) at energy levels orders of magnitude higher than ambient background turbulence. In systems where boat traffic is prevalent, where severe storms are common, or where waves break, phytoplankton experience turbulence orders of magnitude higher than background turbulence. In this study I addressed four key questions to assess the effects of episodic turbulence on diatoms.

Does intense, episodic turbulence cause phytoplankton mortality? Episodic turbulence has been shown to increase zooplankton mortality. In particular, the presence of zooplankton carcasses was higher inside boat wakes than in the water immediately outside the boat wakes; zooplankton carcasses were also more prevalent along a navigation channel than neighboring quiescent waters (Bickel et al. 2011). Phytoplankton are, on average, orders of magnitude smaller than zooplankton, meaning that the magnitude of turbulence required to cause mortality to phytoplankton is expected to be much higher.

The majority of turbulence-related phytoplankton studies focus on dinoflagellates. When dinoflagellate data are separated from those synthesized across many turbulence experiments, trends predicting the effects of turbulence can reverse, highlighting the need for more species-specific studies (Peters and Marrasé 2000). The purpose of this study is to determine the effects of intense, episodic turbulence on two diatom species of different size classes.

To investigate the lethal effect of episodic turbulence, it was necessary to develop a method for differentiating live from intact, dead cells. I therefore tested the mortal stain Evans Blue (EB) to determine its efficacy in making live-dead determinations on marine phytoplankton. The stain was tested on *Thalassiosira weissflogii*, *Skeletonema costatum*, *Rhodomonas salina*, and *Dunaliella tertiolecta*. The EB stain is both visible (it stains dead cells a deep blue color) and fluorescent and is simple to apply. Testing its efficacy across different species and killing methods was necessary to ensure that the method produced replicable results.

Does turbulence affect large phytoplankton to a greater extent? Larger phytoplankton will potentially be affected to a greater extent by turbulence than smaller phytoplankton. This is because intense turbulence produces shear force that may be destructive if the turbulence is strong enough to produce eddies on a length scale comparable to the phytoplankton's length. The Kolmogorov microscale (L_K) is the theoretical length of the smallest eddy size a given turbulence intensity can produce and the length at which inertial forces overcome viscous forces; L_K was calculated for each turbulence level used in these experiments (Tennekes and Lumley 1976). L_K becomes smaller as turbulence intensity increases, creating smaller turbulent eddies. Unless L_K is at or below their cell length, phytoplankton do not experience velocity gradients from the inertial forces of turbulent shear. L_K is useful in describing physical regimes, but it is not the only driver of small-scale shear fields. Local velocity may fluctuate if the direction of the shear field changes rapidly (as is often the case in turbulence).

It is possible that turbulence will affect nutrient fluxes to phytoplankton cells. The Batchelor microscale index, which describes the smallest distance across which a gradient in substrate will exist before diffusive molecular forces dominate, changes with turbulent kinetic energy in a system. Depending on the length of the Batchelor index relative to cell size, phytoplankton may experience an increase in nutrient supply.

Does turbulence increase the release of nutrients and organic compounds by phytoplankton? Sub-lethal shear forces have been shown to cause nutrient extrusion (Saiz and Alcaraz 2002). Enhanced release of nutrients and organic compounds by phytoplankton in turbulent environments may benefit the smaller phytoplankton and

other microbes. This may represent a flux of organic material to the microbial loop and could also represent a disadvantage to phytoplankton in the form of biomass, nutrient, or trace metal loss. I investigated whether diatoms released trace metals and dissolved organic matter when exposed to different turbulent energy levels.

Does turbulence decrease photosynthetic efficiency? A variety of stressful conditions affect phytoplankton photosynthesis, such as irradiance, CO₂ concentration, and nutrient fluxes (Caperon and Smith 1978, Harrison and Platt 1986). Stress responses are defined here as reductions in the ability of a phytoplankton to photosynthesize, produce organic material, assimilate nutrients, or grow and reproduce. Shear forces that do not kill a cell may still produce stress responses, such as decreased photosynthetic efficiency (Thomas et al. 1995). Phytoplankton in the ocean experience varying irradiance regimes primarily due to vertical mixing, and the time scale for their chlorophyll responses to changes in irradiance is on the order of hours rather than seconds (Franks and Marra 1994).). Photo-protective carotenoids, such as diatoxanthin, respond to high irradiance on the order of minutes to protect the cell from photo-oxidative damage (Lavaud et al. 2003). This suggests that phytoplankton may have the ability to photoacclimate rapidly to episodic turbulence, but it is unknown if turbulence itself will elicit this response. However, other mechanisms, such as modulation of light harvesting and Calvin cycle activity (MacIntyre 2000), have been identified in estuarine phytoplankton, which experience much shorter mixing time scales, suggesting that decreased photosynthetic efficiency is a response that may occur on the time scale of episodic turbulence.

Chapter Two is formatted in a “manuscript style” intended for submission for publication in a peer-reviewed journal. It contains Introduction, Materials and Methods, Results, Discussion, and Conclusions encompassing the turbulence experiments and Evans Blue stain trials. Additional comments and concluding remarks follow the manuscript conclusion in Chapter 3. Data not included in the manuscript portion are in Appendices.

Chapter 2: Effects of Episodic Turbulence on Diatoms, with Comments on the Use of Evans Blue Stain for Live-Dead Determinations

1. Introduction

Phytoplankton mortality in the marine environment is largely attributed to zooplankton grazing (Lenz et al. 1992, Cullen et al. 1992) and viral lysis (Brussaard 2004, Suttle 1990). However, the extent to which the physical environment is a source of mortality for phytoplankton is unknown. The physical environment imparts a variety of stresses on phytoplankton. Some phytoplankton mortality due to fluctuations in nutrient availability, UV radiation, and temperature extremes has been documented, but only in instances where fluctuations are rapid and acute (Llabrés and Agustí 2006). Episodic turbulence is ephemeral, intense turbulence produced by events such as storms, boat propellers, and breaking waves. Intense, episodic turbulence has the potential to cause mortality and impart physical stress on phytoplankton as it is by definition a sudden and extreme physical forcing.

Turbulence in the environment spans many orders of magnitude, with measured energy dissipation rates (ϵ) ranging from 10^{-4} to 10^4 $\text{cm}^2 \text{s}^{-3}$ (Peters and Marrasé 2000). Even weak turbulence alters phytoplankton assemblage composition and physiology. Chain-forming diatoms produce thicker tests and more flexible mucous filaments when exposed to bubble-derived turbulence with a relatively weak energy dissipation rate of 10^{-1} $\text{cm}^2 \text{s}^{-3}$ (Clarson et al. 2009). With increasing level of turbulence, diatoms become

dominant relative to dinoflagellates and chlorophytes (Estrada and Berdalet 1997; Thomas and Gibson 1990; Sullivan et al. 2003).

Models of turbulent energy have traditionally considered the air-sea interface as an inverted wall model (Terray et al 1996), but observations of turbulent energy using acoustic instrumentation has revealed energy dissipation rates up to 70 times higher than inverted wall model output (Melville 1994), indicating that turbulence in surface waters has been under-estimated. Observed dissipation energy under wind-driven, breaking surface waves can reach $10^2 \text{ cm}^2 \text{ s}^{-3}$, and those for waves breaking on shore $10^4 \text{ cm}^2 \text{ s}^{-3}$ (Agrawal et al. 1992), and intermittently stormy conditions $10 \text{ cm}^2 \text{ s}^{-3}$ (Gargett 1989). The intermittency of episodic turbulence may also enhance its effects on phytoplankton. Phytoplankton exposed to intermittent turbulence experienced decreased growth rates compared to those exposed to constant turbulence (Gibson and Thomas 1995), and frequency of waves with significant height has been negatively correlated with rates of dinoflagellate growth (Tynan 1993).

Besides natural events, intense episodic turbulence can be generated by boat activities. Damage to marine megafauna due to recreational boat propellers has been well-documented (Beck et al. 1982, Cannon 1994). A single outboard motor propeller may produce turbulence in excess of $3 \times 10^2 \text{ cm}^2 \text{ s}^{-3}$ (Loberto 2007). Propeller turbulence generated by shipping vessels can be up to $5 \times 10^4 \text{ cm}^2 \text{ s}^{-3}$ (Killgore 1987), well above natural turbulence intensities. Larval perch experimentally exposed to turbulence dissipation shear of 35 N m^{-2} experienced 38% mortality after just one minute (Morgan et al. 1976). Alterations in the hydrological regime caused by shipping vessels also affect

fish behavior and riverine migration patterns (Odeh et al. 2002). Recent studies have shown that boat turbulence may also impact even small marine plankton. Episodic turbulence may cause mortality in excess of 30% in natural copepod populations exposed to high volume boat traffic. Zooplankton carcasses are more prevalent inside boat wakes than immediately outside of them, and increased turbulence intensity is correlated with increased percentage of copepod carcasses (Bickel et al. 2011). The presence of copepod carcasses due to episodic turbulence may locally alter water column microbial activities and biogeochemical cycling (Tang et al. 2009, Bickel and Tang 2010).

Phytoplankton cell size is an important factor in predicting the effects of turbulence (Kiørboe 1993). Intense turbulence produces shear forces that may be destructive, but only if the turbulence is strong enough to produce eddies on a scale comparable to the phytoplankton's cell length. The Kolmogorov microscale (L_K) is defined as:

$$L_K = \left(\frac{\nu^3}{\varepsilon} \right)^{1/4}$$

(Tennekes and Lumley 1972) where ν is kinematic viscosity of water ($10^{-2} \text{ cm s}^{-1}$) and ε is the turbulent kinetic energy dissipation rate. L_K describes the scale at which inertial forces dominate over viscous forces; it becomes smaller as turbulence intensity increases. In theory, phytoplankton, which generally have a very small Reynolds number, do not experience damaging velocity gradients from the shear forces generated by turbulence unless L_K is at or below their cell length.

. The Batchelor index describes the smallest distance across which a gradient in substrate will exist before diffusive molecular forces dominate, and like L_K is determined in part by turbulence energy dissipation rate:

$$L_B = \left(\frac{\nu D^2}{\varepsilon} \right)^{1/4}$$

where ν is the kinematic viscosity of water at 19 C (10^{-2} cm s⁻¹), D is mass diffusivity of a given scalar (For transition metals in 18 C water, $5.8 \pm 0.1 \times 10^{-6}$ cm²s⁻¹; Li and Gregory 1974), and ε is the turbulent energy dissipation rate calculated at each experimental turbulence level. The Batchelor index is typically smaller than L_K and may affect organisms even when $L_K >$ cell size. This means that although inertial fluctuations from turbulent eddies may be too large to influence a phytoplankton, the chemical gradients may vary on a scale fine enough for a phytoplankton cell to experience.

It is important to also consider the sub-lethal effects of turbulence on a phytoplankton cell. Even shear forces that do not kill the cell may still produce stress responses such as decreased photosynthetic efficiency (Thomas et al. 1995) and cellular extrusion (Saiz and Alcaraz 2002). Combined, these lethal and sub-lethal effects have important ramifications for phytoplankton composition and functions. For example, if larger cells are affected by a certain turbulence intensity threshold, smaller cells may benefit from the reduced competition and nutrients extruded by the larger cells. This mechanism is one of many that may affect phytoplankton composition following episodic turbulence events. Laboratory studies are suited for investigating turbulence effects on phytoplankton because turbulence exposure (intensity and duration) can be manipulated

and the specific responses of the phytoplankton cells can be observed under controlled conditions.

I conducted experiments to study both the lethal and sublethal effects of intense, episodic turbulence on two diatom species of different sizes. For sublethal effects I measured cellular extrusion of dissolved organic material (DOM) and trace metals, as well as photosynthetic efficiency (F_v/F_m), before and after turbulence exposure.

The study of lethal effect presented a challenge because, unlike unarmored and motile cells, dead but intact diatoms may appear the same as live diatoms. It was therefore my goal to first develop a simple and reliable method to distinguish between live and dead diatoms. Staining is a simple, cost-effective way to make visual live/dead determinations. Staining protocols have been successfully developed for marine and freshwater zooplankton (Bickel et al. 2009, Elliot and Tang 2009). Evans Blue (EB) is a mortal stain that is effective in making live/dead determinations in vascular plant cells, and has been tested on marine phytoplankton (Crippen and Perrier 1974). The stain penetrates cells with compromised plasma membranes and binds to the cytoplasm, producing a clear, deep blue color in dead cells that is visible under light microscopy. In a preliminary trial, EB was compared with the vital stain Neutral Red, but the former proved more effective in distinguishing between live and dead phytoplankton.

This study consisted of two parts. In the first part I assessed the efficacy of EB stain for determining live/dead phytoplankton cells, and developed a simple staining protocol for measuring diatom mortality in my turbulence experiments. I also tested the stain in conjunction with flow cytometry and preservation method to further expand the

application of the protocol for future work. In the second part I conducted laboratory experiments in which I exposed the diatoms to different levels of turbulence and measured both lethal and sublethal effects.

2. Materials and Methods

2.1 Evans Blue Stain

Preparation of Stain Stock Solution

0.25 mg Evans Blue powder (Sigma Aldrich 206334) was added to 25 ml DI water to give a concentration of 1% solution. The mixture was stirred overnight to ensure complete dissolution. The stock solution was then transferred to a borosilicate amber glass bottle and stored at room temperature until use. EB stock remained usable for at least 5 months under these conditions.

Algal cultures

Four species of marine phytoplankton were used in EB stain trials. They included two diatoms [*Thalassiosira weissflogii* (CCMP 1336) and *Skeletonema costatum* (CCMP 1332)], one cryptomonad [*Rhodomonas salina* (CCMP 1319)], and one chlorophyte [*Dunaliella tertiolecta* (CCMP 1320)]. Diatoms were grown in f/2+Si media, *R. salina* in L1, and *D. tertiolecta* in f/2. New cultures were started with fresh media every 7 days. All cultures were grown at 19° C on a 12 h: 12 h light-dark cycle. Cells were taken from cultures in log phase for testing EB stain.

