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ATP as a Quantitative Proxy for Living Microbial Biomass: Cellular ATP Content Under Stress

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ATP AS A QUANTITATIVE PROXY FOR LIVING MICROBIAL BIOMASS: CELLULAR ATP CONTENT UNDER STRESS

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Presented to the

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California State University Monterey Bay

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

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by

Natalia Yingling

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ATP AS A QUANTITATIVE PROXY FOR LIVING MICROBIAL BIOMASS: CELLULAR ATP CONTENT UNDER STRESS

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ABSTRACT

ATP AS A QUANTITATIVE PROXY FOR LIVING MICROBIAL BIOMASS:

CELLULAR ATP CONTENT UNDER STRESS

by
Natalia Yingling

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

Science

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Accurate measurements of phytoplankton biomass can help provide information to oceanographers necessary to understanding the foundations of various marine food webs. In addition, such measurements can offer further insight into their life cycles and their impact on the global biogeochemical carbon cycle. For several decades, the activity or concentration of ATP has been used as a proxy of biomass in oceanography of micro-organisms, however, little is known about the lower limits of ATP signals, their interpretation, and the processes that drive this signal down to zero. Thus, the primary goal of this study was to determine the time scale of ATP depletion under various disinfecting treatments. The secondary goal was to determine the validity of ATP as a phytoplankton biomass indicator in natural systems. ATP measurements were made on a transect line across the Northern Pacific Ocean in order to determine if diel variability could be detected and separated from the influence of zooplankton vertical migration. Four species of phytoplankton; *Thalassiosira weissflogii*, *Amphidinium carterae*, *Dunaliella salina* and *Isochrysis galbana* in addition to a natural community sampled from Moss Landing harbor, were treated with heat, chlorination or glutaraldehyde, and ATP measurements were analyzed overtime. Heat was the most effective treatment followed by chlorination and then glutaraldehyde. In the field, ATP concentrations were higher at midnight and lower at noon in oligotrophic regions on the Pacific Ocean, suggesting a net accumulation from photoautotrophic processes and degradation by grazing and/or viral lysis. This study elucidates the beneficial use of ATP and its role as a proxy of biomass in the fields of ballast water testing and oceanography in general.

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Introduction

Measuring micro-organism biomass

Phytoplankton form the bases of almost every oceanic food web and are responsible for producing approximately half of the total photoautotrophic oxygen on earth. Accurate measurements of biomass aid oceanographers in quantifying the foundation of marine trophic food webs and provide insight into the role of phytoplankton in the biogeochemical carbon cycling. Numerous methods of measuring phytoplankton were developed to quantify biomass such as direct counts, measuring chlorophyll a in-situ or extracted, grow out techniques, particulate organic carbon measurements and staining cells with fluorescein diacetate. Each method has particular strengths and weaknesses.

Direct counts, either by microscopy or a flow cytometer, are an effective way to visually quantify the zooplankton, phytoplankton and bacterial cells in a sample. However, direct counting can be tedious and time consuming. Non-living cells can be counted as living unless a stain is applied to clearly mark living cells. In addition, cells can form colonies leading to an overestimation or underestimation of cells (Todar, 2008). Chlorophyll *a* is a pigment that is possessed by all photoautotrophs and is responsible for the absorption of light that in turn provides energy for the photosynthetic carbon fixations. Thus, chlorophyll *a* serves as one of the most widely used proxies for autotrophic biomass in marine science. However, chlorophyll *a* can lead to an overestimation of biomass since detritus or senescent cells can contain chlorophyll *a* compromising the estimate of active living autotrophic biomass (Lionard et al., 2008, Kruskopft and Flyn, 2005). Although grow-out techniques using serial dilutions, which examine reproduction and growth of phytoplankton over time, are effective, they also require multiple days to provide results and are very time consuming to prepare (Wright and Welschmeyer, 2015). Measuring particulate organic carbon (POC) is time efficient, simple and applicable to biomass as carbon is the basic currency for food webs. However, it is difficult to assess which portion of a carbon measurement belonging to living plankton and bacteria or detritus (Fiedler et al., 2008).

Measurement of adenosine triphosphate (ATP) in oceanography has been of interest and importance since the development of the ATP assay, specifically for marine science, by Holm-Hansen and Booth (1966). The method was developed as an easy and more reliable technique to measure microbial biomass in marine waters than the standard and tedious microscopic counting technique, to enumerate cell biomass (Holm-Hansen and Booth 1966). Since then, ATP has been successfully used as a proxy for biomass in phytoplankton in the ocean (Holm-Hansen, 1970, Holm-Hansen and Booth, 1966), under nitrogen limitation (Eppley et al., 1977), under phosphate limitation (Cavari, 1976, Perry, 1976) and the combination of nutrient and light limitation (Hunter and Laws, 1981). Moreover, ATPase possesses esterase activity that correlates well with the metabolic activity of all living microbial activity in marine areas and the techniques that have been used to extract ATP has been rapid, simple and reproducible (Kuo, 2015).

Quantitative Applications of ATP in Oceanography

ATP is known as the energy currency of the cell since all organisms, from bacteria to multi-cellular animals, contain and produce ATP that is necessary for various chemical reactions. Microorganisms are able to capture and store energy that is metabolized from food or converted from light in the form of ATP. Without ATP, cells would not be able to transfer energy making it impossible for organisms to grow and reproduce (Zhang *et al.*, 2010). ATP is depleted in many biological reactions such as bioluminescence and therefore in order to quantify ATP, we can use an enzyme such as luciferase to produce a bioluminescent reaction. Bioluminescence is useful for a variety of purposes in biology including camouflage, attracting a mate, communication, and defense. The conceptual equation describing the bioluminescence reaction is listed below.

$$ATP + O_2 + \text{Luciferase} \rightarrow \text{Oxyluciferin} + AMP + PPi + CO_2 + \text{Light}$$

Equation 1: Bioluminescence equation that uses ATP to produce light

ATP that is extracted will be hydrolyzed by the enzyme luciferase in the presence of oxygen to produce oxyluciferin, adenosine monophosphate (AMP), pyrophosphate (PPi), carbon dioxide and light, as shown in Equation 1. We are able to measure the light

that is caused by this reaction with a Luminometer and are able to compute ATP concentration if all other reactants are in excess and ATP is the limiting component in the reaction mixture (Kuo, 2015). It is useful and significant to understand the total biomass to be measured that is present in all living cells but is not present in dead cells (Holm-Hansen, 1971).

Since the initial application of the ATP assay for total biomass in a water sample by Osmund Holm-Hansen in the mid-20th century, ATP has been applied to measure micro-organism biomass in wastewater treatment and drinking water facilities, inspecting fermentation tanks for contamination in brewing beer and examining contamination on counter surfaces, medical devices and surgical instruments in hospitals (Lewis *et al.*, 2008). However, in order for ATP to be properly used as a proxy of living biomass, certain assumptions must be met: 1.) All living organisms have reasonably constant ATP content, 2.) ATP can be quantitatively extracted from living cells, 3.) the ratio of ATP to cell carbon is reasonably constant, and 4.) dead cells rapidly lose ATP. Assumption number 1 has been fairly well accepted in the science realm as a true fact. Past research has indicated that assumption number 2 has also been validated (Holm-Hansen & Booth, 1966, Holm-Hansen, 1970, Karl, 1980, Sinclari *et al.*, 1979). Likewise, assumption number 3 has been substantiated with previous research (Holm-Hansen, 1971, Hunter and Laws, 1981). However, there exists contradictory information in the literature regarding the organism ATP content upon death. Some research has shown full ATP depletion in only a few minutes, other cases show depletion over many hours and not necessarily to undetable levels (Eguchi *et al.*, 1997, Knechtges, 2012).

In an oceanography context, data on ATP depletion is further limited and inadequate. A study done by First and Drake (2014) claimed ATP begins to decrease following 10 minutes under UV light or chlorination methods of cell extermination. However, these authors kept exposure time constant and altered UV dosage and chlorine dosage as shown in Figure 1, therefore omitting the timescale for ATP loss. Although it is widely assumed that dead cells do not contain ATP, it is not known how this transition in living cell ATP quotas decreases with time following cell death.

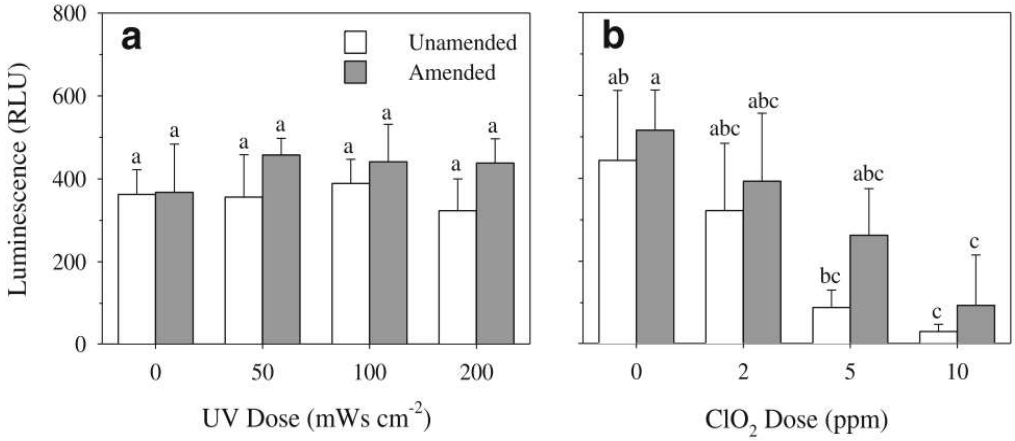

Figure 1. ATP luminescence in samples of mixed cultured organisms for two different treatments, a.) UV irradiation b.) ClO_2 dose. Darker bars represent treatments with amendments of organic carbon and mineral matter and light bars represent unamended treatments. Bars show the mean ± standard deviation (n=3) and different letters indicate significant differences (p>0.05). Letters are displayed in plot where measurements are not significantly different (First and Drake, 2014).

Practical Application of Using ATP as a Proxy for Biomass: Ballast Water Testing

More than 3 to 5 billion metric tons of ballast water are discharged in coastal ports of the world every year, with at least 8 million metric tons of non-endemic water being discharged into U.S. waters (Hunt *et al.*, 2005). The associated invasive species have the ability to reproduce and spread quickly, degrade habitats, impact water quality, out-compete native species and short circuit food webs (Sakai *et al.*, 2001). Furthermore, invasive species have the capability to threaten human health and hurt the economy by damaging critical industries such as fisheries, aquaculture and tourism (Wittenberg and Cock, 2001). As a result, the International Maritime Organization (IMO) and Management of Ship's Ballast Water and Sediments Convention (BWM Convention) now require that all ships treat and manage their ballast water before discharging. It is essential that all treatment systems meet all regulations and standards set by the IMO and the U.S. Coast Guard and be type approved, meaning that in order for these ballast treatment systems to be certified, they must meet a minimum set of regulations, standards

and safety policies. The discharge water regulations can be found in Table 1 for each of the three typical marine size classes.

Table 1: Maximum concentrations of various size classes for discharge water set by IMO regulations (Regulation D-2 of the BWM Convention) (IMO, 2008a).