Since phytoplankton cells from the cultures were almost 100% live, it was necessary to produce dead cells by artificially killing a subsample of the cultures. Several killing methods were used:

- a. Heat: 1 ml samples from phytoplankton cultures were incubated in a 30°C convection oven for 15 minutes.
- b. Salinity shock: 1 ml samples from phytoplankton cultures grown at ~20 psu, were gently filtered with 20 µm nylon mesh and rinsed into 27 ASW. Tests were conducted with cells stained before and after filtration to ensure that a significant amount of cells were not killed by handling during filtration.
- c. UV radiation: 1 ml samples were exposed to intense UV A/B radiation by placing a UV lamp directly above sample cuvettes for 25 minutes.
- d. Biocide: 0.975 mg ml⁻¹ sodium azide was added to 1 ml phytoplankton samples for a final concentration of 15 mmol L⁻¹ (Pett 1989).

Live and dead cells were mixed in ratios of 100:0, 75:25, 50:50, 25:75 and 0:100. The mixtures were treated with the stain and the observed percentage of live (unstained) cells was compared to the expected percentage of live cells per sample.

Stain Protocol

An aliquot of 15 ml was gently pipetted out from each of the live:dead mixtures, and EB stock solution was added in the amount of 0.02 ml EB per ml sample. The sample was stained for at least 15 minutes. Three 1 ml subsamples were transferred from the

stained sample to Sedgewick Rafter counting slides. Each subsample was allowed to settle for 2 minutes prior to microscopy.

Microscopic Enumeration

Cells were counted at 200x using a Nikon TS100 inverted microscope. About 600 cells per sample were randomly selected and counted on a Sedgewick-Rafter slide. Live cells appeared light green or colorless and dead cells appeared deep blue due to EB uptake (Figure A1).

Flow Cytometry

EB fluoresces at ~680 nm when excited at 620 nm (Saria and Lundberg 1983). The stain protocol was tested to determine if it enabled the flow cytometer to make live-dead differentiations. Flow cytometry was tested on samples of *T. weissflogii* and *S. costatum*. For comparison of a 100% 'live' sample before and after staining, 1.5 ml diatom culture was analyzed by an Accuri C6 Flow Cytometer. The C6 was set to run at high speed for two minutes, consuming a 131 μ l subsample. Triplicate samples were stained and analyzed by the C6. In another trial, 1.5 ml of a diatom culture was analyzed; then, half of the remaining sample was killed by either heat or biocide. The 'killed' subsample was mixed with the untreated sample to create a 1:1 live/dead mixture and analyzed. Afterward the mixture was stained with EB and three replicates re-analyzed. Lastly, 1.5 ml of diatom culture was killed by either heat or biocide and analyzed before and after staining; three replicates per killing method were evaluated. Differences in

fluorescence and side scatter were evaluated using the Batch Analysis tool in the BD Biosciences C6 software.

Preservation Method

To expand the future usability of the staining technique, EB was tested in conjunction with refrigeration or formalin preservation (Weber 1968). Heat- or azide-killed phytoplankton cells were mixed with live cells in triplicate, and then stained with EB for 20 minutes. The stained samples were counted immediately then stored at room temperature, or refrigerated without preservative, or preserved in 5% formalin. The samples were re-counted after 24 and 48 h. The formalin-preserved samples were also counted after 7 d. Cell counts at each time point were compared using ANOVA.

2.2 Turbulence Experiments

Experimental Unit Construction

The experimental unit consists of a rectangular 10-gallon glass aquarium, a turbulence generation device, and a Nortek Acoustic Doppler Velocimeter (ADV). The turbulence generation device was constructed using the motor of a 6-speed hand mixer, non-toxic marine epoxy, hollow styrene tubing, and a 5 inch diameter, 4-blade plastic propeller. The ADV and the propeller were positioned at opposite ends of the aquarium such that there was a 15-cm space on all sides of the ADV and a 19-cm space between the ADV and the bottom of the tank (Figure A2).

Experimental Procedure

The aquarium, propeller apparatus, and sample bottles were washed with HCl and DI water prior to each experiment. After rinsing and drying, the tank was filled with seven L 70 psu trace-metal-clean seawater (Sunda et al. 2005) and 21 L Milli-Q water for a total volume of 28 L and salinity of 22. Blanks were taken from the tank at this point. 4 L of diatom culture was gently filtered from growth media into 4 L ASW. After filtering, the diatoms in ASW were added to the tank for a final volume of 32 L. The turbulence generation device was run for 45 s at different speeds to generate different turbulence energy levels. The actual turbulence levels, measured by the ADV, were designated as high, medium, and low (Figure A3). I conducted four replicate experiments per turbulence level for *T. weissflogii* and triplicates per turbulence level for *S. costatum*. One or more samples for each of the analyses below were taken before and after each turbulence trial, except for ICPMS samples, which were only collected for two of the *T. weissflogii* experiments due to logistical constraints. Trace-metal-clean seawater+Milli-Q blanks were collected for each of the chemical analyses described below.

Mortality and Cell Size

The EB stain protocol was applied to three 10-ml samples taken before and after each turbulence trial for live/dead determinations. Stained samples were counted on a Sedgewick-Rafter counting slide (a minimum of 180 cells were counted). The C6 gave live/dead counts as well as cell size and abundance.

Trace Metal Content of Diatoms

Inductively coupled plasma mass spectroscopy (ICPMS) was used to measure cellular trace metal and phosphorus content before and after turbulence. My analysis focused on changes in Mn, Fe, Co, Cu content of *T. weissflogii*. Due to logistical constraints, the analyses were only performed for two *T. weissflogii* replicate experiments at each turbulence level. Two 125-ml samples from the experimental tank were taken in LDPE bottles and vacuum filtered in a trace-metal-clean room through acid washed, pre-weighed 8 μm polycarbonate filters. Algae cells were washed of surface-complexed metals and metal hydroxide precipitates using established methods (citrate/oxalate/EDTA wash) followed by an ultrapure, pH-buffered salt rinse (Tang and Morel, 2006; Tovar-Sanchez et al., 2003). Filters were dried at 60°C for at least 24 h and weighed. Dried filters were refrigerated until acid digestion prior to ICPMS analysis. Filters were placed in pre-weighed LDPE lock-top centrifuge tubes to which 0.67 ml HCl and 0.33 ml HNO₃ were added. After 12 h samples were shaken and capped, weighed, then incubated in acid for seven days. Samples were diluted 1:11 (200 ml sample:2000 ml acid+Indium standard) in trace-metal-clean sample cups and assessed using the ICPMS. Metal concentrations in algal biomass digests were measured by high resolution inductively coupled plasma mass spectrometry (ICPMS; ThermoFisher Element2) using indium as an internal standard. Digests were diluted 11-fold and injected directly into the ICPMS. Concentrations were externally calibrated using NIST-traceable multi-element standard solutions (Inorganic Ventures). Accuracy was confirmed using standard reference material NIST 1643e, which gave recoveries within 10% of the certified value.\

DOM Content of Water

A Shimadzu RF-1501 Spectrophotofluorometer was used to qualitatively characterize optically reactive DOM in the ambient water (Stedmon et al. 2003). It was not intended that this method would yield the exact quantity of each DOM species in the samples; rather, a qualitative comparison of the fluorescence spectrum before vs. after turbulence would indicate if turbulence led to the release of DOM. A control experiment without phytoplankton was conducted to test whether the propeller apparatus introduced DOM into the tank.

Photosynthetic Efficiency

A Pulse Amplitude Modulating (PAM) fluorometer (Walz Company) was used to assess photosynthetic efficiency of the diatoms. 25-ml samples were taken before and after each turbulence trial. Samples were diluted with artificial seawater (10 ml sample: 40 ml seawater) and injected into the PAM. Three separate F_o/F_m pulse readings and one saturation pulse reading per sample were obtained before draining the sample. The instrument was rinsed with distilled water between samples. The following equation was used to calculate the photosynthetic efficiency of the cells:

$$F_v/F_m = (F_m - F_o)/F_m$$

where F_m = maximum fluorescence, F_o = minimum fluorescence and F_v = variable fluorescence (Juneau et al 2005). F_v/F_m is widely used as a proxy for the efficiency of Photosystem II (PS II) and is proportional to the quantum yield (i.e., molecules of CO_2 fixed per photon absorbed).

Calculating Turbulent Energy Dissipation Rate

Turbulent kinetic energy is the mean kinetic energy per unit mass produced by turbulent flow. Turbulence kinetic energy (k) in the aquarium is given as:

$$k = \frac{1}{2} \left((\overline{u'_1})^2 + (\overline{u'_2})^2 + (\overline{u'_3})^2 \right)$$

where u'_1 , u'_2 , and u'_3 are deviations from mean velocity measurements in the x, y and z directions (Libby 1996). Once k is calculated, the value is used to calculate turbulent energy dissipation rate (ε) in the aquarium:

$$\varepsilon = c_\mu^{3/4} k^{3/2} l^{-1}$$

where c_μ is assumed to be 0.09 (Libby 1996) and l is the maximum size of an eddy possible given the physical constraints of the system:

$$l = Kz$$

where z is the length of the water mass monitored by the ADV and K represents von Karman's constant (0.41), a dimensionless value that describes the fluid velocity gradient from a no-slip boundary layer. In this study $z=15$ cm. All calculations were performed in MATLAB.

3. Results

3.1 Evans Blue Stain Protocol

A significant linear relationship between expected percentages of live cells and observed percentages of live cells was found for all four phytoplankton species and different killing methods (Figure 1). The flow cytometer was unable to distinguish a sample of live cells from a 1:1 live/dead mixed sample without staining (Figure 2a, 2c). When Evans Blue was applied, stained cells fluoresced at a different frequency than unstained cells, creating a second peak in the emission spectrum and enabling live/dead differentiation of *T. weissflogii* (Figure 2d). A sample of only dead stained cells produced a peak comparable to the second peak in the 1:1 live/dead mixture (Figure 2e).

EB stained samples stored at room temperature or refrigerated showed a significant decrease in the percentage of unstained cells after 24 ($p=0.01$) and 48 hr ($p=0.03$). (Figure 3 a, b). EB stained samples preserved with formalin showed no significant change in the percentage of live cells counted 24, 48, or 7 d after staining ($p=0.7$; Figure 3c), indicating good stain retention by dead cells.

3.2 Turbulence Experiments

Energy dissipation rate (ϵ), Kolmogorov length scale (L_K), and Batchelor microscale index were calculated for each turbulence level from the ADV data (Table 1).

High, medium and low turbulence had energy dissipation rates of 4.0, 2.5, and 1.1 $\text{cm}^2\text{sec}^{-3}$ respectively. The smallest L_K , 224 μm , and the smallest Batchelor microscale index, 55 μm , were produced by high turbulence (Table 1).

In the low turbulence treatment, percentage live cells decreased 1% after turbulence in *T. weissflogii* (paired t-test; $p=0.703$), and decreased 2% in *S. costatum* ($p=0.092$), indicating that turbulence did not cause a significant increase in mortality (Figure 4). Medium turbulence caused a 20% decrease in live cells in *T. weissflogii* ($p=0.002$) and a 5% statistically insignificant decrease in percentage live *S. costatum* ($p=0.085$). High turbulence caused a significant 15% decrease in percentage live *S. costatum* ($p=0.005$), but only a 1% decrease in *T. weissflogii* ($p=0.391$).

In addition to relative proportions of live/dead cells, the duration of turbulence exposure on cell abundance suggested that overall cell abundance decreased after exposure to high or medium turbulence (Figure 5). After high turbulence, cell concentration decreased 17% in *T. weissflogii* (paired t-test; $p=0.002$) and 30% in *S. costatum* ($p=0.028$), indicating disruption of cells by turbulence. Medium turbulence also caused a significant decrease in total abundance of 15% in *T. weissflogii* ($p=0.023$) and 13% in *S. costatum* ($p=0.021$). There was no significant difference in the cell count for either *T. weissflogii* ($p=0.418$) or *S. costatum* ($p=0.918$) at low turbulence.

Active fluorescence showed no significant differences in quantum yields before and after turbulence at low or medium turbulence intensity (Figure 6 a, b, d, e). After low turbulence, F_v/F_m increased from 0.42 to 0.45 in *T. weissflogii* ($p=0.05$) and decreased from 0.54 to 0.52 ($p=0.3$) in *S. costatum*, but neither change was significant. After

medium turbulence, F_v/F_m decreased from 0.54 to 0.53 in *T. weissflogii* ($p=0.05$) and remained at the same value, 0.54, in *S. costatum* ($p=0.7$). High turbulence was the only level that caused a significant reduction in photosynthetic efficiency. F_v/F_m significantly decreased by 25% from 0.50 to 0.37 in *T. weissflogii* ($p=0.02$) and by 12% from 0.56 to 0.5 in *S. costatum* ($p=0.01$; Figure 6 c, f).

Spectrofluorometry showed changes in the fluorescence signatures of reactive DOM after turbulence (Figure 7, Figure 8). Reactive DOM prior to turbulence was low in fluorescence intensity and number of peaks detected. At low turbulence, the DOM spectra for both *T. weissflogii* and *S. costatum* were similar to the spectra detected before turbulence; the only major difference was the appearance of a peak at 315 nm after turbulence that was small compared to medium and high turbulence peak heights. Medium turbulence generated two peaks in both species: 318 nm and 684 nm in *T. weissflogii* and 319 and 686 nm in *S. costatum*. High turbulence also produced two peaks after turbulence that were similar wavelengths to the peaks produced at medium turbulence in both species. High turbulence peak heights were higher than medium-turbulence peak heights in *S. costatum*, but not in *T. weissflogii*. The fluorescence spectrum showed little change in the control experiment, indicating that the propeller apparatus did not release reactive DOM into the water (Figure A4).

ICPMS results showed varying levels of metal reduction between trials and the metals evaluated (Figure 9). At high and medium turbulence, the Mn, Fe, and Co content per gram dry weight of *T. weissflogii* decreased, while Cu content per gram dry weight decreased at medium turbulence and increased at high turbulence. Low turbulence either

produced no effect or a positive effect on cellular Mn, Fe, Co, and Cu content. One medium turbulence replicate and one high turbulence replicate produced below-detection-limit values for Cu and Fe, so statistical comparison was not performed for those replicates.