	Organisms greater or equal to 50 microns	Organisms greater or equal to 10 micros but less than 50 microns	Indicator microbes may not exceed:
IMO guidelines for Ballast Water Exchange standard in regulation D-2	Must contains less than 10 viable organisms per cubic meter	Must contain less than 10 viable organisms per milliliter	*Vibrio Cholerae* - less than 1 CFU (colony forming unit) per 100 mL *E. Coli* – less than 250 CFU per 100 mL *Enterococci* – less than 100 CFU per 100 mL

Requirements for discharged ballast water are rigorous and detailed, therefore methods of testing ballast water biomass must be comprehensive and confirmed through scientific research (Werschkun *et al.*, 2014). Currently there are various methods of testing for microorganism biomass including epifluorescence and stereoscopic microscopy for phytoplankton and zooplankton, flow cytometry, dissolved organic carbon, pre-packaged kits that estimate colony-forming units for indicator organisms such as *Vibrio cholera*, measurements of chlorophyll *a* and staining methods to mark "live cells". Recently, measurements of ATP have also been incorporated in ballast water testing as a proxy of living biomass that is user-friendly and accurate. A model for

calculating live cell concentrations from ATP was developed for the size group of 10 to 50 microns, coinciding with the IMO regulated size class identified for protists (Welschmeyer and Kuo, 2016). That study determined that the mean expected ATP cellular quote for the 10-50 micron size class was 2.62 pg ATP per cell. Therefore, a ballast water sample with a size fractionated, 10-50 microns, ATP concentration of 26.2 ng ATP/L would indicate approximately 10 live cells/mL, corresponding to the ballast water standard limit for the 10-50 micron size class as shown in Table 1. ATP concentration of unknown ballast water samples can then potentially be compared to the equivalent ATP-based ballast water stand limit of 26.2 ng ATP/L for the purpose of quantitative compliance monitoring (Welschmeyer and Kuo, 2016). In order to confirm that ATP is a proper proxy of living biomass, the death-induced loss of cellular ATP must be confirmed. Three methods of achieving cell death, glutaraldehyde, chlorination and heat, were applied in this project in attempt to verify the extent of ATP degradation in marine phytoplankton.

Glutaraldehyde

Glutaraldehyde is a toxic chemical that is primarily used in oceanography as a fixative to preserve samples, but can also be used as a disinfectant and cleaner in the health field. It's an effective preservative because it kills cells by cross linking proteins therefore the cell that is treated would be dead but not destroyed, intact and useful for microscopy (Sournia, 1978).

Chlorination

Several ballast treatment systems incorporate chemical treatments to disinfect ballast water, such as the addition of chlorine to the water to kill pathogens by breaking the chemical bonds in their molecules through strong oxidation. Chlorination has been well studied and has provided insight into how successful treatment is in killing microbes, especially in chlorine application to wastewater treatment and drinking water preparation. A study done by Frist and Drake examined the physiological and metabolic impacts of various dosages of chlorination on various algal species. It was discovered that

ATP concentrations significantly decreased at an elevated chlorination dose compared to the control sample, as shown in Figure 1 (First and Drake, 2014).

Heat

Phytoplankton, depending on species, have a range of temperatures that are tolerated for optimal growth. When temperature starts to increases to intolerable levels, proteins in the cell begin to denature. Above this threshold, heat is often used in to kill phytoplankton in grazing experiments and therefore can be used as a method of achieving cell death while still maintaining intact cellular structure (Zetsche *et al.*, 2012).

OBJECTIVES

The following set of objectives were set to guide this thesis:

1. Determine the time scale of ATP degradation in phytoplankton cells and microbial communities that are stressed and dying

2. Investigate when ATP concentrations reach near zero concentrations after a variety of treatments that induces cell death

3. Investigate the use of ATP as a proxy of biomass in the field to determine if diel variability is observed

The ultimate goal is to determine how ATP concentrations quickly or slowly deplete after cell death under various disinfecting treatments and if diel variability can be detected by using ATP as a proxy of biomass.

METHODS AND MATERIALS

I. LABORATORY METHODS

Phytoplankton Cultures

Four phytoplankton species were used during this study; *Thalassiosira weissflogii*, *Amphidinium carterae*, *Dunaliella salina* and *Isochrysis galbana*. Cultures were either isolated from Monterey Bay, CA or acquired from local colleagues. Media cultures were made following Guillards f/2 recipe (Guillard and Ryther, 1962, Guillard, 1975). Media was filtered through a 0.45 μ filter cartridge with a peristaltic pump and filtrate was collected into a 2 L polycarbonate bottle. Bottles were then autoclaved for 20 minutes on liquid setting to heat kill any micro-organisms in the media. Media was sealed and stored in a 13° C walk in freezer until ready to use. Glassware used to grow phytoplankton cultures was cleaned and autoclaved for 30 minutes on glassware setting before used. Cultures were maintained at room temperature on a north-facing window shelf in the Biological Oceanography lab and grown in natural light/dark cycle and in ambient room temperature (18-22°C).

Daily Measurements

In order to understand how ATP and cell densities fluctuated under standard conditions; *Thalassiosira weissflogii*, *Amphidinium carterae*, *Dunaliella salina* and *Isochrysis galbana* were also grown in f/2 medium, similar to methods described under Phytoplankton Cultures, in an incubator under continuous light. ATP measurements and cells densities measurements were made daily at the same time to minimize error and standardized to photoperiod effects, for 16 consecutive days.

Methods of Achieving Cell Death

Glutaraldehyde

Samples were prepared by aliquoting 30 mL of each culture into separate testing tubes. Then the addition of glutaraldehyde was calculated to achieve a final concentration of 0.25%, 0.5% and 1%; samples without glutaraldehyde served as controls. Time series sampling followed immediately after dosing with glutaraldehyde and continue 10 minute intervals for 30 minutes. At each given time point, the 30 mL sample was filtered on a 0.7 micron glass fiber filter (GFF) and immediately submerged in a 1 mL snap cap vial filled with 1 mL of P-BAC, phosphoric acid benzalkonium chloride, as shown in ATP protocol list below (Kuo 2015).

Harbor water sample was collected from Moss Landing harbor, California and used as a representative microbial community sample. Seven mL of sample was dispersed into small test tubes and 50% glutaraldehyde was added as required to achieve a final concentration of 1%. Before filtration, all samples were mixed by gentle inversion with a rubber stopper. Control samples were filtered on a Whatman GFF and were labeled as either a control sample with no additional glutaraldehyde or a control sample with glutaraldehyde. Samples that were spiked and filtered immediately were designated as the t-zero sample. All other samples were spiked with glutaraldehyde at a given time and were filtered at 30 minutes, 1 hour, 2 hour, 4 hour, 6 hour.

Chlorination

The same four species of phytoplankton cultures as listed above; *Thalassiosira weissflogii*, *Amphidinium carterae*, *Dunaliella salina* and *Isochrysis galbana,* were utilized for experiments with chlorination as the toxic agent. Four to six days after inoculation, cell counts were obtained using an Attune flow cytometer using red fluorescence from chlorophyll *a* as the optical discriminator for phytoplankton particles. Each culture was split into 4 separate, acid washed containers. One container was used as a control with no added bleach (hypochlorite). The second container was used as a time-zero, where bleach was added and the sample was filtered immediately. The remaining two containers were treatment samples, one at 6 ppm and one at 12 ppm. One sample aliquot was withdrawn from each treatment container at time intervals of 10, 20, 30, 60, 90 and 120 minutes and filtered onto a GF/F filter. In addition to measuring ATP, chlorophyll *a* was also measured for 3 different treatment concentrations; 4 ppm, 8 ppm

and 12 ppm. The protocol for measuring chlorophyll *a* can be found in section "*Chlorophyll a*" section listed below (First and Drake, 2014).

Heat

Four species of phytoplankton cultures as listed above; *Thalassiosira weissflogii*, *Amphidinium carterae*, *Dunaliella salina* and *Isochrysis galbana,* were grown in f/2 medium on the window sill. Four to six days after inoculation, cytometric cell counts were recorded. Cultures were then split into 9 clear plastic 15 mL sample falcon tubes, 36 samples in total. A water bath was heated to 50 °C served as the means for producing heat-filled cells. A control was filtered (GF/F) just prior to heat treatment; all other samples were fully submerged in the water bath and filtered at 15, 30, 45, 60, 75, 90, 105, and 120 minutes.

A natural community sample was collected from Moss Landing Harbor, California that served as a representative microbial sample that is similar to samples that are used in ship ballast operations. Samples were distributed into 9 different 50 mL falcon tubes. A temperature controlled water bath was heated to 55 °C and used for the thermal treatments. One sample was the control and filtered on a GFF filtered with zero time spent in the water bath. All other samples were fully submerged in the water bath and filtered every 15 minute at the same time-series as listed above.

Cell Counts

Cultures were grown for 4-6 days until color was visible. Cell counts were performed using an Attune™ flow cytometer after cells were stained with fluorescein diacetate (FDA). FDA is initially non-fluorescent but is converted enzymatically within live cells to the green-fluorescent by product fluorescein which serves as the indicator for live vs. dead phytoplankton cells; native red chlorophyll *a* fluorescence served as the optical discriminator for cytometric identification of both live and dead phytoplankton cells. Cell counts as above were used to calculate ATP per cell, as listed in Equation 2.

ATP Protocol

The method of extracting and calculating ATP was based on the phosphoric acid and benzalkonium chloride (PBAC) ATP assay optimized by Kuo (2015). The PBAC

method was shown to give higher final ATP extraction yields by 2-5x (Kuo, 2015) relative to the traditional boiling Tris buffer extraction technique of Holm-Hansen and Booth (1966).

I. Sampling

A known volume of the sample was vacuum filtered on a 0.7 micron glass fiber filter (GFF) using a peristaltic pump vacuum that did not exceed 1/3 atmosphere. The filter was then submerged immediately into 1 mL of phosphoric acid benzalkonium chloride (PBAC) for thirty minutes to one hour to release and fully extract ATP from cells. The samples could then be processed immediately or frozen in -20° C freezer for future processing (Kuo, 2015).

II. Luminescence ATP determination

ATP was analyzed by diluting the P-BAC extracted sample fluid 100-fold with 25 mM tricine (pH = 7.75) to reduce the light-quenching effects of the PBAC solution on the chemiluminescent light reaction. Luciferase enzyme was then added into the diluted sample, 50 μL of enzyme to 100 μL of diluted sample, mixed immediately and inserted into the cuvette chamber of a PhotonMaster™ Luminometer (LuminUltra Technologies, Ltd., Canada) to read the resulting luminescence signal over a fixed 6-second integration time. The signals were record as Raw Luminescence Units (RLU). The light-emitting reaction produced RLU responses directly proportional to the concentration of ATP contained in the sample cuvette. The final ATP concentration was computed from an empirically-determined instrument response factor (RLU/(ng ATP) calculated, as described below, from ATP standard solutions of known concentration provided by Promega, Inc. All ATP reagents were stored frozen and standardized again on days of sample analysis. Response factors were determined daily before each sample batch.