4. Discussion

4.1 Turbulence Experiments

At low turbulence, the percentage of intact, live *T. weissflogii* and *S. costatum* cells and cell abundance were not significantly changed after episodic turbulence, indicating that low turbulence did not cause mortality (Figure 4). At medium turbulence, the percentage of dead *S. costatum* increased significantly by 10%, and despite the fact that *T. weissflogii* cells are smaller than *S. costatum* cell chains, medium turbulence also produced a 20% increase in intact dead *T. weissflogii* cells. High turbulence did not result in an increase in percentage of dead *T. weissflogii* cells, but there was a significant decrease in abundance at high and medium turbulence, suggesting that high turbulence primarily caused mortality via cell destruction. High turbulence caused a significant increase in dead cells and a decrease in total cell abundance in *S. costatum*. Medium and high turbulence produced a 15 and 17% decrease in *T. weissflogii* cell abundance and a 13 and 30% decrease in *S. costatum* abundance, respectively (Figure 5). Both changes were significant when compared with a paired t-test ($p < 0.05$).

Differences in the effects of turbulence on the two diatom species could be due to size differences. *S. costatum* is a chain-forming diatom (9-12 μm) and was consistently observed in chains of three to four cells, making their effective chain size 36-48 μm . *T. weissflogii* single cells averaged 21 μm . After high and medium turbulence chain size

was typically reduced to two cells. Low turbulence did not produce a significant decrease in cell abundance in either species. Since the effective size of *S. costatum* chains was larger than individual *T. weissflogii*, they may have experienced shear from turbulent eddies or velocity fields that were too large to directly impact *T. weissflogii*, causing a higher disruption of cells at high turbulence. However, *S. costatum* experienced a lower rate of cell destruction at medium turbulence, which may be attributed to the breakage of cell chains rather than destruction of individual cells.

Episodic turbulence appeared to affect the larger phytoplankton to a greater extent, causing a preferential reduction of the abundance of larger cells. Phytoplankton size is important in determining the structure of marine food webs. A system dominated by small cells will recycle most biomass in surface waters as part of the microbial loop (Azam et al. 1983, Legendre and Le Fevre 1995), whereas a system dominated by larger cells allows a greater transfer of energy to higher trophic levels via grazing by size-specific predators such as mesozooplankton (Cushing 1989). Phytoplankton patchiness at small scales (<20 cm) is a feature of many marine environments (Owen 1989). The water volume searched by zooplankton is often even smaller than this scale, so episodic turbulence may cause reduction of available food or food encounter rates if phytoplankton cells of a certain size class are destroyed. Conversely, turbulent advection may provide a pulse of nutrients or phytoplankton cells across stratified vertical or horizontal gradients (Kjørboe 1993).

In both *T. weissflogii* and *S. costatum*, active fluorescence showed that F_v/F_m was significantly reduced at high turbulence and was not reduced at low or medium

turbulence (Figure 6). These findings are consistent with the few reports concerning the direct effects of turbulent shear on photosynthesis. For example, in a study of *Gonyaulax polyedra*, photosynthesis was not as sensitive to turbulent shear as population growth rate; chlorophyll-specific photosynthetic rate ($\mu\text{mol C } \mu\text{mol chl}^{-1} \text{ h}^{-1}$) only decreased at the highest turbulence level tested ($0.18 \text{ cm}^2 \text{ sec}^{-3}$), while cell division rates sharply declined at lower turbulence levels, insinuating that the mechanical action of turbulence, not its influence on photosynthesis, was preventing cell division (Thomas et al. 1995). Rapidly changing physical regimes reduce photosynthetic efficiency transiently (Lewis et al. 1984), but when the regime change or perturbation persists over a period of hours phytoplankton are able to acclimate their photochemical apparatus to optimize photosynthetic yield (Cullen and Lewis 1988). The duration of episodic turbulence is short—on the order of seconds to minutes in the case of waves and boat propeller disturbances. Phytoplankton may be unable to adapt on this timescale, which may explain the reduced photosynthetic capacity observed at high turbulence intensity. Moreover, whether or not phytoplankton are able to adapt on this timescale, it may not be energetically advantageous for them to alter their photosystems in response to such a transient disturbance.

Spectrofluorometer results showed an increase in optically reactive DOM at wavelengths 319-325 nm, a signature consistent with tyrosine-like amino acids or small proteins (Figure 7, Figure 8; Fellman 2010, Ferrari 2010). Such material is extremely labile and may promote bacteria-phytoplankton associations (Capone et al. 1994). DOM excretion is common in healthy phytoplankton and typically consists of photosynthates that are rapidly consumed by bacteria (Björnson 1988). Exudates from healthy, growing

phytoplankton are often low molecular weight molecules like amino acids, some polysaccharides, and nitrogen compounds (Watt 1969, Mague et al. 1980, Mykelstad et al. 1989), which is consistent with the spectral signature of DOM released during high and medium turbulence experiments. A second peak at ~688 nm appeared after both medium and high turbulence; this might be humic-like compounds with low reactivity that may be derived from larger cell fragments (Coble 1996, Matthews et al. 1996).

One proposed mechanism for this increase in DOM release is the elimination of boundary layers by turbulent shear; in the absence of a stable boundary layer, diffusion gradients between the cell and ambient water increase and cause elevated DOM extrusion (Malits et al. 2004). Changing photosynthetic efficiency may also cause DOM release if photosynthetic carbon fixation generates more organic carbon than is required for cellular metabolism, growth and reproduction, resulting in excretion of excess material (Smith and Wiebe 1976). I did not monitor the fate of material released via cell destruction and DOM extrusion, but it is probable that this labile material would be taken up by bacterial cells (Malits et al. 2004, Arin et al. 2002). Episodic turbulence may therefore cause increased flux of phytoplankton-derived organic material to the microbial loop.

The maximum amounts of metals per gram of dry weight of *T. weissflogii* cells are: 916 nmol Mn, 290 nmol Fe, 20 nmol Co, and 30 nmol Cu (Finkel et al. 2006, Ho et al. 2003). Mn, Co and Cu levels detected were both well below their calculated maximum amounts. Fe values were consistently higher than the calculated maximum, suggesting that an external source, possibly residual f/2 medium in the experimental unit,

contaminated the results. Future experiments will require more stringent trace-metal-clean technique to reduce possible contaminations.

While the absolute amounts of cellular metals should be viewed with caution, comparison of before- and after-turbulence treatments did reveal some effects of turbulence on cellular trace metal content. *T. weissflogii* cells displayed significantly lower Mn, Co, and Fe concentrations after high and medium turbulence treatments (Figure 9 a,b,c), suggesting that cells expelled compounds containing trace metals during turbulence. In diatom cells, Mn and Fe are primarily located in chloroplasts as part of the light machinery (Nuester et al. 2012), while Co, a carbonic anhydrase, is located in the cytoplasm (Twining and Baines 2013). If episodic turbulence destroys a diatom cell or if cellular matter is extruded as a stress response, one or all three of these metals will be released, resulting in a decrease in metal content per gram dry weight.

Cu content per gram dry weight, on the other hand, decreased after medium turbulence, but *increased* after high turbulence (Figure 9d). Unlike Mn, Fe, and Co, Cu is located in the cell membrane as part of a high-affinity iron uptake molecule (Twining and Baines 2013), meaning it will not be released concomitantly with the other metals if turbulence results in loss or extrusion of cellular content (cytoplasm, organelles, etc.). Accumulation of frustule and membrane fragments (which were observed after high turbulence) containing Cu-rich compounds during filtering may have resulted in a higher amount of Cu per gram dry weight after high turbulence, in other words, if the cells lost biomass but the remaining membrane and frustule fragments were collected during filtering, there may have been an inflation in the amount of Cu relative to filter dry

weight. Examination of metal:Phosphorus (M:P) ratios (P measured by the ICPMS is a proxy for biomass) of these measurements will provide insight into the mechanisms behind trace metal release.

Trace metal release is particularly relevant in surface waters, where high phytoplankton abundance may reduce the concentrations of essential trace metals to nearly zero (Morel and Price 2003). At low turbulence, metal content increased. This could be due to shear from turbulence interrupting the boundary layer microzone around the phytoplankton cell, replacing nutrient-deficient water with nutrient-replete water (Peters et al. 1998). Bioavailable forms of Mn, Fe, Co, and Cu are rare in the euphotic zone due to relatively low supply and high uptake by phytoplankton (Coale and Bruland 1988, Danielsson et al. 1985, Saito and Moffett 2002). Bioavailable metals released from cells during turbulence may readily be removed by phytoplankton and microbes, which also benefit from release of labile DOM during turbulence (Malits et al. 2004). Metals that remain within intact cells can have extremely slow release rates, which results in transport of metals to depth rather than remineralization in euphotic waters (Fisher and Wente 1993). Recently, iron incorporation into biogenic silica produced by diatoms has been implicated as a mechanism of iron removal to depth; iron in broken or intact dead diatom frustules may still remain inaccessible to phytoplankton and other microbes in surface waters (Ingall et al. 2013).

4.2 Evaluation of the Use of Evans Blue

A diverse suite of stains are available for analysis of marine plankton: SYBR-green for assessing cell cycle stage and other parameters, SYTOX-green for viability of

phytoplankton >50 μm , and Nile red for lipid evaluation (Yentsch and Campbell 1991, Zetsche and Maysman 2012). There are many stains available for plankton work, but these stains are often only applicable to larger size classes of phytoplankton and mesozooplankton, and may exhibit high interspecific variability (Buma et al. 1995). Evans Blue was anecdotally tested earlier for differentiating live and dead phytoplankton, but the efficacy of the stain was not fully explored (Crippen and Perrier 1974). To confirm usability across phytoplankton taxa, I tested the stain on two diatoms and two non-siliceous species. I also tested several killing methods (biocide, osmotic stress, UV, and heat) to ensure that the cause of death would not alter EB stain efficacy. Overall the staining protocol yielded consistent and reliable results across all trials, making it particularly useful for environmental monitoring where mixed phytoplankton taxa and multiple mortality factors are expected.

My results showed there was a significant linear relationship between expected and observed phytoplankton mortality; the correlation also showed a positive y-intercept, suggesting that the stain underestimated mortality (Figure 1). That is, when no live cells were expected, the stain still showed ~15% live cells. Therefore, the results of EB staining should be treated as conservative estimates of phytoplankton mortality assumed to have negative error.

Cells as small as 6 μm produced visible stain color, but smaller cells were not tested. It is possible that smaller cells would be difficult to differentiate with the EB stain if their cytoplasm is not readily visible via light microscopy. When photographing stained samples, it is recommended that the samples be rinsed with filtered seawater after

staining (Figure A1). The residual EB stain in the water decreases the amount of light that reaches the camera, producing a darkened image. EB staining in combination with flow cytometry provides fast quantification of live/dead phytoplankton. A portable flow cytometer may be used to directly assess the stained samples in the field, but EB-stained samples preserve well with formalin, allowing stained samples to be preserved and assessed at a later date.

EB has potential for use in a variety of studies concerning phytoplankton mortality (e.g., ballast water treatments) especially as automated methods of enumerating and evaluating phytoplankton become increasingly available (Veldhuis and Kraay 2000). Ecological monitoring and water quality monitoring are two potential applications of the stain, as it stains both diatoms and non-diatoms regardless of the mortality source (so long as the cell remains intact). EB can provide insights into the live/dead status of natural plankton assemblages that would be extremely difficult to quantify without the use of a stain.

5. Conclusions

Although turbulence impacts on phytoplankton are known, most studies have focused on sustained low (background) level turbulence and how that affects growth, nutrient uptake and inter-specific competitions (Peters and Redondo 1997, Peters and Marrasé 2000). Intense, episodic turbulence is orders of magnitude stronger than background turbulence. This study showed that exposure to episodic turbulence could directly destroy phytoplankton cells or produce dead but intact cells. Even at sublethal levels, episodic turbulence may also increase trace metal extrusion, DOM extrusion, and reduction in photosynthetic efficiency.

I successfully developed a protocol using the mortal stain Evans Blue to visually differentiate live and dead phytoplankton cells. This protocol is a simple and inexpensive way to quantify live/dead phytoplankton, and to study phytoplankton mortality due to different factors. The staining method, when combined with preservation and flow cytometry, should greatly expand its applications for laboratory and field studies.

As storms and other extreme weather events are predicted to increase due to global climate change (Meehl et al. 2000), the results of this study will improve our understanding of how these events may affect phytoplankton. Rising sea surface temperature, decreasing pH (due to higher CO_2), and changing nutrient cycles related to climate change will all impact the ocean's phytoplankton (Karl 2003; Doney et al. 2009).

Episodic turbulence may have an even stronger effect on phytoplankton when acting synergistically with these stressors. I suggest that more studies of sublethal and lethal effects, with the aid of Evans Blue staining, of episodic turbulence in synergy with other climate-related stressors will be a valuable addition to our understanding of the impacts of climate change.

Conventionally, dinoflagellates are considered to be the primary phytoplankton group negatively influenced by turbulence. However, the turbulence levels tested in this study—1.1 to 4.0 $\text{cm}^2\text{sec}^{-3}$ —produced dead cells and lowered cell abundance in both *T. weissflogii* and *S. costatum*, indicating that turbulence may also represent a source of mortality to diatoms. Furthermore, photosynthetic efficiency dropped 20-30% after high turbulence, indicating that photosynthesis may be inhibited by intense turbulence. DOM and trace metal release stimulated by turbulence may represent a flux of nutrients to heterotrophic bacteria, shunting organic material into the microbial loop. In localities that frequently experience intense turbulence, diatom mortality and nutrient release should be taken into consideration when evaluating the ecology and primary production of the system.