The following procedure was used to quantitatively dilute (100x) PBAC extraction fluid to remove luminescence quenching effects. The same protocol was used in diluting ATP standard solutions and blanks so that samples, standards and blanks were all prepared with identical reagent mixtures yielding a 100x final dilution of PBAC extraction fluid. Quantitative solutions of ATP standard were prepared in clean snap-cap

microvials by adding 10 µL ATP standard (1x10-7 Molar; Promega, Inc.) with 10 µL PBAC and 980 µL 25 mM tricine buffer, followed by mixing. Sample mixtures were prepared in identical vials by adding 10 µL sample extract (in PBAC) to 990 µL tricine. Blanks were prepared by combining 10 µL PBAC with 990 µL tricine. The blank background signal was subtracted from each raw sampling reading as well as from each ATP standard reading. The instrument response factor was calculated as the blank-adjusted ATP standard RLU reading divided by the know ATP standard concentration (blank adjusted measurement was divided by the ATP standard, 507.18 ng/L. The final sample concentration was calculated from the equation below.

III. Calculations

To calculate the luminescence of a known amount of ATP, as listed in equation 2, 10 µL of the ATP standard, 10 µL of PBAC and 980 µL of tricine were mixed and slowly inverted. Next, 100 µL of this mixed sample was withdrawn and added into a new cuvette and 50 µL of luciferase was measured and the raw RLU was recorded. To adjust for background noise, the standard blank was subtracted out of the ATP standard measurement to calculate an ATP standard blank adjusted measurement. To calculate the response factor, the ATP-standard blank-adjusted RLU measurement was divided by the known ATP standard concentration (507.18 ng/L). The response factor can change with each new mixture of P-BAC, tricine, or luciferase and therefore a new response factor was calculated before every new sample batch and after any changes in assay reagents, as necessary. As shown below, every sample was blank adjusted to account for any background noise in the reagents. Extraction volume was the volume of P-BAC used, usually 0.1 L, and the volume filtered was the volume of the untreated or treated sample that was filtered onto the GF/F filter. The sample dilution was 100-fold in all cases; The Luciferase and extract volumes were always held constant (50 µL and 100 µL, respectively) since linearity of the optical cuvette reading was volume dependent.

$$\text{ATP Concentration (ng/L)} = \frac{\text{Blank Adjusted RLU}}{\text{Response Factor}} * \frac{\text{Extraction Volume (L)}}{\text{Volume Filtered (L)}} * \text{Sample Dilution}$$

Equation 2: Explanation of how ATP concentration (ng/L) was calculated in all samples

Chlorophyll a

Chlorophyll *a* measurements were made as a comparable and commonly used oceanography biomass estimator for reference against the measured ATP concentrations. Three species of phytoplankton cultures as listed above; *Thalassiosira weissflogii*, *Amphidinium carterae*, and *Dunaliella salina* were the tested for variability in the chlorophyll *a* to ATP ratio. Samples were treated with four different bleach treatments; Control, 4 ppm, 8 ppm, and 12 ppm. A known volume of sample was filtered onto a 0.7 μ GF-F filter using vacuum filtration. The filter was then placed into 1.2 mL of acetone. The sample tube was inverted and mixed to ensure proper extract and frozen until ready for analysis. Two hundred μL of the sample was transferred into a PCR tube and processed using a handheld Qubit 4 fluorometer having similar excitation and emission wavelengths described by Welschmeyer (1994). Using a response factor calculated from a standard curve with pure chlorophyll *a*, the following equation was used to calculate chlorophyll *a* concentration, as shown below in equation 3.

$$\text{Chlorophyll } a \text{ Concentration (μg/L)} = \frac{\text{Blank Corrected RFU} * \text{Response Factor ((μg Chl. } a\text{/mL)/RF)} * \text{Extraction Volume (mL)}}{\text{Volume Filtered (L)}}$$

Equation 3: Explanation of chlorophyll *a* concentration calculations for all samples

II. FIELD METHODS

Water surface samples were collected on a transect line from Honolulu, Hawaii to Seattle, Washington from June 4 to 13, 2017 on Cal Maritime's T/S *Golden Bear*. There were 55 sampling locations with samples were collected every 4 hours; 4:00, 8:00, 12:00, 16:00, 20:00, and 24:00 as shown in Figure 2 and Figure 3. The sampling scheme was an effort to detect potential diel fluctuations in surface ATP concentrations that might

indicate fluctuations in planktonic biomass resulting from the balance between photoautotrophic production and heterotrophic consumption.

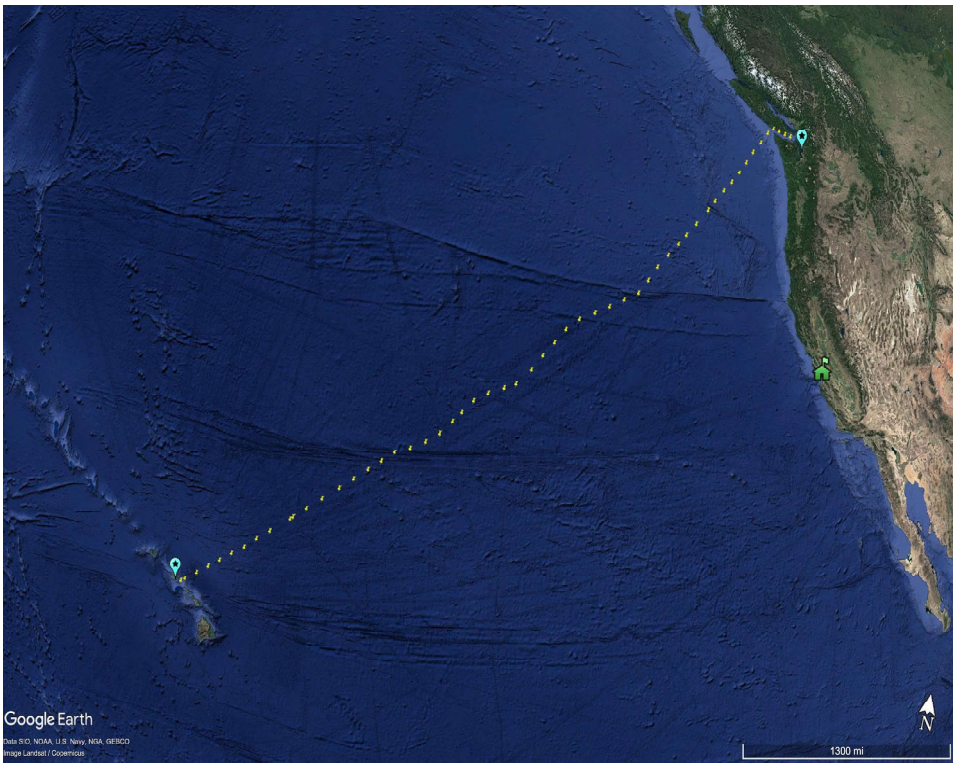

Figure 2. Sampling stations between Honolulu, HI and Seattle, WA. Total of 55 sampling locations on a transect line.

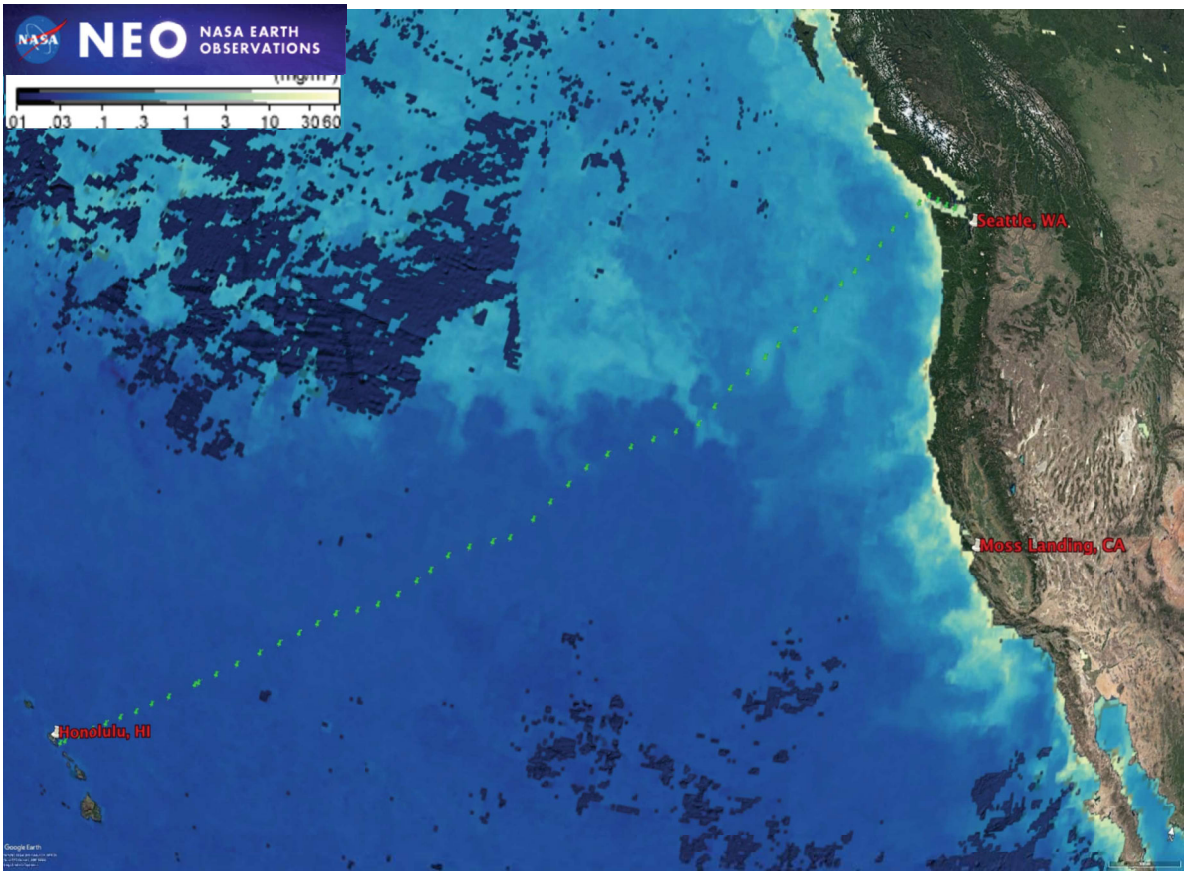

Figure 3. Sampling stations between Honolulu, HI to Seattle, WA overlayed on satellite chlorophyll *a* data retrieved from June 2017 from NASA's database.

Sampling water was collected while every approximately 20 nautical miles/hour using a sturdy, capped PVC pipe with a rope attached to the open side. Date, time, temperature and GPS coordinates were recorded for every station. Water was filtered through a 333 μm mesh to remove larger zooplankton before being added into final sample collection bottles (2 L, polycarbonate bottles) however captured zooplankton were rarely observed

At every sampling location, ATP measurements were made in triplicate following the procedure previously stated (Kuo, 2015). In addition to ATP measurements; bulk esterase activity measurements, chlorophyll *a*, temperature and nutrients were also taken at every station.