Chapter 3: Conclusions and Future Research Directions

The goal of this research was to assess the lethal and sub-lethal effects of episodic turbulence on two diatoms, *Skeletonema costatum* and *Thalassiosira weissflogii* to answer four key questions about turbulence-derived mortality, size-specific effects, nutrient loss, and decreased photosynthetic efficiency. I used a propeller to induce episodic turbulence and measured turbulence intensity using an Acoustic Doppler Velocimeter (ADV). Photosynthetic efficiency, extrusion of trace metals and dissolved organic matter (DOM), and mortality were all measured before and after episodic turbulence using Pulse Amplitude Modulation (PAM) fluorometry, spectrofluorometry, Inductively Coupled Plasma Mass Spectroscopy (ICPMS), and Evans Blue (EB) mortal stain. I found that episodic turbulence $> 2.0 \text{ cm}^2 \text{ sec}^{-3}$ produced **mortality**, **nutrient loss** of cellular materials, and reduced **photosynthetic efficiency** in both diatom **size classes** (20 and 40 μm). This level of turbulence is on the order of breaking surface wave and stormy conditions (Agrawal et al. 1992, Gargett et al. 1989); other sources, such as boat propellers and breaking shore waves, may produce turbulence orders of magnitude higher than those tested in these experiments.

As Earth's climate continues to change, a myriad of stressors, such as rising sea surface temperature, decreasing pH and changing nutrient cycles, will impact marine phytoplankton (Karl 2003; Doney et al. 2009). The combination of biotic and abiotic factors that affect phytoplankton make it difficult to predict their response to climate forcings; therefore, more empirical data are necessary if we are to make realistic

forecasts. By quantifying the response of stressed phytoplankton to turbulence events, I hope to gain insights into the mechanistic drivers underlying the effects that climate change may have on phytoplankton assemblages and their biogeochemical cycling.

Mesocosm experiments containing a mix of phytoplankton species would be an ideal follow-up study of my research findings. While not a perfect imitation of natural conditions, mesocosms or wave tanks could be used to more closely approximate the turbulence created by storms or breaking waves, and provide more precise insights into how *in situ* turbulence affects phytoplankton and other microbes. It would also be interesting to compare the responses of natural phytoplankton assemblages from typically quiescent environments to phytoplankton assemblages from more turbulent environments to see how their responses to episodic turbulence differ. In both mesocosm and *in situ* experiments, identifying *specific* DOM compounds released would be informative.

Performing repeated, intermittent episodic turbulence experiments on the same group of phytoplankton over days or weeks would be useful for monitoring changes in physiology over subsequent generations of phytoplankton. With the increase of storm events and more frequent overseas cargo shipping, phytoplankton will be exposed to episodic turbulence more frequently. Evaluating their ability to adapt to episodic turbulence events could be an informative line of inquiry that would shed light on intergenerational tolerance of turbulence events.

Table 1. Turbulence intensities and theoretical characteristics as generated by different propeller speeds. The Batchelor microscale describes the smallest distance across which a gradient in substrate will exist before being dominated by molecular diffusion.

Propeller Speed	ε (cm ² sec ⁻³)	L_K (μ m)	Batchelor microscale, metals (μ m)
High	4	224	5
Medium	2.5	251	6
Low	1.1	309	8

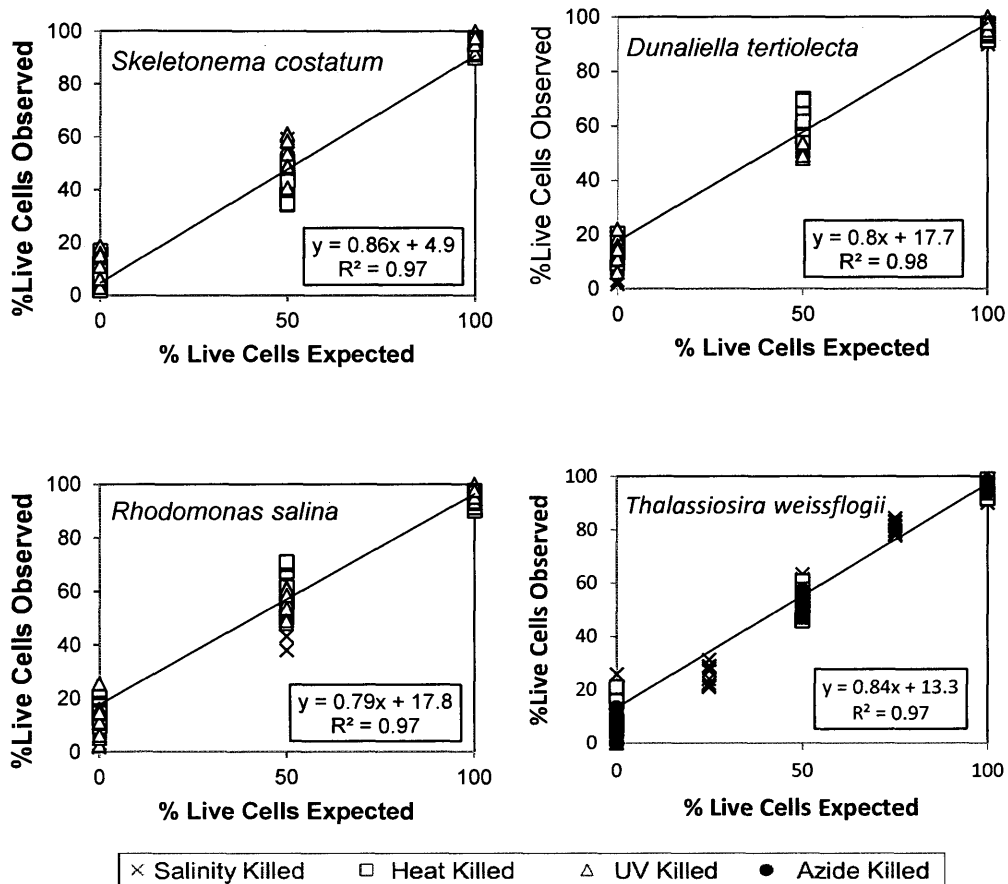


Figure 1: EB stain trials showed a linear relationship between expected percentage of live cells and observed percentage of live (unstained) cells. EB staining results were consistent across several killing methods and phytoplankton species (n=10 per killing method). Linear regressions significantly described the data in each species (*S. costatum* p<0.01, *D. tertiolecta* p<0.01, *R. salina* p<0.01, *T. weissflogii* p<0.01).

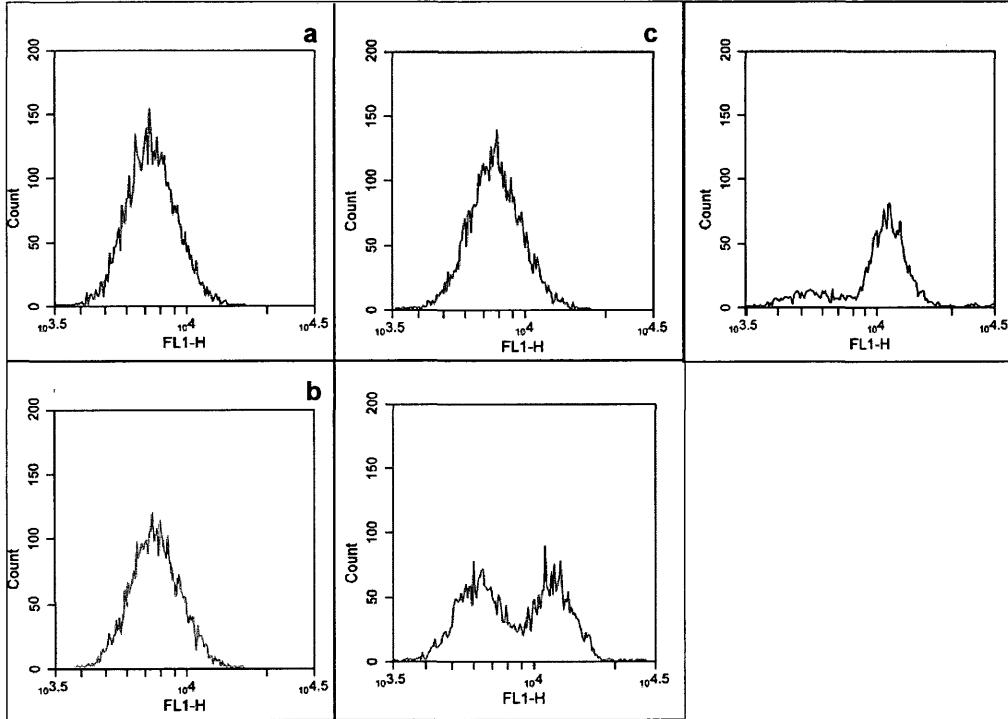


Figure 2: Flow cytometer plots of fluorescence wavelengths detected by the 530 nm band filter (FL1-H) and cell count for (a) live *T. weissflogii* cells (b) dead (azide-killed) *T. weissflogii* cells (c) Unstained 1:1 live/dead mixture (d) Stained 1:1 live/dead mixture and (e) Stained dead cells.

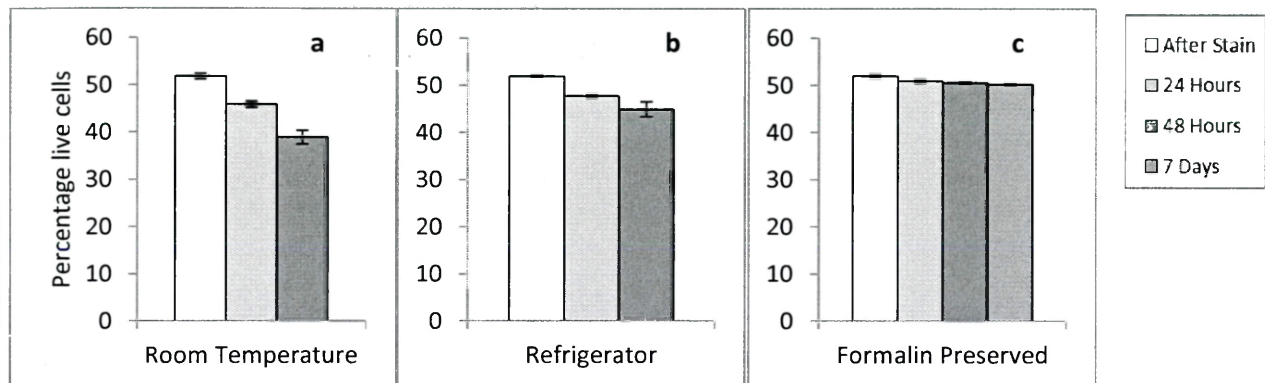


Figure 3: Percentage of live cells counted in stained samples that were (a) kept at room temperature, (b) refrigerated without preservative, or (c) preserved in formalin. Over 48 hours, ANOVA indicated that there was a significant decrease in the percent live cells in the room temperature samples ($n=3$, $p=0.01$) and refrigerated samples ($n=3$, $p=0.03$), but the observed percent live cells in formalin preserved samples did not change significantly over 7 days ($n=3$, $p=0.7$).

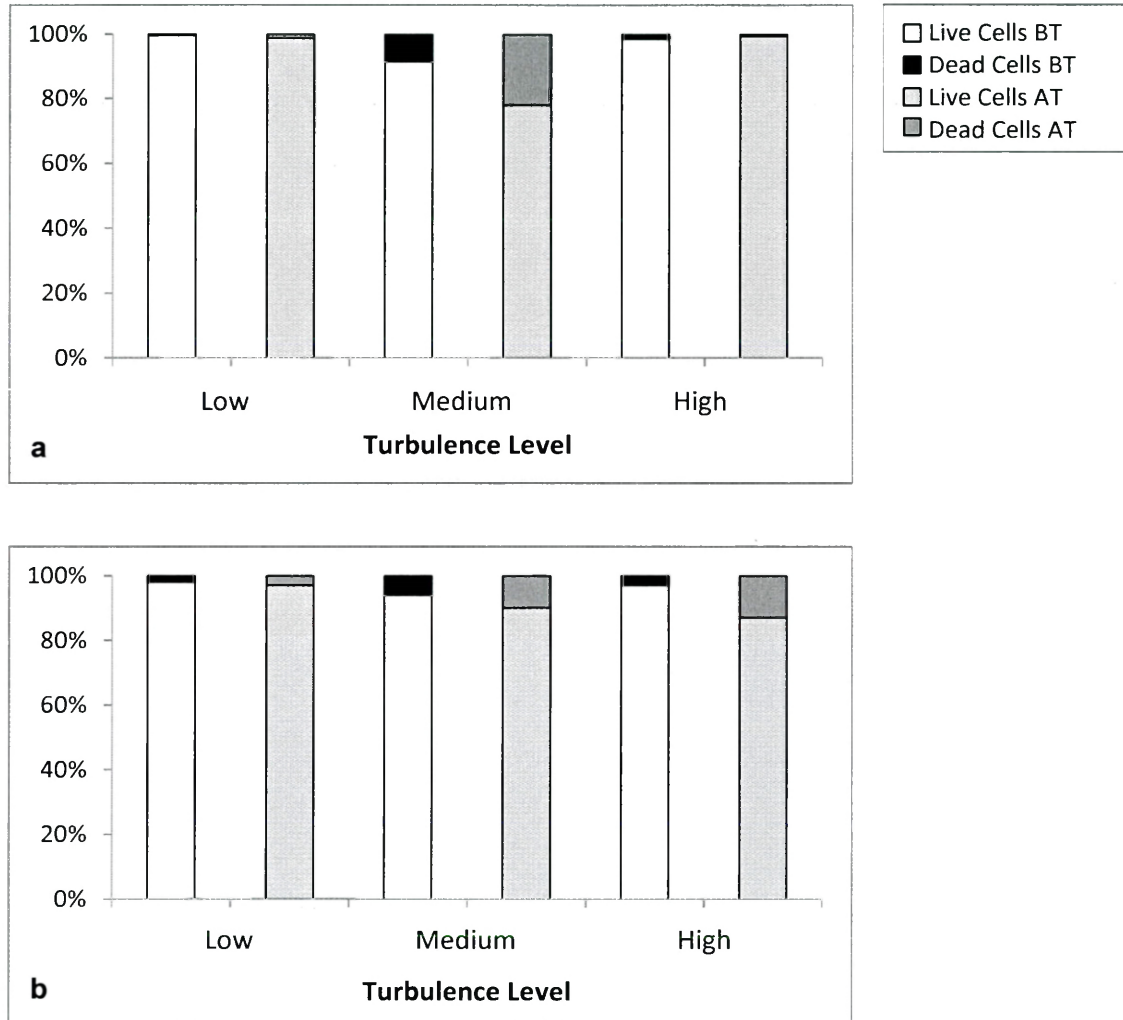


Figure 4: Percentage live and dead cells before and after turbulence for (a) *T. weissflogii* (n=4) and (b) *S. costatum* (n=3) at low, medium and high turbulence. * indicates significant difference before (BT) and after (AT) turbulence treatment (paired t-test; $p < 0.05$).