Bulk Esterase Activity using Fluorescein Diacetate (FDA)

The bulk FDA method described by Welschmeyer (2013) and optimized by Mauer (2015) and Welschmeyer and Mauer (2015) was performed at all sampling stations. The bulk FDA method uses FDA, fluorescein diacetate, a non-fluorescent compound that freely crosses the cell membrane of all live organisms. Once FDA is added to a sample and enters living cells, intracellular esterases will convert FDA into fluorescein. Fluorescein can readily be quantified using a fluorometric technique. Over a prolonged incubation in the presence of FDA (typically one hour) fluorescein will diffuse out of the cells and into the surrounding buffer. The esterase activity, measured as fluorescein production rate, has been shown to be proportional to living biomass. This bulk FDA method was used on the cruise as proxy of living biomass to complement simultaneous measurements of ATP made at every station.

The protocol used in the research cruise was as followed: a known volume of sample, 12 mL for chlorophyll and 610 mL for ATP and Bulk FDA, was concentrated onto a 0.7 μm pore-size GF/F filter. The bulk FDA filter was immediately placed into 1 mL of incubation buffer, referred to as 'reagent A' consisting of 500 mM d-Sorbitol and 20mM MES-KOH, pH 6.5 (Maurer, 2013). Immediately afterwards, 4 μL FDA, 2.5 mM was added into the sample for a final FDA concentration of 10 μM. The sample was then incubated in the dark for thirty minutes. After thirty minutes, 200 μL of the sample was transferred into a PCR tube and fluorescence was measured using a handheld Qubit® 4 fluorometer under blue light excitation and green light emission. The amount of FDA produced by metabolically active cells in the sample was proportional to fluorescence; the fluorescein production rate was calculated as shown in equation 4. with an empirically-determined standard curve and response factor. For each station, these measurements were made in triplicate.

$$\text{Bulk FDA or Production Rate ng/(L*hr)} = \frac{((\text{Blank Adjusted RFU} * \text{Response Factor (ug/L)}) * \text{Reagent A volume (L)}) \div \text{Volume Filtered (L)}}{\text{Incubation Time (hour)}} * 1000$$

Equation 4: Explanation of bulk FDA production calculations for all samples

A nutrient sample was collected twice a day at noon and midnight. Nutrient samples were collected by pre-filtering 50 mL of seawater using a 0.2 µ syringe filter. Samples were then frozen for later analysis. Nutrients were processed at MLML by the trace metals lab on a Lachat® nutrient analyzer.

RESULTS

I. LABORATORY RESULTS

Daily Growth

Daily growth was monitored in four phytoplankton cultures, *Thalassiosira weissflogii*, *Amphidinium carterae*, *Dunaliella salina* and *Isochrysis galbana* (Figs. 4-7). *Thalassiosira weissflogii* (Fig. 4) cells grew until day 6 and then plateaued. ATP concentration was scaled well to the live count data, however values began to plateau around day 4. *Amphidinium carterae* (Fig. 5) cell abundance irregularly increased until day 7 when reaching peak cell abundance then decreased on day 8 and remained constant. ATP concentration for *Amphidinium carterae* increased until day 5, decreasing on day 6 then continued to increase. *Dunaliella salina* (Fig. 6) cell abundance increased to day 6 then slowly began to decrease while ATP concentrations slowly increased overall with a substantial increase from day 8 to day 9. *Isochrysis galbana* (Fig. 7) cell abundance increased up to day 6 and then plateaued while ATP concentration increased the entire length of the experiment with the highest concentration on day 10. The data suggest that live cell counts are coupled fairly well with ATP concentration in a culture setting, showing an overall increasing trend as cell counts increase. However, a decrease in ATP when cell reproduction ceased was not evident.

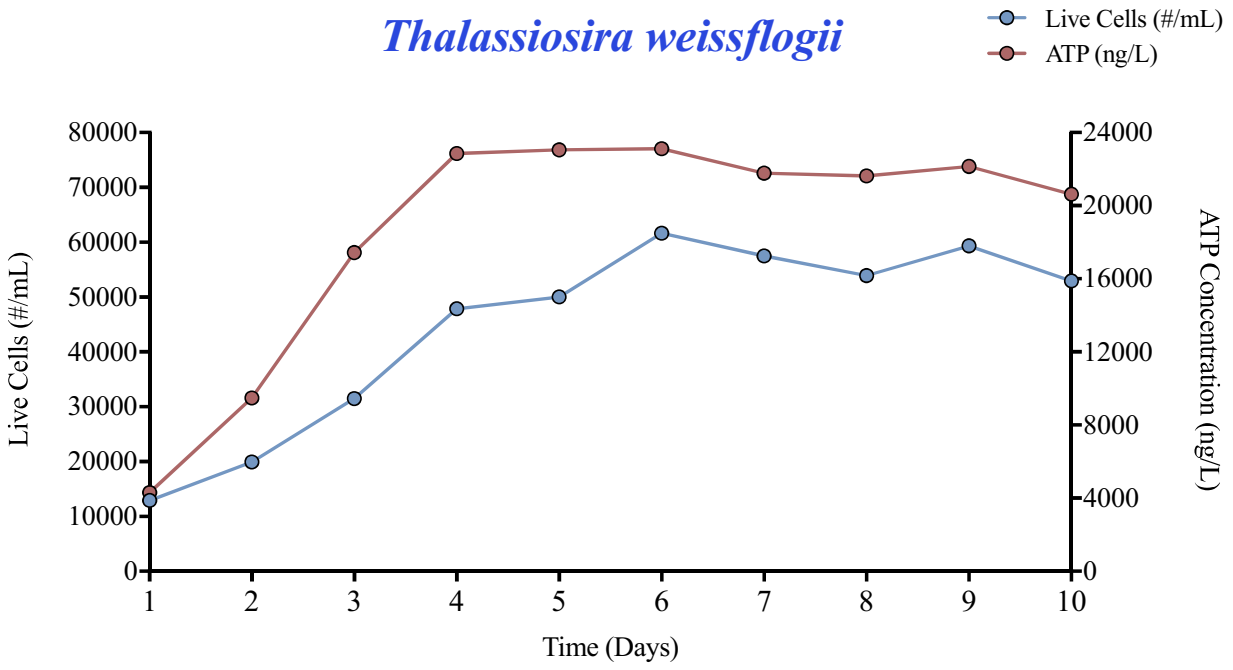

Figure 4. *Thalassiosira weissflogii* daily growth over time. Live cells (#/mL) displayed on the left y-axis and ATP concentration (ng/L) displayed on the right y-axis.

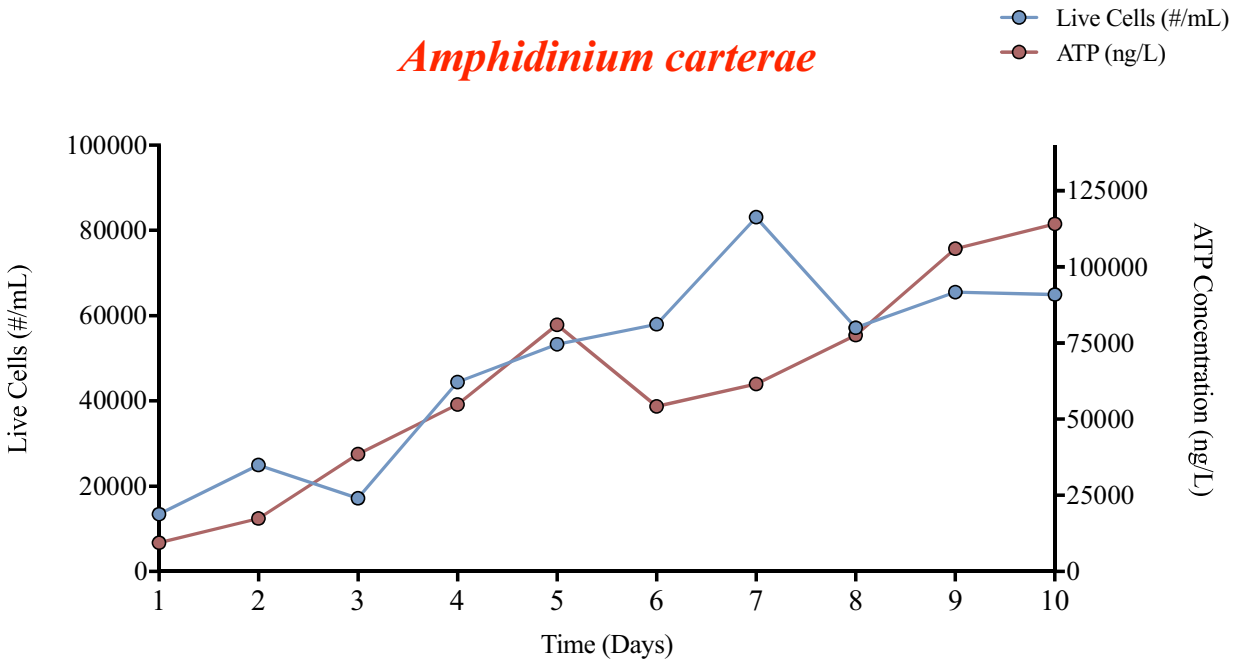

Figure 5. *Amphidinium carterae* daily growth over time. Live cells (#/mL) displayed on the left y-axis and ATP concentration (ng/L) displayed on the right y-axis.

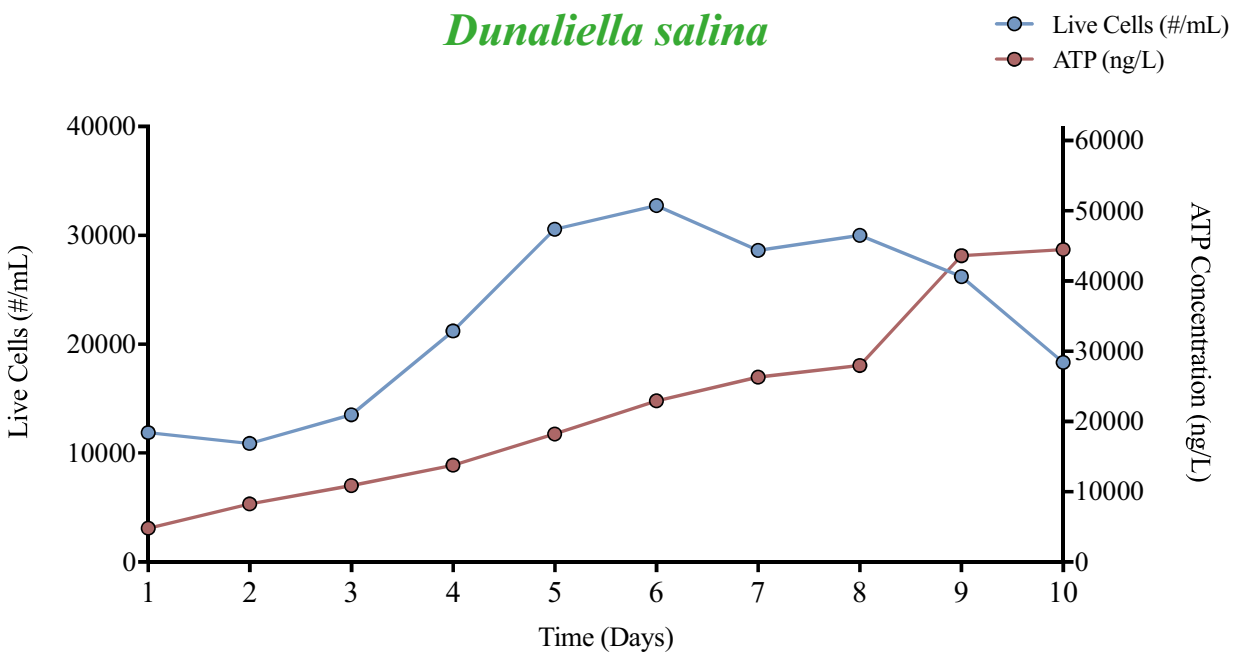

Figure 6. *Dunaliella salina* daily growth over time. Live cells (#/mL) displayed on the left y-axis and ATP concentration (ng/L) displayed on the right y-axis.

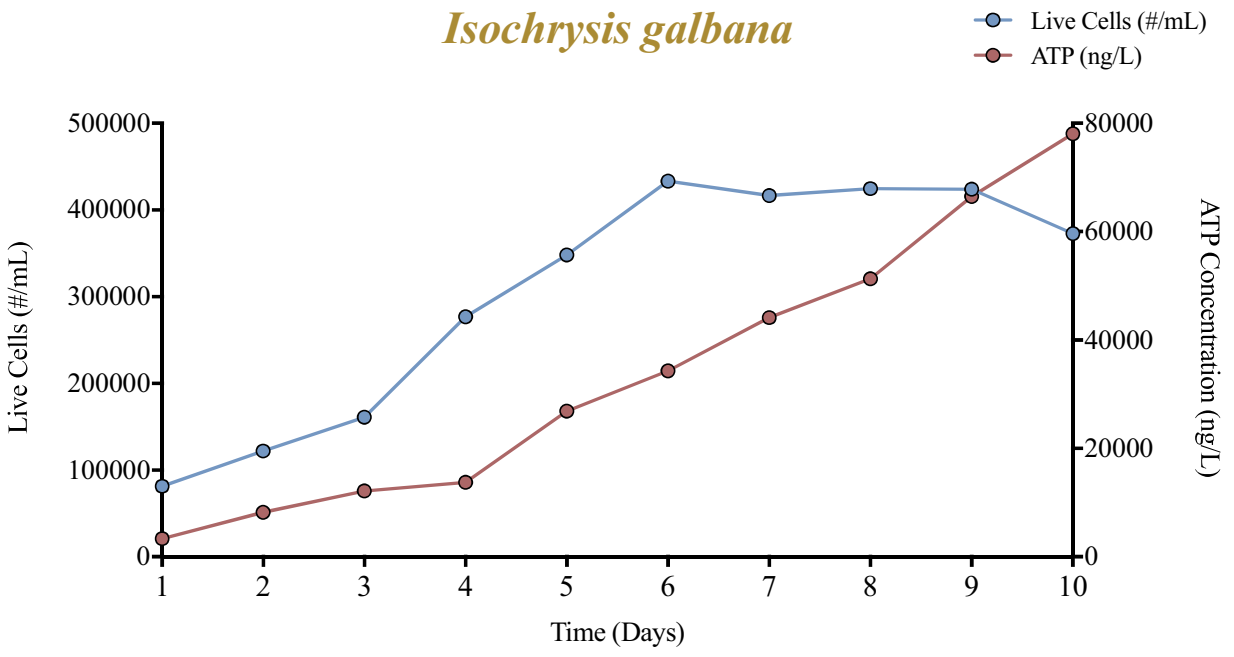

Figure 7. *Isochrysis galbana* daily growth over time. Live cells (#/mL) displayed on the left y-axis and ATP concentration (ng/L) displayed on the right y-axis.

Glutaraldehyde

The time series of ATP following treatment of Moss Landing Harbor water with 1% final concentration of glutaraldehyde as shown in Figure 8. The blue circle shows the average ATP concentration of the sample with no added glutaraldehyde while the red circle, also at time zero, shows the immediate impact of glutaraldehyde. Overall, ATP concentrations slowly decreased with time over the 6-hour observation period. All treated samples, excluding the time zero control, were significantly different than the control. However, ATP concentrations did not approach zero and data suggests that the ATP signal was not yet leveled by the end of the experiment. Phytoplankton cultures under the same glutaraldehyde treatments show mixed results. *Thalassiosira weissflogii* (*Fig. 9)* and *Isochrysis galbana (Fig. 12)* had similar trends with control samples having the highest ATP concentrations than the three other treatment samples over time. *Amphidinium carterae (Fig. 10)* plot shows 0.5% and 1% had higher ATP concentrations than the control at time zero, however at the end of the sampling series, control clearly had the highest ATP concentration followed by 1%, 0.5% and 0.25%. *Dunaliella salina (Fig. 11)* ATP concentrations were slightly higher at 0.25% concentration than the control at time zero, however, similar to *Dunaliella salina*, control ATP was highest at the end of sampling followed by 1%, 0.5% and 0.25%. The results suggest that the impact of glutaraldehyde was stronger on the species, *Thalassiosira weissflogii*, *Dunaliella salina* and *Isochrysis galbana*, where ATP concentrations of treated samples at the highest glutaraldehyde concentrations were reduced by approximately 10-fold relative to the control. *Amphidinium carterae* showed only a 2-fold reduction in ATP relative to the control.

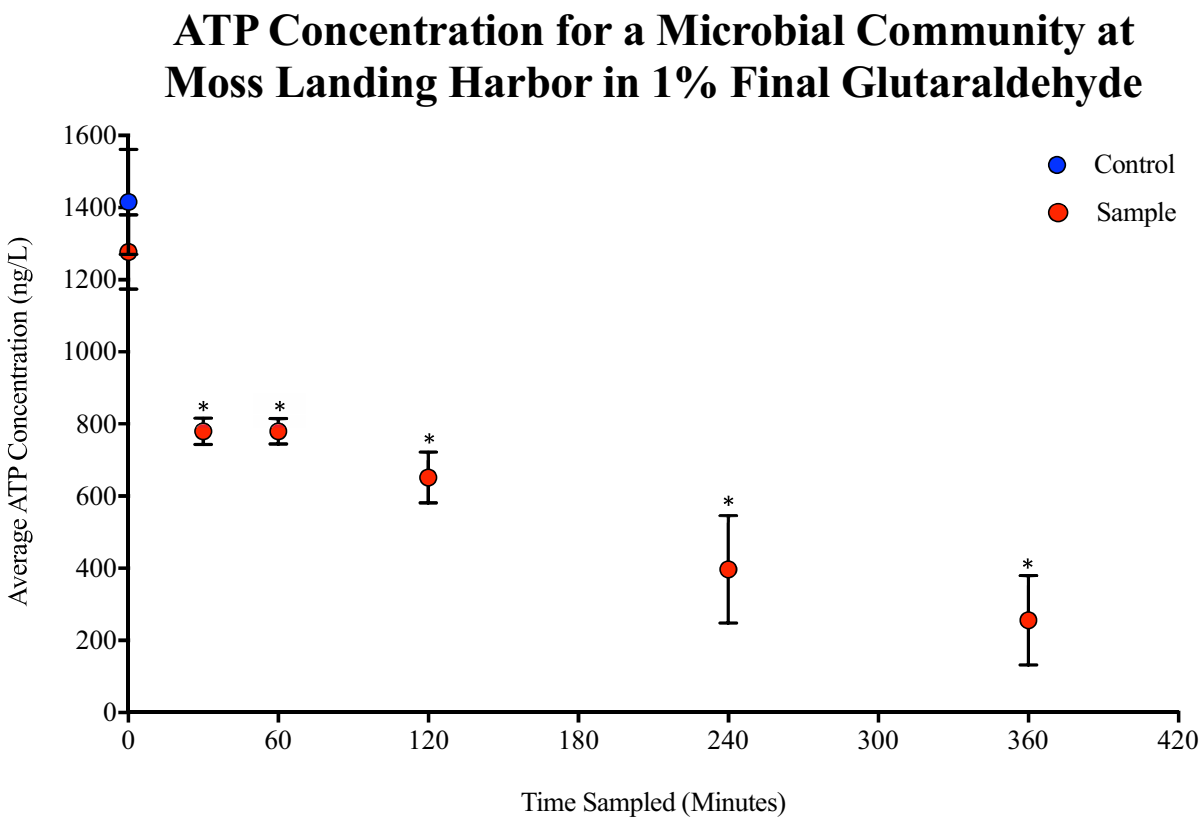

Figure 8. Average ATP concentration (ng/L) of a natural plankton community over time in 1% glutaraldehyde. Samples with asterisks next to them indicate samples that are significantly different from the control as indicated by one-way ANOVA with a Tukey's multiple comparisons test (*Alpha is 0.05, p-value is <0.0001)

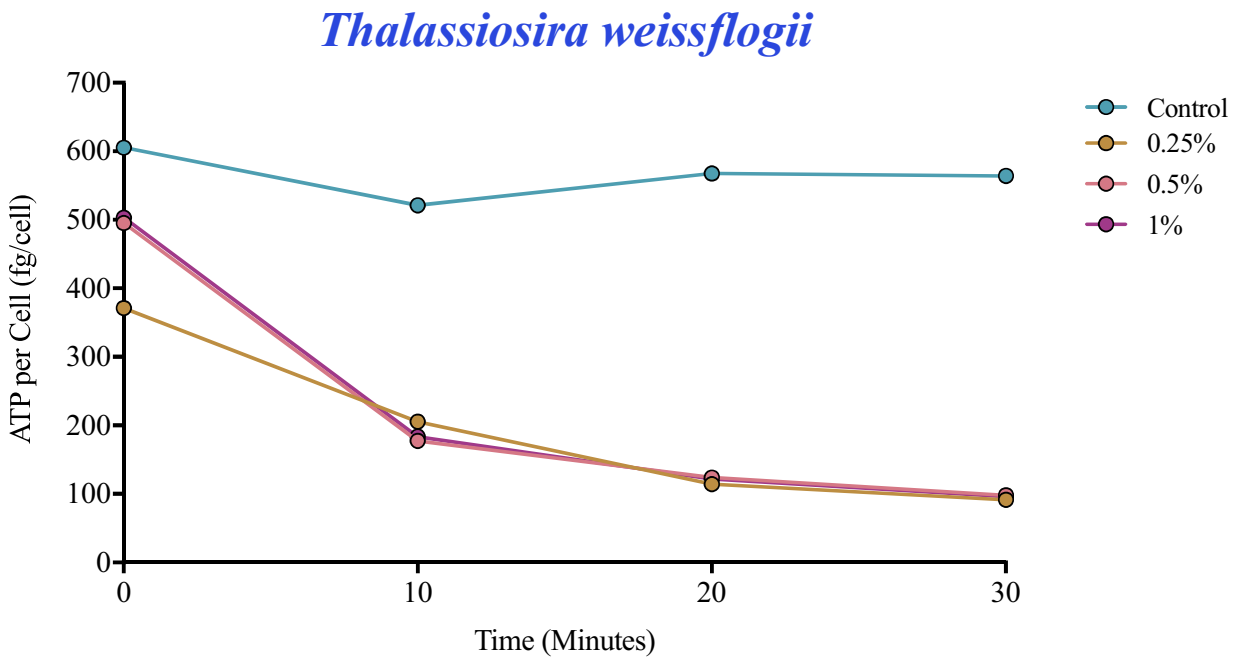

Figure 9. ATP per cell of *Thalassiosira weissflogii* in four various glutaraldehyde treatments over time