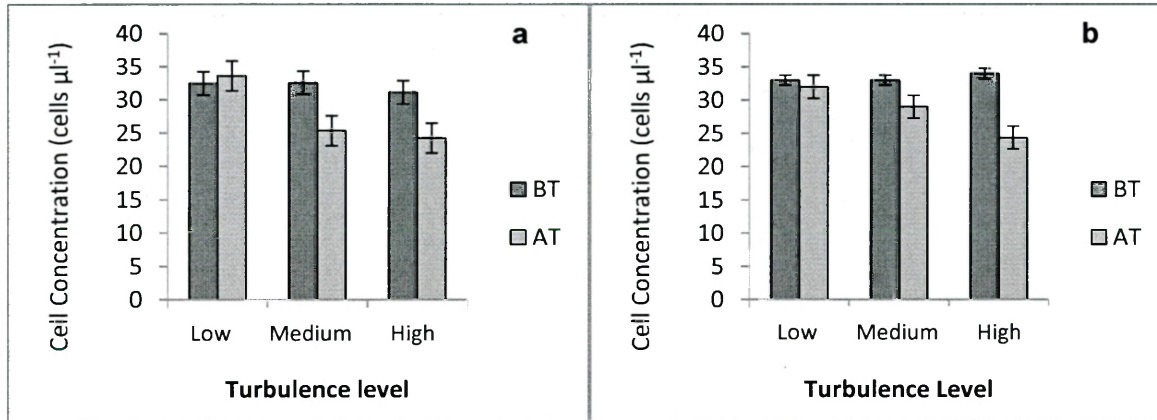


Figure 5: Total cell concentrations before (BT) and after (AT) exposure to low, medium, and high turbulence for (a) *T. weissflogii* and (n=4) (b) *S. costatum* (n=3) (error bars=1 SD; n=4). * indicates significant difference before and after turbulence treatment (paired t-test; $p < 0.05$).

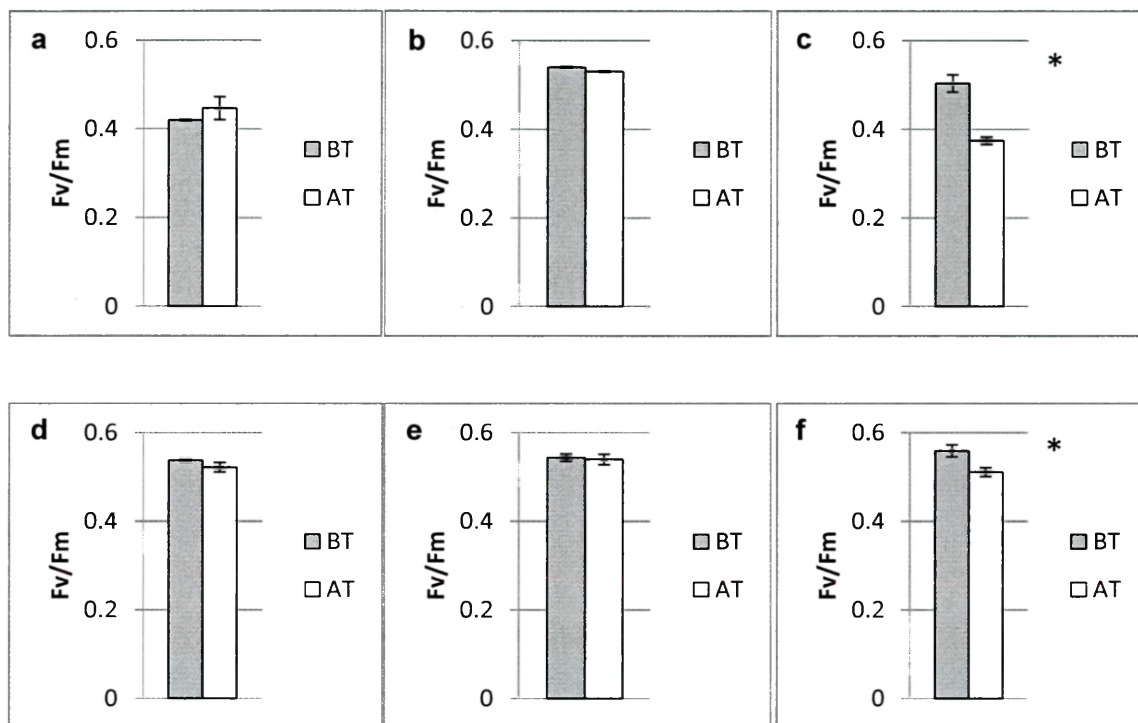


Figure 6: PAM fluorometer readings for *T. weissflogii* (n=4) before (BT) and after (AT) turbulence exposure in (a) low (p=0.05) (b) medium (p=0.05) and (c) high (p=0.02) experiments, and *S. costatum* (n=3) before (BT) and after (AT) (d) low (p=0.30) (e) medium (p=0.70) and (f) high (p=0.01) turbulence (error bars=1 SD). * indicates significant difference before and after turbulence treatment (paired t-test; p<0.05).

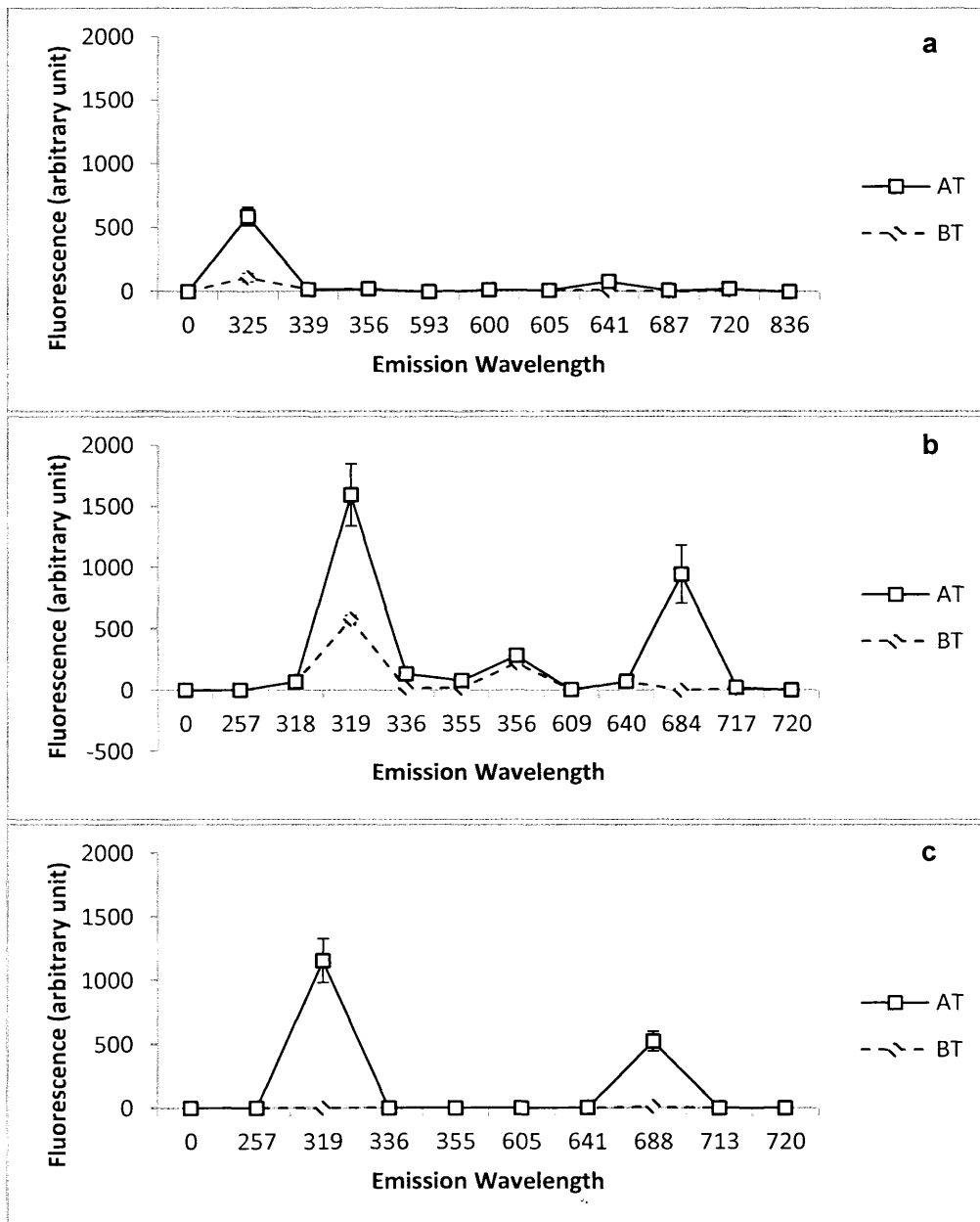


Figure 7: Spectral emissions of ambient water in the *T. weissflogii* experiments (n=4) before (BT) and after (AT) exposure to (a) Low, (b) Medium and (c) High turbulence (ex=280 nm). Error bars indicate 1 SD. Wavelengths obtained by auto-scanning emissions from 220-900 nm.

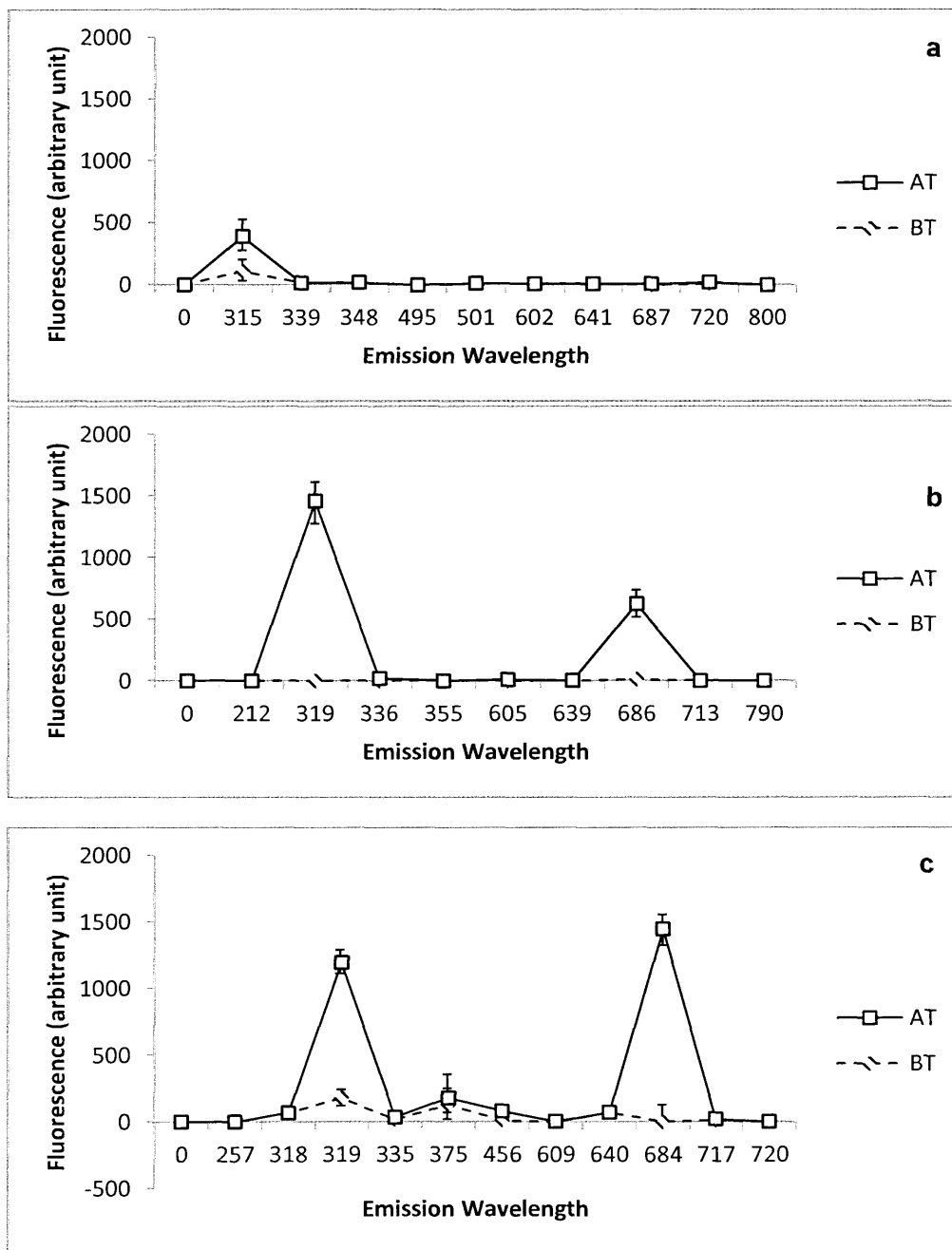
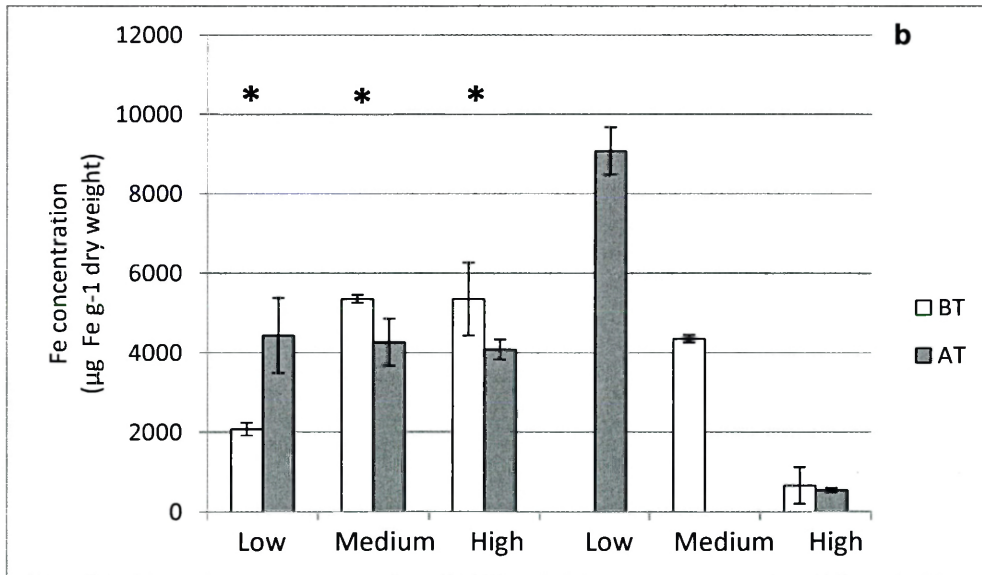
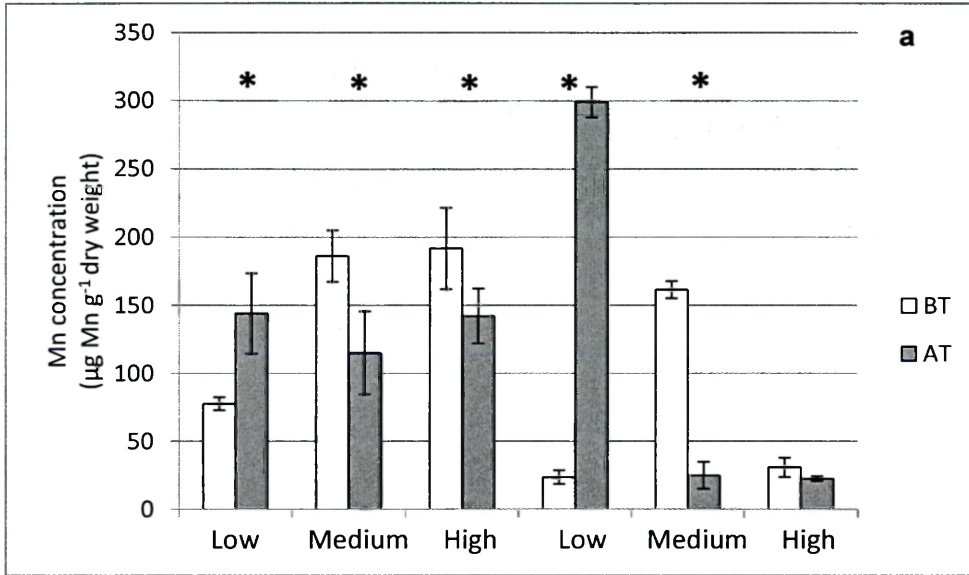