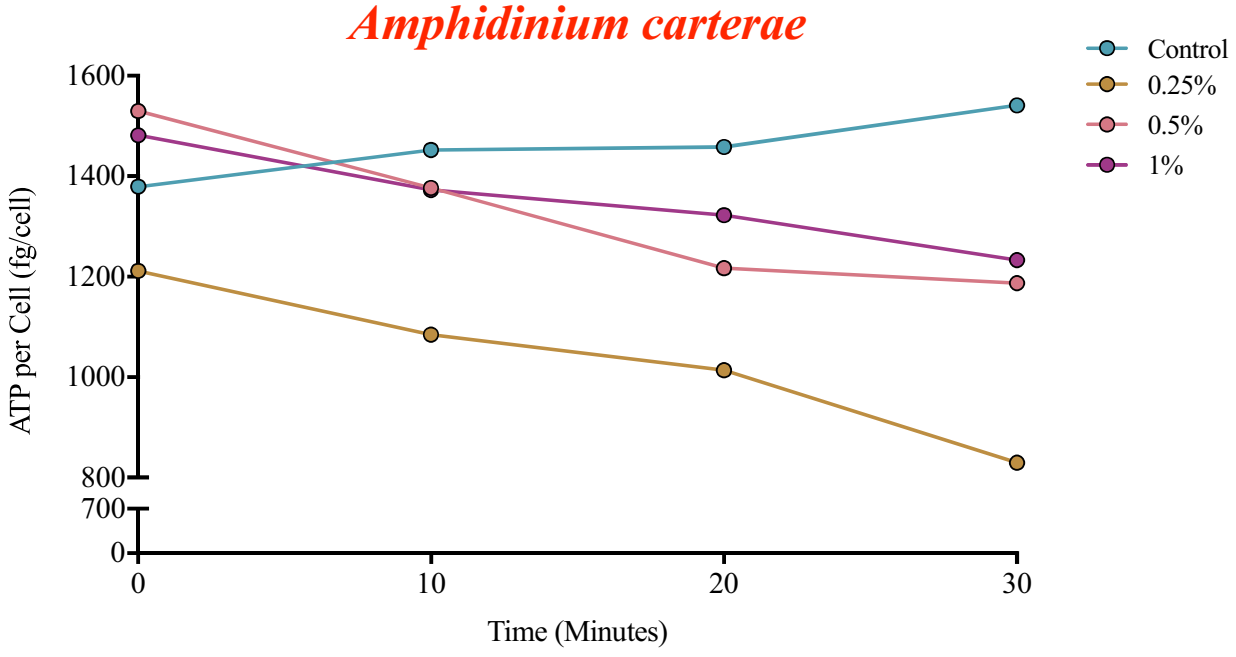

Figure 10. ATP per cell of *Amphidinium carterae* in four various glutaraldehyde treatments over time

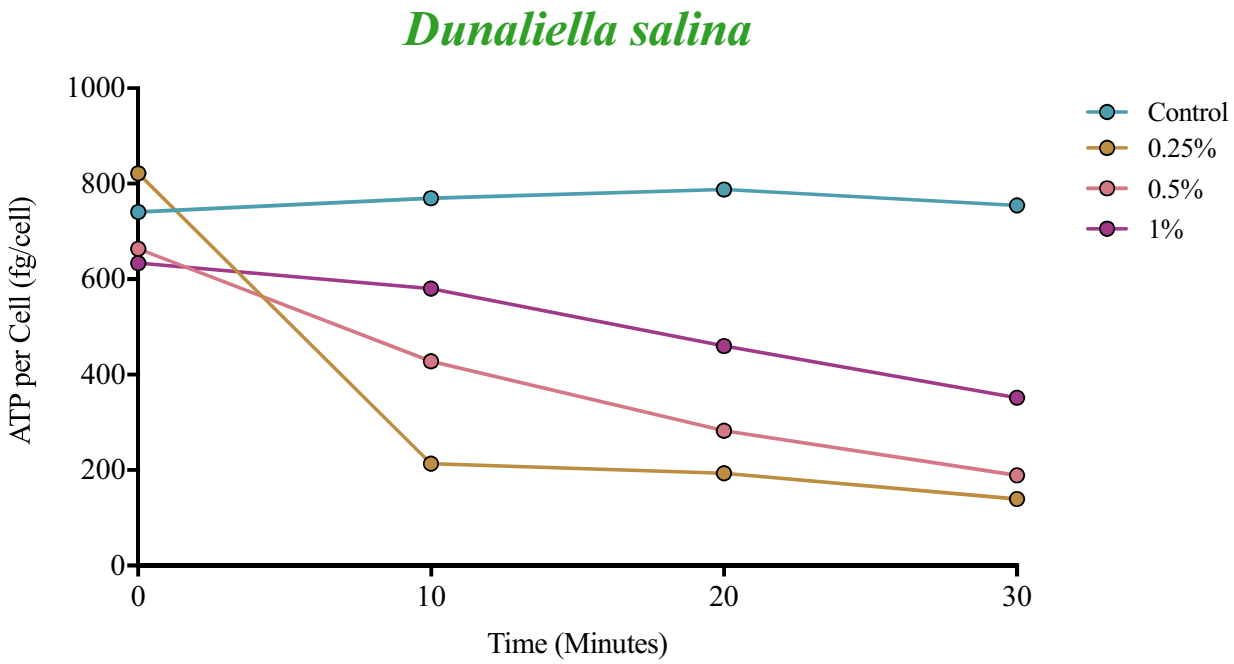

Figure 11. ATP per cell for *Dunaliella salina* under four various glutaraldehyde treatments over time

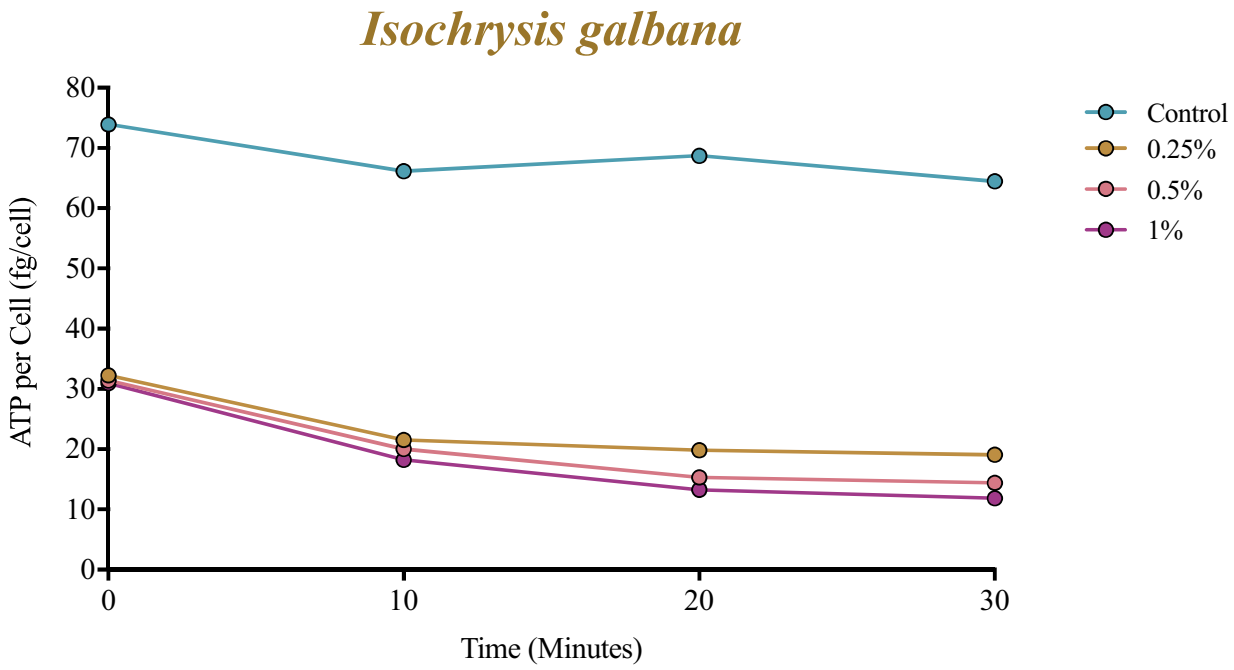

Figure 12. ATP per cell of *Isochrysis galbana* in four various glutaraldehyde treatments over time

Chlorination

Four phytoplankton cultures, *Thalassiosira weissflogii*, *Amphidinium carterae*, *Dunaliella salina* and *Isochrysis galbana*, were treated with two different hypochlorite levels, 6 ppm and 12 ppm, and were monitored over time (*Fig. 13-16)*. The pink circles indicate the control samples, where no bleach was added, whereas treated samples at one and two minutes are t-zero samples show the immediate impact of hypochlorite on ATP concentration per cell. *Thalassiosira weissflogii (Fig. 13)* ATP concentration per cell drastically decreased after 10 minutes, 13-fold decrease, and a gradual decline after. The effect of hypochlorite was clearly more potent than that of glutaraldehyde as noted by ATP reductions of approximately 50-fold to 100-fold over 2 hours in 3 of the 4 species. All treated samples were statistically significantly lower than the control. *Amphidinium carterae (Fig. 14)* ATP per cell concentration had a similar trend to *Thalassiosira weissflogii*, with one t-zero sample that was not statistically significant from the control, and a dramatic decrease was observed after 10 minutes with a gradual decline after 10 minutes. All samples, with the exception of t-zero for 6 ppm, were statistically significantly lower than the control. *Dunaliella salina (Fig. 15)* ATP per cell concentrations had a dramatic decrease after 10 minutes, 3 to 5-fold decrease from t-zero to 10 minutes, followed by a gradual decline. All samples were statistically significantly lower than the control. *Isochrysis galbana (Fig. 16)* ATP per cell concentrations had a contradictory trend from the three other phytoplankton species. Samples tend to general decrease over time compared to the control, however only three samples were statically significantly lower than the control suggesting no clear trend was observed. The results indicate that chlorination was an effective strategy in substantially decreasing ATP per cell concentration in *Thalassiosira weissflogii*, *Amphidinium carterae* and *Dunaliella salina*, however chlorination did not have as strong of an impact in *Isochrysis galbana* culture. The data also suggest that the concentration did make a significant difference in the decrease of ATP concentration, with 12 ppm having lower ATP per cell than 6 ppm for a given phytoplankton culture. ATP concentration per cell did not reach zero in any experiment, although the reductions reached levels approximating 1% of the initial values. All phytoplankton cultures, with the exception of *Isochrysis galbana*, experienced a substantial decrease in ATP per cell. *Thalassiosira weissflogii* had a 66-fold decrease

from control to 120 minute samples, *Amphidinium carterae* had a 61-fold decrease from control to 120 minute samples and *Dunaliella salina* had a 53-fold decrease from control to 120 minute samples.