Figure 8: Spectral emissions of ambient water in the *S. costatum* experiments (n=3) before (BT) and after (AT) exposure to (a) Low, (b) Medium and (c) High turbulence (ex=280 nm). Error bars indicate 1 SD. Wavelengths obtained by auto-scanning emissions from 220-900 nm.



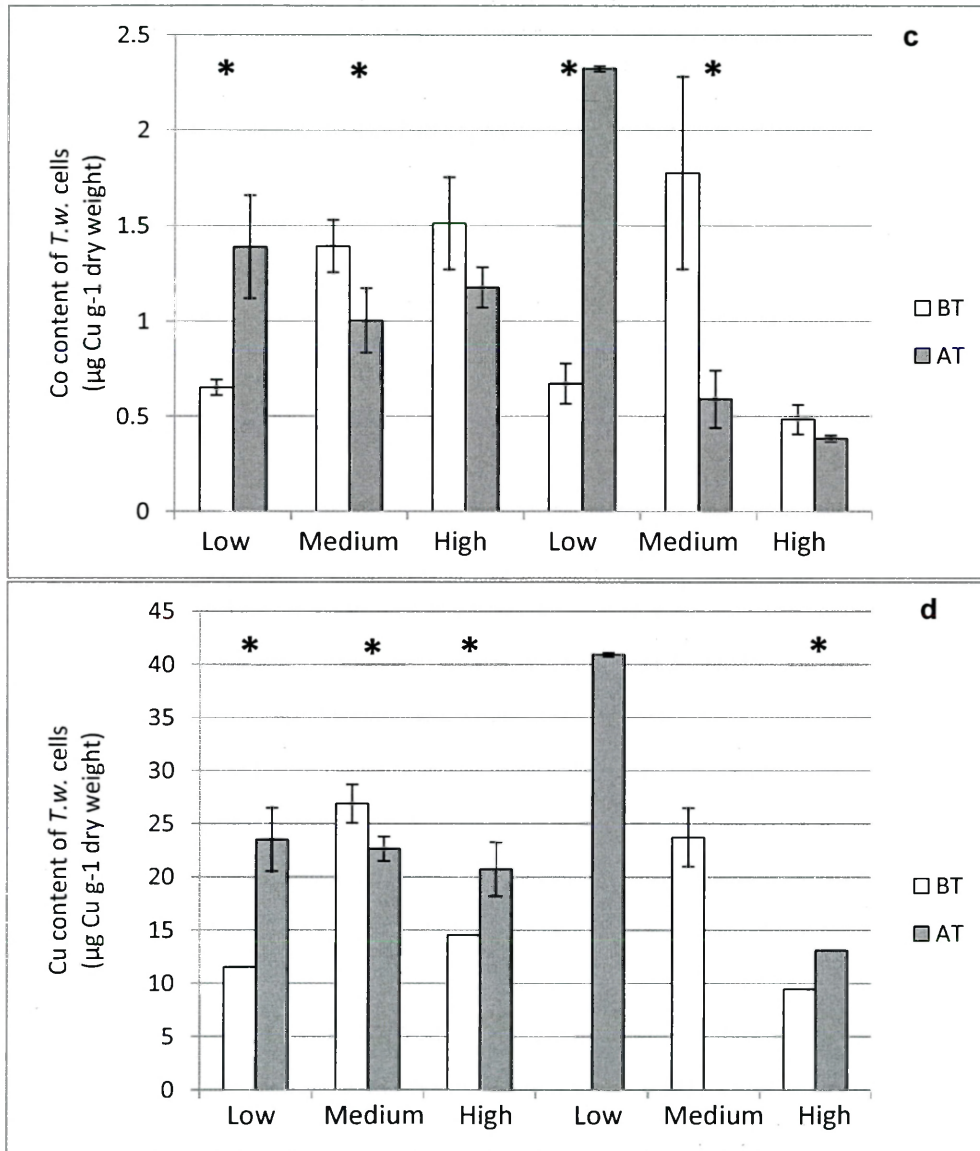


Figure 9: ICPMS Analysis results for (a) Manganese (b) Iron (c) Cobalt and (d) Copper content of *T. weissflogii* cells before (BT) and after (AT) turbulence. Each turbulence level was tested twice, and error bars indicate one standard deviation of duplicate samples. * indicates significant difference before and after turbulence treatment (paired t-test; $p < 0.05$).

APPENDICES

Appendix 1: Live/Dead EB Microscope Photos

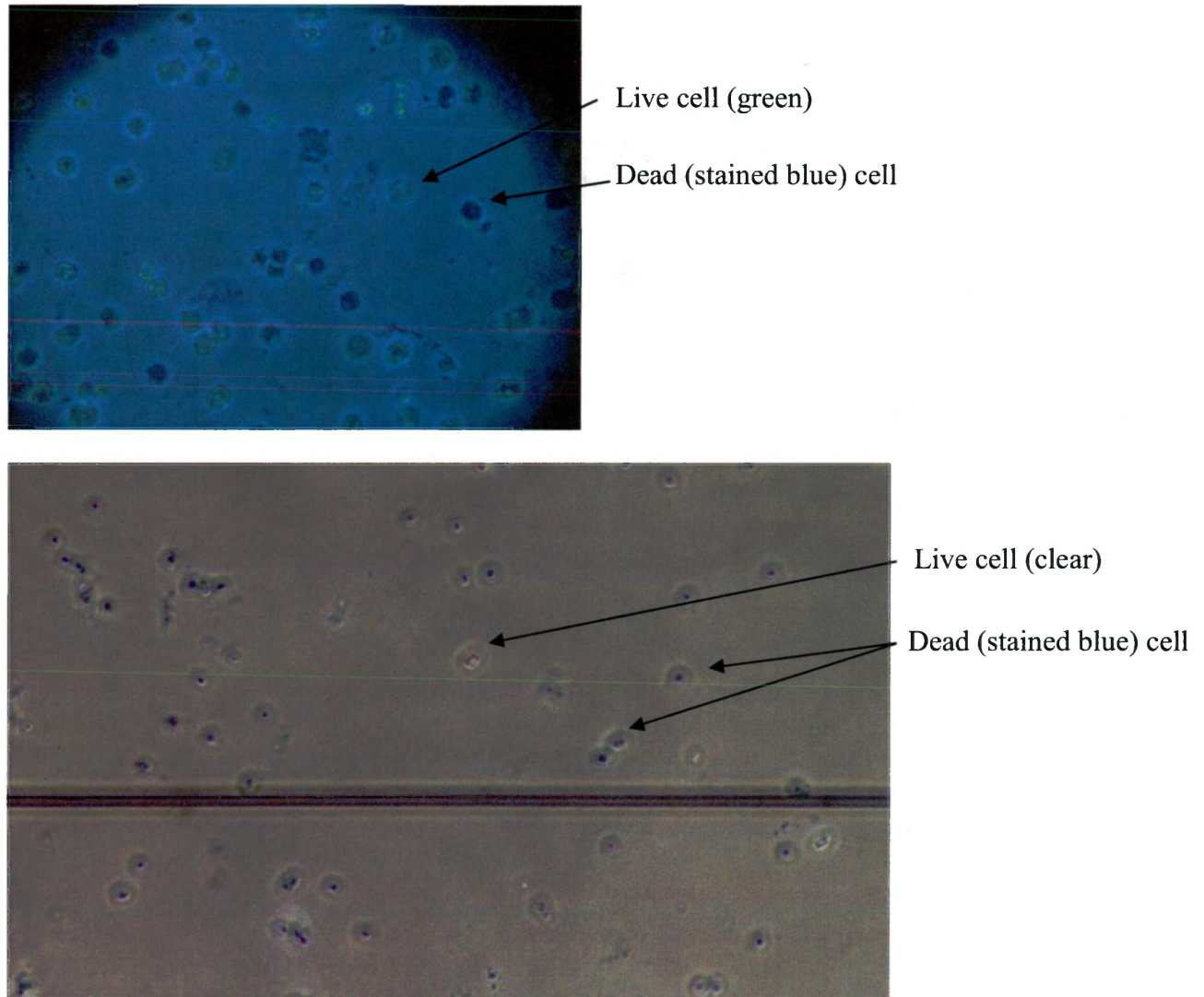


Figure A1: *T. weissflogii* cells (a) treated with Evans Blue and (b) treated with Evans Blue and rinsed.