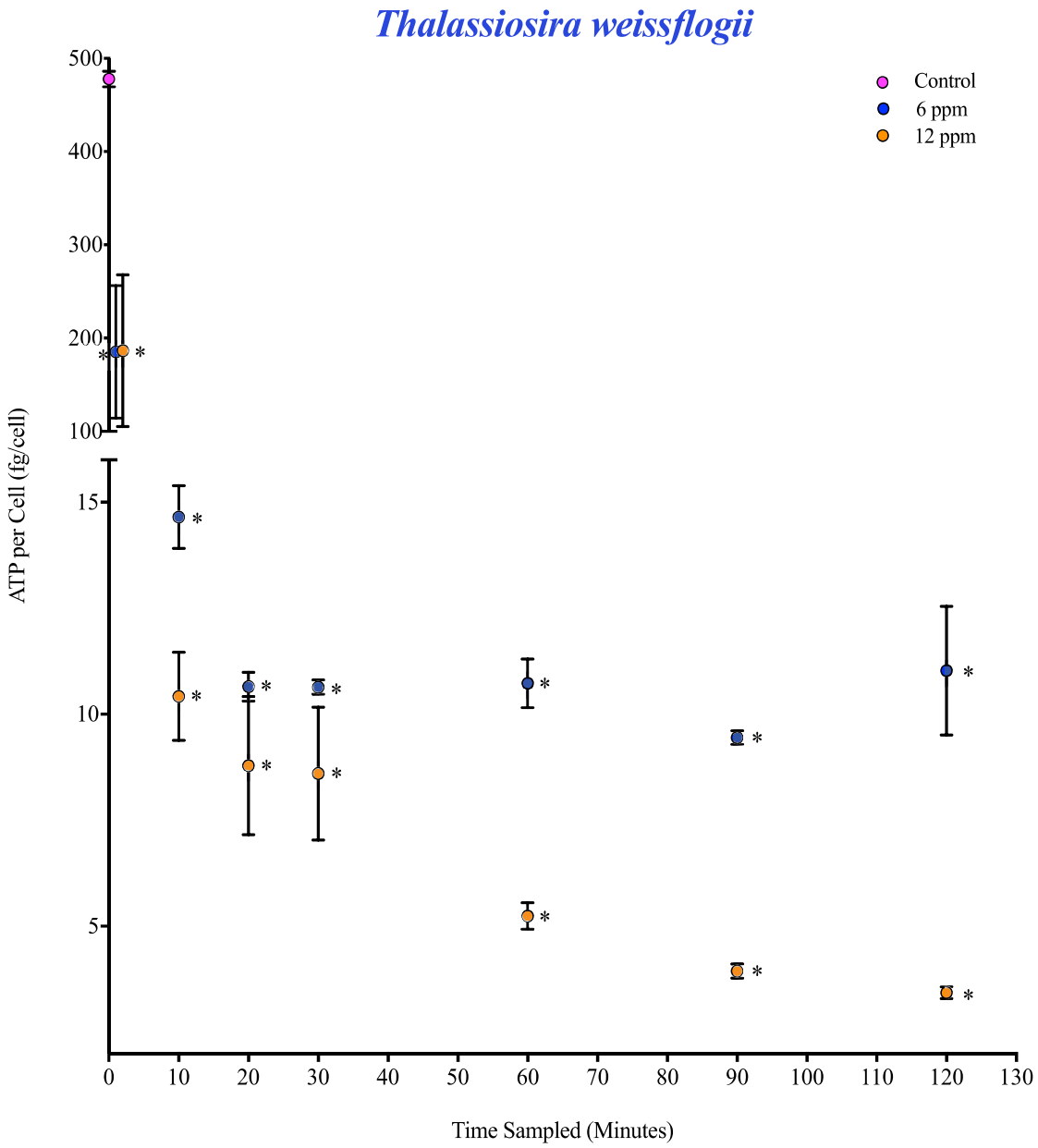

Figure 13. *Thalassiosira weissflogii* ATP concentrations (fg/cell) exposed to different concentrations of bleach and increasing exposure times. Samples with asterisks next to them indicate samples that are significantly different from the control as indicated by one-way ANOVA with a Tukey's multiple comparisons test (*Alpha is 0.05, p-value is <0.0001)

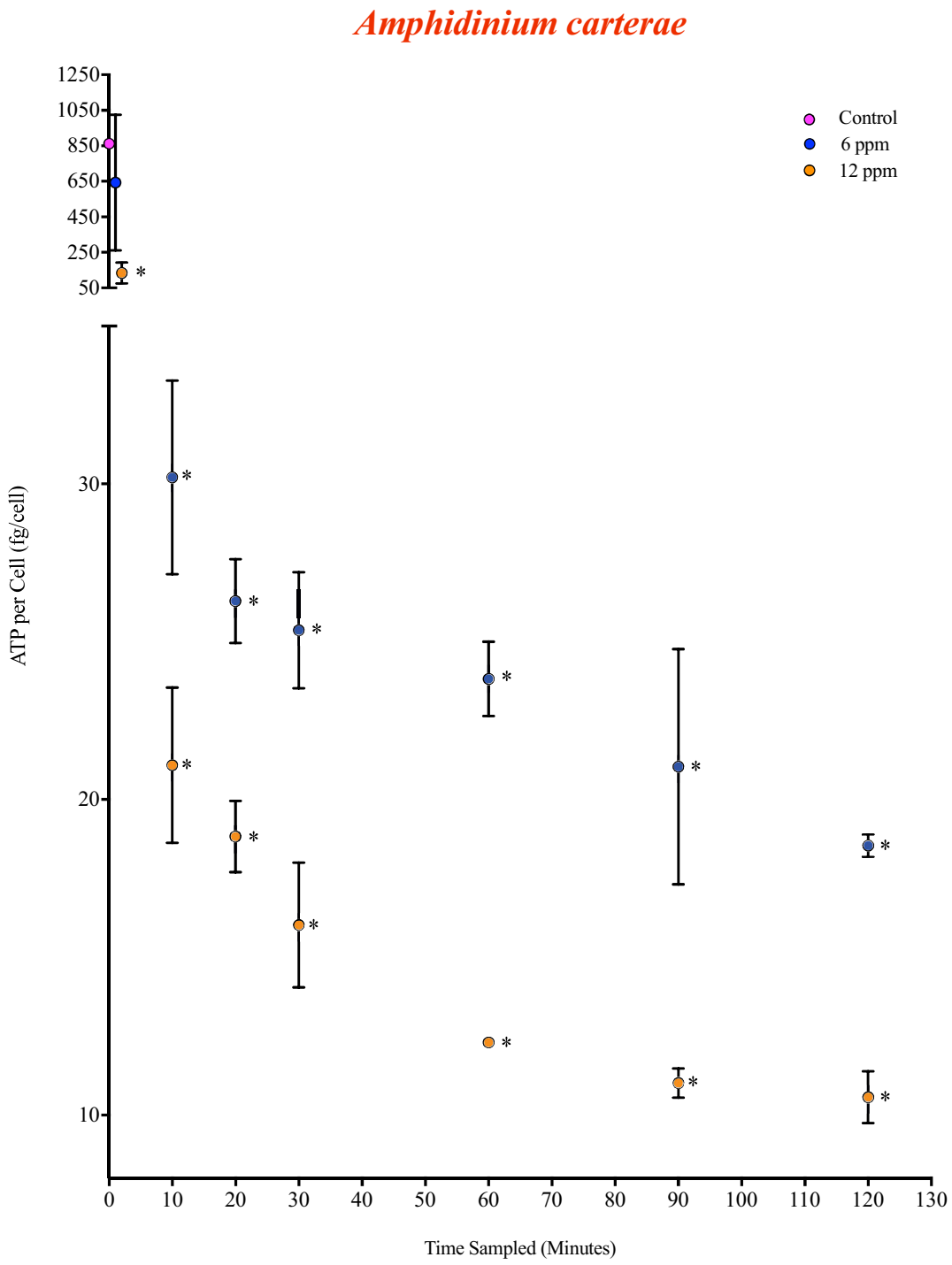

Figure 14. *Amphidinium carterae* ATP concentrations (fg/cell) exposed to different concentrations of bleach and increasing exposure times. Samples with asterisks next to them indicate samples that are significantly different from the control as indicated by one-way ANOVA with a Tukey's multiple comparisons test (*Alpha is 0.05, p-value is <0.0001)

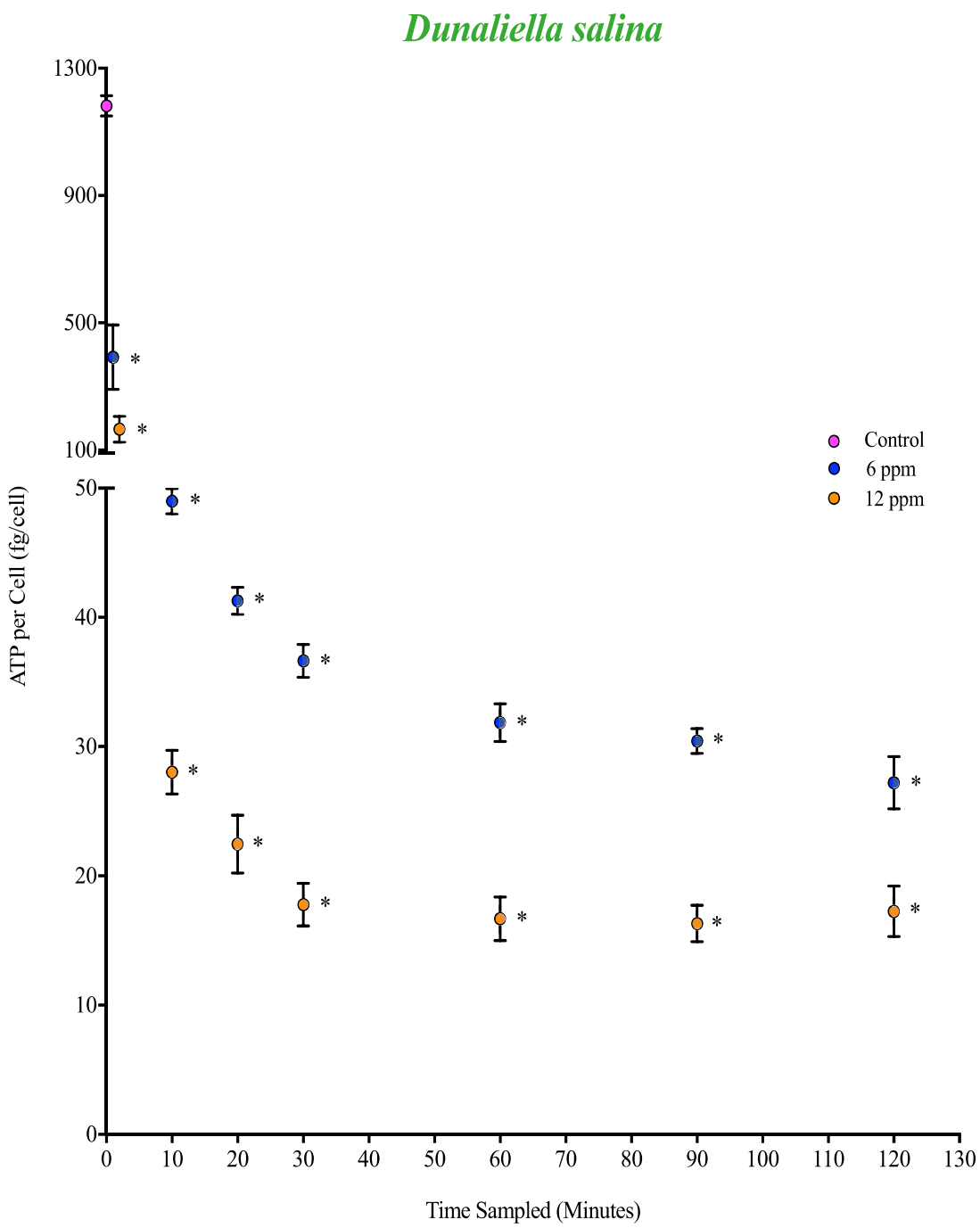

Figure 15. *Dunaliella salina* ATP concentrations (fg/cell) exposed to different concentrations of bleach and increasing exposure times. Samples with asterisks next to them indicate samples that are significantly different from the control as indicated by one-way ANOVA with a Tukey's multiple comparisons test (*Alpha is 0.05, p-value is <0.0001)