Appendix 2: Turbulence Experiment Layout

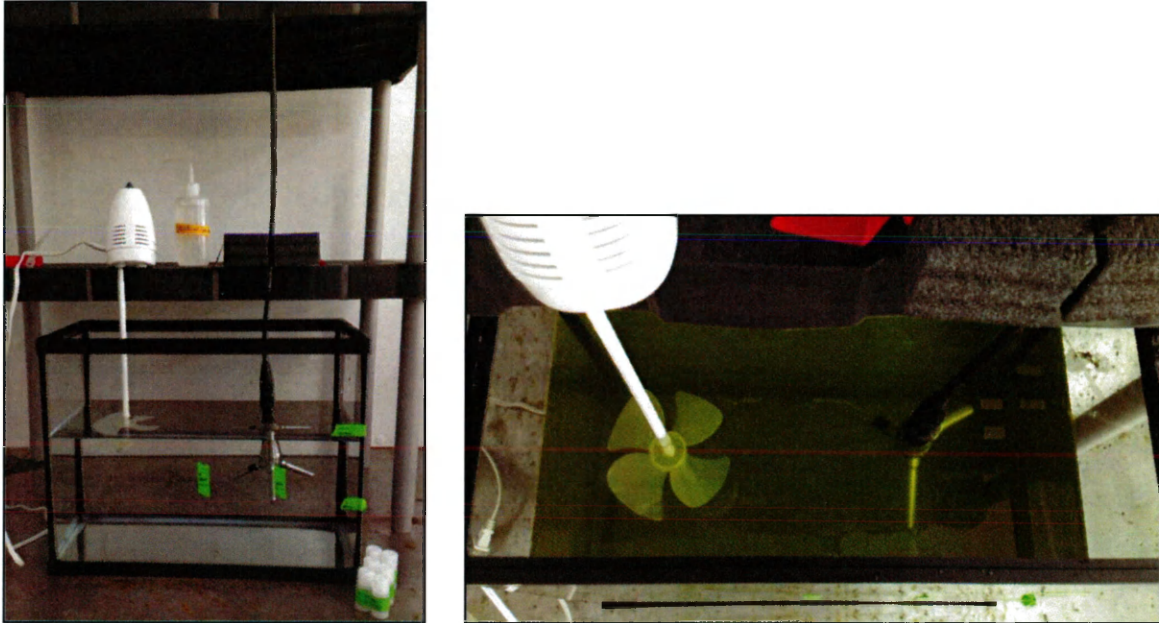
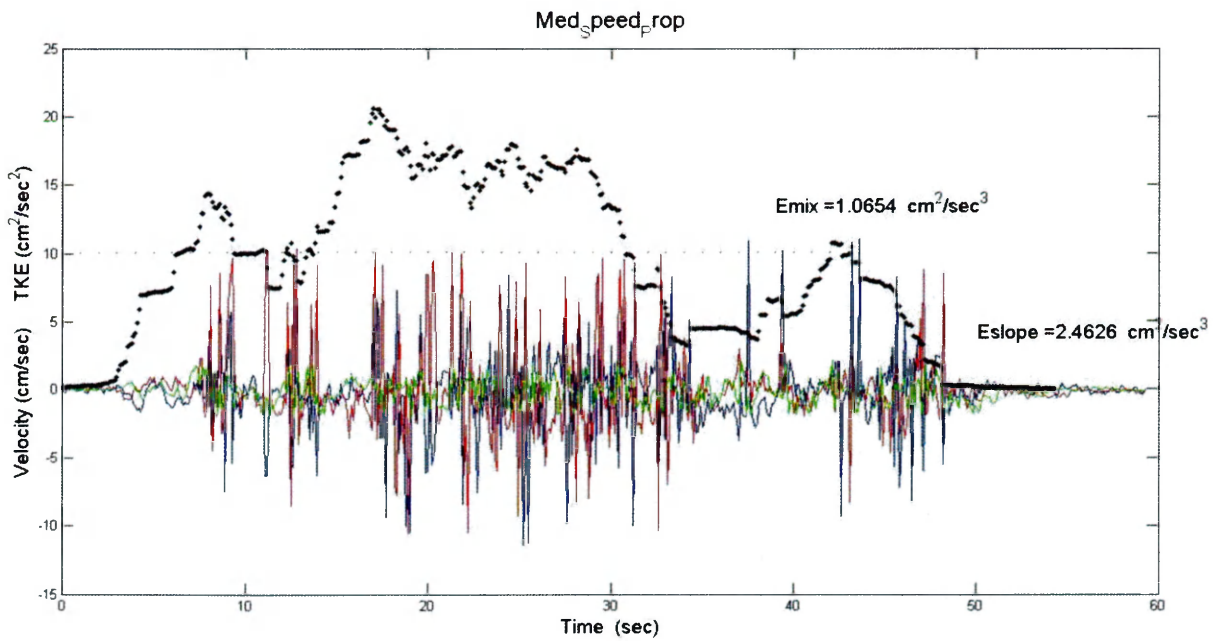
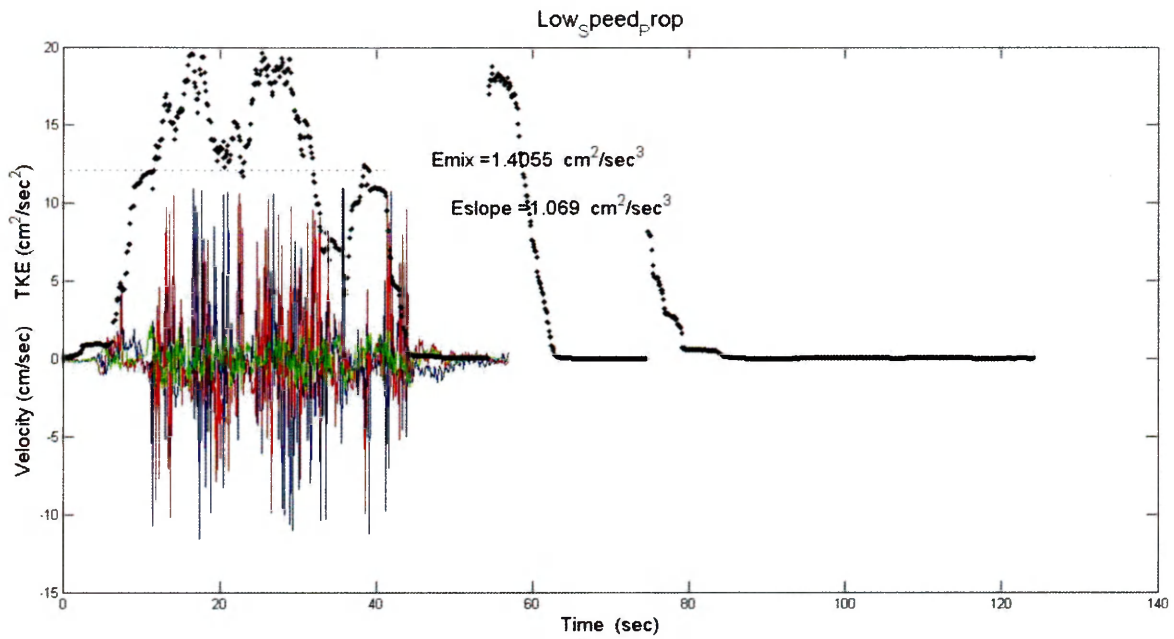


Figure A2 (a): Experimental aquarium with propeller and ADV. The ADV and propeller were placed at opposite ends of the tank to reduce the possibility of the ADV becoming entangled in the propeller blade. **(b):** Top view of aquarium after addition of *T. weissflogii* culture. 4L of dense culture was added to the tank for a final volume of 32 L.

Initially, the ADV was placed 12 cm from the bottom, side walls, and propeller to minimize mechanical interference with the ADV readings. However, the ADV sampling geometry is such that the ADV samples a 18 mm high x 15 mm diameter volume at a point 15 cm below its three prongs. The ADV was re-positioned 18 cm from the tank bottom and 12 cm each from the walls of the aquarium and the propeller.

Appendix 3: ADV Data



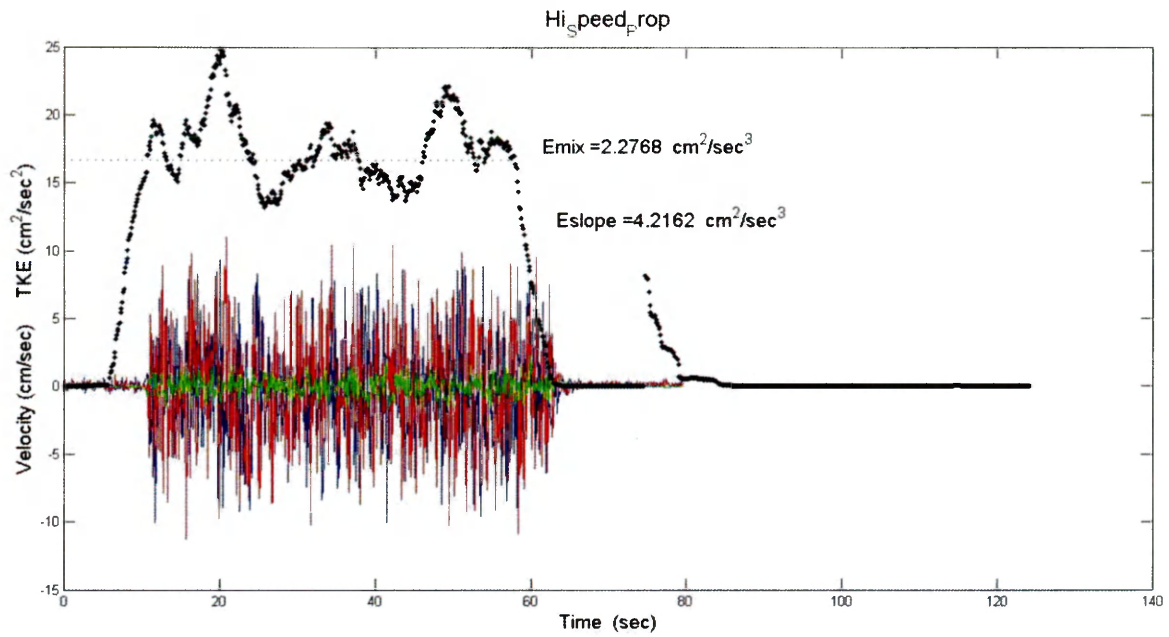


Figure A3: Representative examples of (a) Low, (b) Medium and (c) High turbulence ADV measurements in the x, y, and z directions (red, green and blue lines). TKE (black line) was calculated and turbulent energy dissipation rate (ϵ) determined from the amount of time it took TKE to reach zero after the propeller was turned off.

Appendix 4: DOM Control Experiment

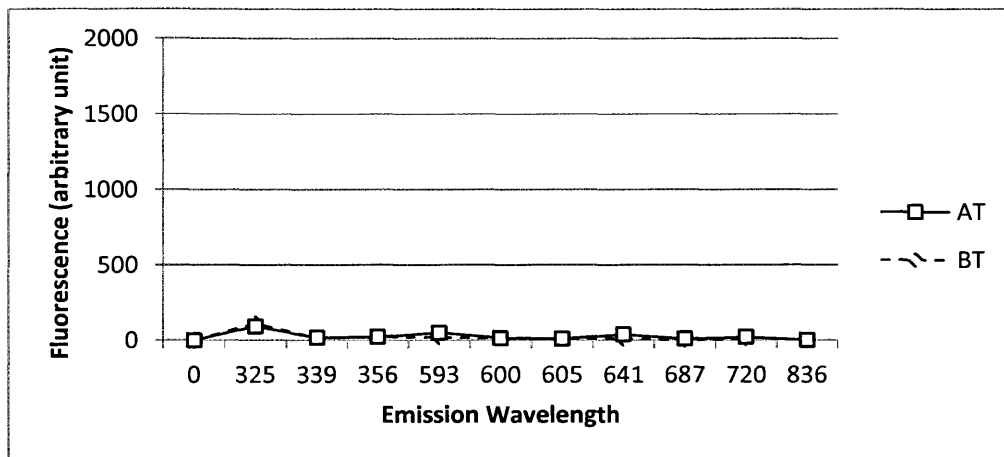


Figure A4: Spectral emissions before and after ‘blank’ run (ex=280nm). There was little difference between the before and after samples, indicating that the propeller was not a source of DOM to the aquarium.

LITERATURE CITED

- Agrawal, Y. C., Terray, E. A., Donelan, M. A., Hwang, P. A., Williams III, A. J., Drennan, W. M., Kahma, K. K., et al. (1992). Enhanced dissipation of kinetic energy beneath surface waves. *Nature*, 359, 219–220.
- Allen, W. E. (1938). “Red Water” along the West Coast of the United States in 1938. *Science*, 88, 55–56.
- Anderson, L. A., & Sarmiento, J. L. (1994). Redfield ratios of remineralization determined by nutrient data analysis. *Global Biogeochemical Cycles*, 8, 65–80.
- Arin, L., Marrasé, C., Maar, M., Peters, F., Sala, M.M., & Alcaraz, M. (2002). Combined effects of nutrients and small-scale turbulence in a microcosm experiment. I. Dynamics and size-distribution of osmotrophic plankton. *Aquatic Microbial Ecology*, 29, 51–61.
- Beck, C. A., Bonde, R. K., & Rathbun, G. B. (1982). Analyses of Propeller Wounds on Manatees in Florida. *The Journal of Wildlife Management*, 46, 531–535.
- Bickel, S. L., Malloy Hammond, J. D., & Tang, K. W. (2011). Boat-generated turbulence as a potential source of mortality among copepods. *Journal of Experimental Marine Biology and Ecology*, 401, 105–109.

- Bickel, S. L., & Tang, K. W. (2010). Microbial decomposition of proteins and lipids in copepod versus rotifer carcasses. *Marine Biology*, 157, 1613–1624.
- Bickel, S. L., Tang, K. W., & Grossart, H.-P. (2009). Use of aniline blue to distinguish live and dead crustacean zooplankton composition in freshwaters. *Freshwater Biology*, 54, 971–981.
- Bjørnson, P. K. (1988). Phytoplankton exudation of organic matter : Why do healthy cells do it? *Limnology and Oceanography*, 33, 151–154.
- Brussaard, C. P. D. (2004). Viral Control of Phytoplankton Populations - A Review. *The Journal of Eukaryotic Microbiology*, 51, 125–138.
- Buma, A. G. J., Hannen, E. J. Van, & Veldhuis, M. J. W. (1995). Monitoring Ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. *Journal of Phycology*, 321, 314–321.
- Cannon, A. C. (1998). *Gross Necropsy Results of Sea Turtles Stranded on the Upper Texas and Western Louisiana Coasts, 1 January-31 December 1994*. R. Zimmerman, Ed. In *Characteristics and Causes of Texas Marine Strandings*, NOAA Report NMFS 143. Seattle, WA (pp. 81–85).
- Caperon, J., & D. F. Smith. 1978. Photosynthetic rates of marine algae as a function of inorganic carbon concentration. *Limnology and Oceanography*, 23,704-708.

- Capone, D. G., Ferrier, M. D., Carpenter, E. J., Ferrier, M. D., & Carpenter, E. J. (1994). Amino Acid Cycling in Colonies of the Amino Acid Cycling in Colonies of the Planktonic Marine Cyanobacterium *Trichodesmium thiebautii*. *Applied Environmental Microbiology*, *60*, 3989-3995.
- Clarson, S. J., Steinitz-Kannan, M., Patwardhan, S. V., Kannan, R., Hartig, R., Schloesser, L., & Hamilton, D. W. (2009). Some Observations of Diatoms Under Turbulence. *Silicon*, *1*, 79–90.
- Coale, K. H., & Bruland, K. W. (1988). Copper complexation in the northeast Pacific. *Limnology and Oceanography*, *33*, 1084–1101.
- Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry*, *51*, 325–346.
- Crippen, R. W., & Perrier, J. L. (1974). The Use of Neutral Red Stain and Evans Blue for Live Dead Determinations on Marine Plankton with Comments on the Use of Rotenone for Inhibition of Grazing. *Stain Technology*, *40*, 97–104.
- Cullen, J. J., & Lewis, M. R. (1988). The kinetics of algal photoadaptation in the context of vertical mixing. *Journal of Plankton Research*, *10*, 1039–1063.
- Cullen, J.J., Lewis, M.R., Davis, C.O., & Barber, R.T. (1992). Photosynthetic characteristics and estimated growth rates indicate grazing is the proximate control of primary production in the equatorial Pacific. *Journal of Geophysical Research: Oceans*, *97*, 639–654.

- Cushing, D. H. (1989). A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. *Journal of Plankton Research*, *11*, 1–13.
- Danielsson, L., Magnusson, B., & Westerlund, S. (1985). Cadmium, copper, iron, nickel and zinc in the North-East Atlantic Ocean. *Marine Chemistry*, *17*, 23–41.
- Doney, S., et al. (2009) Ocean Acidification: the other CO₂ problem. *Annual Review of Marine Science*, *1*, 169–192.
- Elliott, D. T., & Tang, K. W. (2009). Simple staining method for differentiating live and dead marine zooplankton in field samples. *Limnology and Oceanography: Methods*, *7*, 585–594.
- Eppley, R. W., & Peterson, B. J. (1979). Particulate organic matter flux and planktonic new production in the deep ocean. *Nature*, *282*, 677–680.
- Estrada, M., & Berdalet, E. (1997). Phytoplankton in a turbulent world. *Scientia Marina*, *61*, 125–140.
- Falkowski, P. G. (1984). Physiological responses of phytoplankton to natural light regimes. *Journal of Plankton Research*, *6*, 295–307.
- Fellman, J. B., Hood, E., & Spencer, R. G. M. (2010). Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnology and Oceanography*, *55*, 2452–2462.

- Finkel Z.V., Quigg, A., Raven, J.A., Reinfelder, J.R., Schofield, O.E. & Falkowski, P.G. (2006). Irradiance and the elemental stoichiometry of marine phytoplankton. *Limnology and Oceanography*, *51*, 2690–2701.
- Fisher, N.S., & Wentz, M. (1993). The release of trace elements by dying marine phytoplankton. *Deep Sea Research Part I: Oceanographic Research Papers*, *40*, 671-694.
- Franks, P.J.S. & Marra, J. (1994). A simple new formulation for phytoplankton photoresponse and an application in a wind-driven mixed-layer model. *Marine Ecology Progress Series*, *111*, 143–153.
- Gargett, A. (1989). Ocean Turbulence. *Annual Review of Fluid Mechanics*, *21*, 419–451.
- Gargett, A., Tejada-Martinez, A. E., & Grosch, C. E. (2008). Measuring turbulent large-eddy structures with an ADCP-1 vertical velocity variance. *Journal of Marine Research*, *66*, 157–189.
- Gibson, C.H. & Thomas, W.H. (1995). Effects of turbulence intermittency on growth inhibition of a red tide dinoflagellate, *Gonyaulax polyedra* Stein. *Journal of Geophysical Research*, *100*, 24841-24846.
- Harrison, W.G., & Platt, T. (1986). Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. *Polar Biology*, *5*, 153-164.

- Ho, T., Quigg, A., Zoe, V., Milligan, A. J., Falkowski, P. G., & Morel, M. M. (2003). The Elemental Composition of Some Marine Phytoplankton. *Journal of Phycology*, *39*, 1145–1159.
- Ingall, E. D., Diaz, J. M., Longo, A. F., Oakes, M., Finney, L., Vogt, S., & Brandes, J. A. (2013). Role of biogenic silica in the removal of iron from the Antarctic seas. *Nature Communications*, *4*, 1–6.
- Juneau, P., & Harrison, P. J. (2005). Comparison by PAM fluorometry of photosynthetic activity of nine marine phytoplankton grown under identical conditions. *Photochemistry and photobiology*, *81*, 649–53.
- Jackson, G. A. (1990). A model of the formation of marine algal flocs by physical coagulation processes. *Deep-Sea Research*, *37*, 1197–1211.
- Karl, T.R. (2003). Modern global climate change. *Science*, *302*, 1719-1723.
- Karp-Boss, L., Boss, E., & Jumars, P. A. (1996). Nutrient Fluxes to Planktonic Osmotrophs in the Presence of Fluid Motion. *Oceanography and Marine Biology: an Annual Review*, *34*, 71–107.
- Killgore, K. J., Miller, A. C., & Conley, K. C. (1987). Transactions of the American Fisheries Society Effects of Turbulence on Yolk-Sac Larvae of Paddlefish. *Transactions of the American Fisheries Society*, *116*, 37–41.

- Kiørboe, T. (1993). Turbulence, Phytoplankton Cell Size, and the Structure of Pelagic Food Webs. *Advances in Marine Biology*, 29, 2–70.
- Kitaigorodskii, S. A., Donelan, M. A., Lumley, J. L., & Terray, E. A. (1983). Wave-Turbulence Interactions in the Upper Ocean. Part II: Statistical Characteristics of Wave and Turbulent Components of the Random Velocity Field in the Marine Surface layer. *Journal of Physical Oceanography*, 13, 1988–1999.
- Lavaud, J., Rousseau, B., & Etienne, A.L. (2003). Enrichment of the light-harvesting complex in diadinoxanthin and implications for the non-photochemical fluorescence quenching in diatoms. *Biochemistry*, 42, 5802–5808.
- Legendre, L., & Le, J. (1995). Microbial food webs and the export of biogenic carbon in oceans. *Aquatic Microbial Ecology*, 9, 69–77.
- Lenz, J., Morales, A., & Gunkel, J. (1993). Mesozooplankton standing stock during the North Atlantic spring bloom study in 1989 and its potential grazing pressure on phytoplankton : A comparison between low, medium and high latitudes. *Deep-Sea Research*, 40, 559–572.
- Lévy, M., Klein, P., & Jelloul, M. Ben. (2009). New production stimulated by high-frequency winds in a turbulent mesoscale eddy field. *Geophysical Research Letters*, 36, 1–5.

- Lewis, M. R., Horne, E. P. W., Cullen, J. J., Oakey, N. S., & Platt, T. (1984). Turbulent motions may control phytoplankton photosynthesis in the upper ocean. *Nature*, *311*, 49–50.
- Li, Y.-H., & Gregory, S. (1974). Diffusion of ions in sea water and in deep-sea sediments. *Geochimica et Cosmochimica Acta*, *88*, 703–714.
- Libby, P.A., 1996. Introduction to Turbulence. Taylor & Francis Ltd, Washington D.C, pp. 267–275.
- Llabrés, M., & Agustí, S. (2006). Picophytoplankton cell death induced by UV radiation : Evidence for oceanic Atlantic communities. *Limnology and Oceanography*, *51*, 21–29.
- Loberto, A. (2007). *An Experimental Study of the Mixing Performance of Boat Propeller*. M.E. Thesis, Queensland University of Technology.
- Lorke, A., & Wüest, A. (2005). Application of Coherent ADCP for Turbulence Measurements in the Bottom Boundary Layer. *Journal of Atmospheric and Oceanic Technology*, *22*, 1821–1828.
- MacIntyre, H. L., Kana, T. M., & Geider, R. J. (2000). The effect of water motion on short-term rates of photosynthesis by marine phytoplankton. *Trends in plant science*, *5*, 12–7.

- Mague, T. H., Friberg, E., Hughes, D. J., & Morris, I. (1980). Extracellular Release of Carbon by Marine Phytoplankton; Physiological Approach. *Limnology and Oceanography*, 25, 262–279.
- Malits, A., Peters, F., Bayer-Giraldi, M., Marrasé, C., Zoppini, A., Guadayol, O., & Alcaraz, M. (2004). Effects of small-scale turbulence on bacteria: a matter of size. *Microbial Ecology*, 48, 287–99.
- Margalef, R. (1978). Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica Acta*, 1, 493–509.
- Matthews, B. J. H., Jones, A. C., Theodorou, N. K., & Tudhope, A. W. (1996). Excitation-emission-matrix fluorescence spectroscopy applied to humic acid bands in coral reefs. *Marine Chemistry*, 55, 317–332.
- Meehl, G. A., Zwiers, F., Evans, J., Knutson, T., Mearns, L., & Whetton, P. (2000). Trends in Extreme Weather and Climate Events : Issues Related to Modeling Extremes in Projections of Future Climate Change. *Bulletin of the American Meteorological Society*, 81, 427–436.
- Melville, W. K. (1994). Energy Dissipation by Breaking Waves. *Journal of Physical Oceanography*, 24, 2041–2049.
- Morgan, R. ., Ulanowicz, R. E., Rasin, V. J., Noe, L. A., & Gray, G. B. (1976). Effects of Shear on Eggs and Larvae of Striped Bass, *Morone saxatilis*, and White Perch, *M. americana*. *Transactions of the American Fisheries Society*, 105, 37–41.

- Mykkestad, S., Holm-hansen, O., & Varum, K. M. (1989). Rate of release of extracellular amino acids and carbohydrates from the marine diatom *Chaetoceros affinis*. *Journal of Plankton Research*, *11*, 763–773.
- Nuester, J., Vogt, S., & Twining, B. S. (2012). Localization of Iron Within Centric Diatoms of the Genus *Thalassiosira*. *Journal of Phycology*, *48*(3), 626–634.
- Odeh, M., Noreika, J. F., Haro, A., Maynard, A., Castro-Santos, T., & Cada, G. F. (2002). *Evaluation of the Effects of Turbulence on the Behavior of Migratory Fish* (pp. 1–37). Portland, OR.
- Owen, R. W. (1989). Microscale and finescale variations of small plankton in coastal and pelagic environments. *Journal of Marine Research*, *47*, 197–240.
- Pasciack W.J., & Gavis J. (1975). Transport limited nutrient uptake rates in *Ditylum brightwellii*. *Limnology and Oceanography*, *20*, 605-617.
- Peters, F., & Marrasé, C. (2000). Effects of turbulence on plankton : an overview of experimental evidence and some theoretical considerations. *Marine Ecology Progress Series*, *205*, 291–306.
- Peters, F., Marrasé, C., Gasol, J. M., & Arin, L. (1998). Effects of turbulence on bacterial growth mediated through food web interactions. *Marine Ecology Progress Series*, *112*, 293–303.

- Peters, F., & Redondo, J. M. (1997). Turbulence generation and measurement : application to studies on plankton. *Scientia Marina*, *61*, 205–228.
- Peters, H., & Bokhorst, R. (2000). Microstructure Observations of Turbulent Mixing in a Partially Mixed Estuary. Part I : Dissipation Rate. *Journal of Physical Oceanography*, *30*, 1232–1244.
- Pett, R. J. (1989). Kinetics of microbial mineralization of organic carbon from detrital *Skeletonema costatum*. *Marine Ecology Progress Series*, *52*, 123–128.
- Saito, M. A., & Moffett, J. W. (2002). Temporal and spatial variability of cobalt in the Atlantic Ocean. *Geochimica et Cosmochimica Acta*, *66*, 1943–1953.
- Saria, A., & Lundberg, A.M. (1983). Evans blue fluorescence: quantitative and morphological evaluation of vascular permeability in animal tissues. *Journal of Neuroscience Methods*, *8*, 41-49.
- Saiz, E., & Alcaraz, M. (1992). Enhanced excretion rates induced by small-scale turbulence in *Acartia* (Copepoda: Calanoida). *Journal of Plankton Research*, *14*, 681-689.
- Smith, D. F., & Wiebe, W. J. (1976). Constant release of photosynthate from marine phytoplankton. *Applied and Environmental Microbiology*, *32*, 75–79.

Sullivan, J. M., Swift, E., Ba, A. L., Murray, W., Whedon, A., & Balech, K. (2003). Effects of Small-Scale Turbulence on Net Growth Rate and Size of Ten Species of Marine Dinoflagellates. *Journal of Phycology*, 94, 83–94.

Sunda, W. G., Price, N. M., & Morel, F. M. M. (2005). Trace Metal Ion Buffers and Their Use in Culture Studies. R.A. Anderson, Ed. In *Algal Culturing Techniques* (pp. 35–63). Academic Press.

Suttle, C. A., Chan, A. M., & Cottrell, M. T. (1990). Infection of phytoplankton by viruses and reduction of primary productivity. *Nature*, 347, 467–469.

Tang, D., & Morel, F.M.M. (2006). Distinguishing between cellular and Fe-oxide-associated trace elements in phytoplankton. *Marine Chemistry* 98, 18-30.

Tang, K.W., Bickel, S.L., Dziallas, C., & Grossart, H.P. (2009). Microbial activities accompanying decomposition of cladoceran and copepod carcasses under different environmental conditions. *Aquatic Microbial Ecology*, 57, 89–100.

Tennekes, H.H. & Lumley, J.L. (1972). A first course in turbulence. MIT Press, Cambridge, MA.

Terray, E. A., Donelan, M. A., Agrawal, Y. C., Drennan, W. M., Kahma, K. K., Williams III, A. J., Hwang, P. A. (1996). Estimates of Kinetic Energy Dissipation under Breaking Waves. *Journal of Physical Oceanography*, 26, 792–807.

- Thomas, W. H., & Gibson, C. H. (1990). Effects of small-scale turbulence on microalgae. *Journal of Applied Phycology*, 2, 71–77.
- Thomas, W. H., Vernet, P. M., & Gibson, C. H. (1995). Effects of small-scale turbulence on photosynthesis, pigmentation, cell division, and cell size in the marine dinoflagellate *Gonyaulax polyedra* (Dinophyceae). *Journal of Phycology*, 31, 50–59.
- Tovar-Sanchez, A., Sanudo-Wilhelmy, S. A., Garcia-Vargas, M., Weaver, R. S., Popels, L. C., & Hutchins, D. A. (2003). A trace metal clean reagent to remove surface-bound Fe from marine phytoplankton. *Marine Chemistry*, 82, 91–99.
- Twining, B. S., & Baines, S. B. (2013). The trace metal composition of marine phytoplankton. *Annual Review of Marine Science*, 5, 191–215.
- Tynan, C. T. 1993. Effects of small-scale turbulence on dinoflagellates. Ph.D. thesis, Scripps Institution of Oceanography, University of California, San Diego.
- Veldhuis, M. J. W., & Kraay, G. W. (2000). Application of flow cytometry in marine phytoplankton research : current applications and future perspectives . *Scientia Marina*, 64, 121–134.
- Watt, W. D. (1969). Extracellular Release of Organic Matter from Two Freshwater Diatoms. *Annals of Botany*, 33, 427–437.
- Weber, C. I. (1968). The Preservation of Phytoplankton Grab Samples. *Transactions of the American Microscopical Society*, 87, 70–81.

Weller, R. A., Dean, J. P., Marra, J., Price, J. F., Francis, E. A., & Boardman, D. C.

(2013). Three-Dimensional Flow in the Upper Ocean. *Science*, 227, 1552–1556.

Yentsch, C.M., & Campbell, J.W. (1991). Phytoplankton growth: perspectives gained by flow cytometry. *Journal of Plankton Research*, 13, S83–S108.

Zetsche, E.-M., & Meysman, F. J. R. (2012). Dead or alive? Viability assessment of micro- and mesoplankton. *Journal of Plankton Research*, 34, 493–509.

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