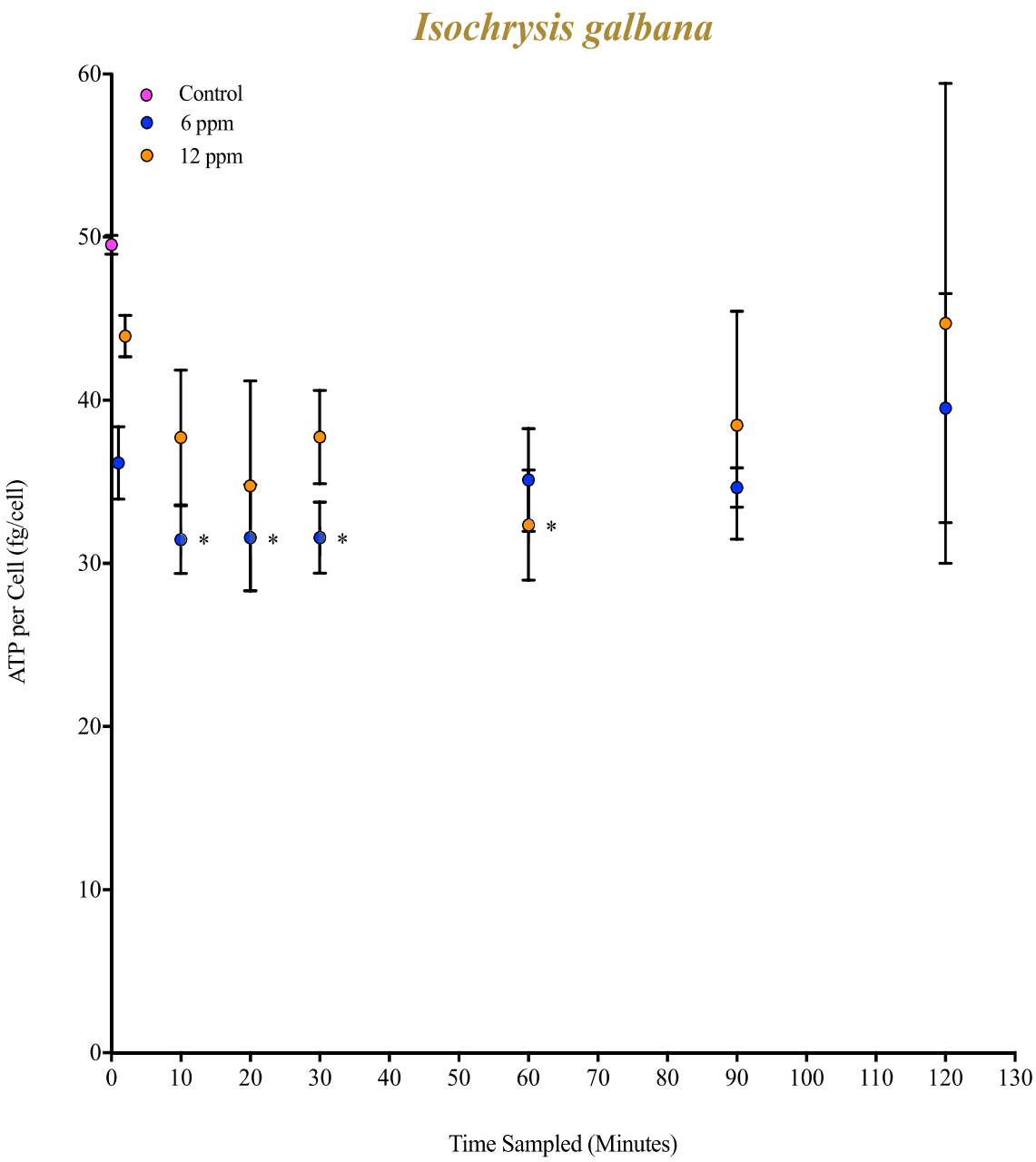

Figure 16. *Isochrysis galbana* ATP concentrations (fg/cell) exposed to different concentrations of bleach and increasing exposure times. Samples with asterisks next to them indicate samples that are significantly different from the control as indicated by one-way ANOVA with a Tukey's multiple comparisons test (*Alpha is 0.05, p-value is <0.0001)

The chlorophyll results after hypochlorite treatment for the same four phytoplankton cultures are shown in Figures 17-20. Chlorophyll *a* measurements were taken every ten minutes for thirty minutes. The control samples are represented by pink circles and lines while the treated samples are represented by various shades of blue circles and lines. The effects of hypochlorite treatment were dramatic in three of the four species, showing approaching zero after 10 minutes. Again, *Isochrysis galbana (Fig. 20*) seemed to be more resistant than the other treated species.

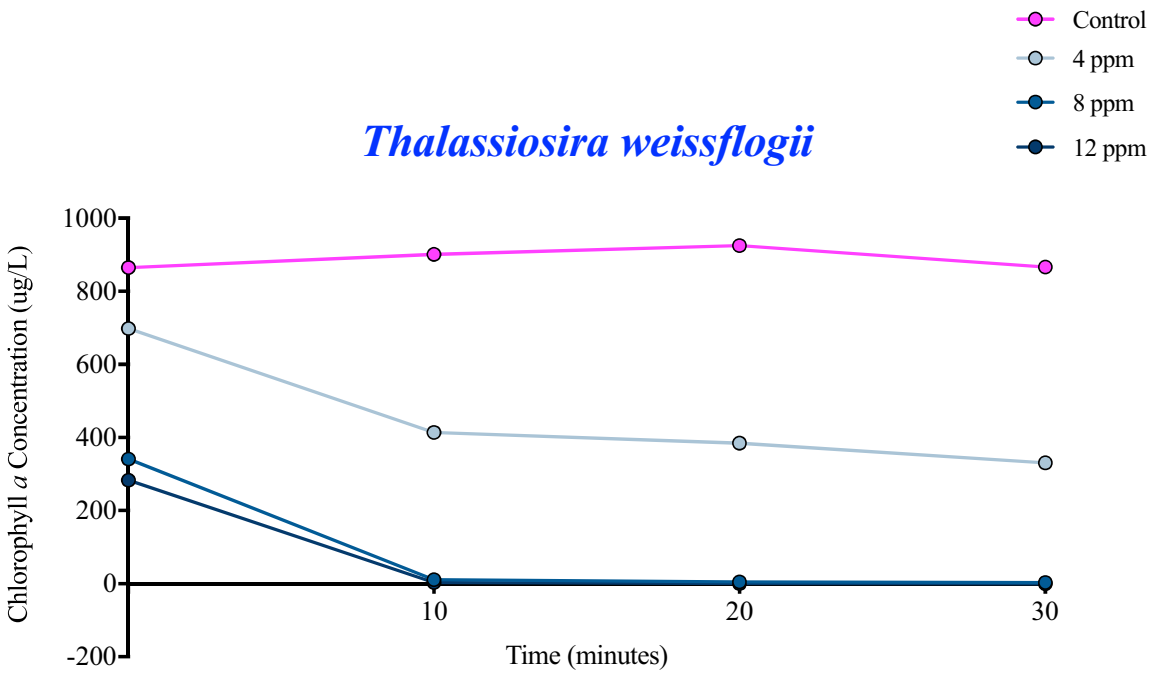

Figure 17. *Thalassiosira weissflogii* chlorophyll *a* concentrations exposed to different concentrations of bleach and increasing exposure times.

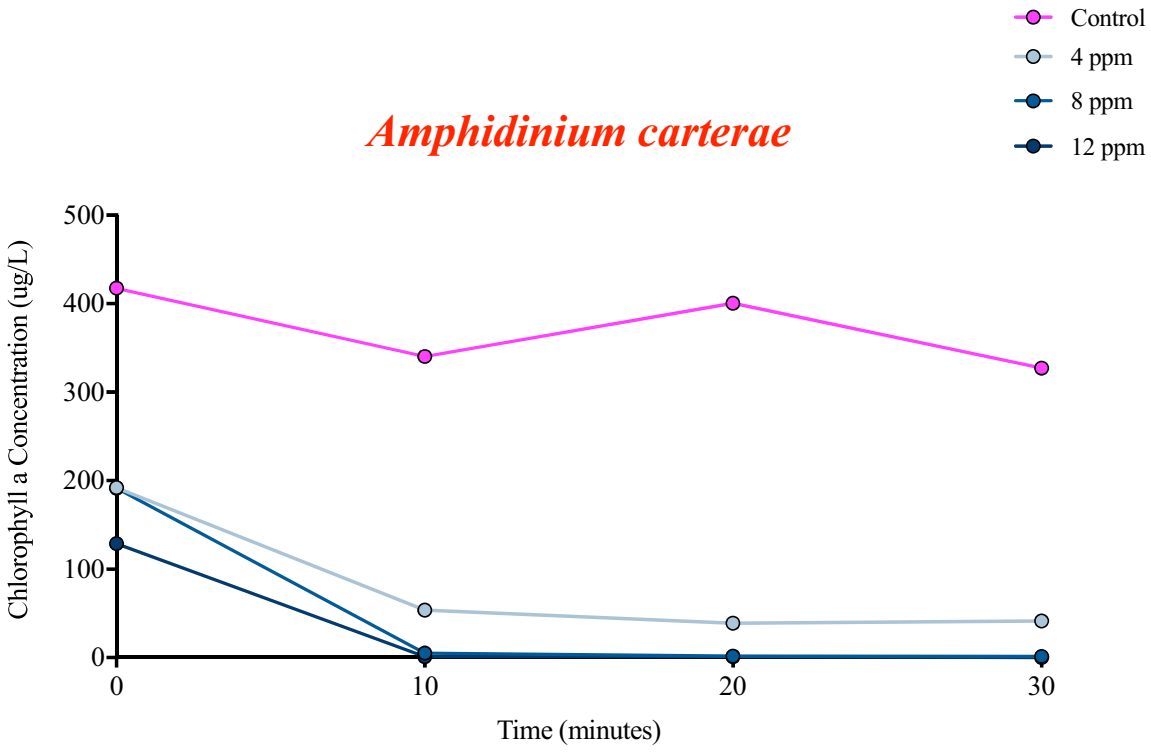

Figure 18. *Amphidinium carterae* chlorophyll *a* concentrations exposed to different concentrations of bleach and increasing exposure times.

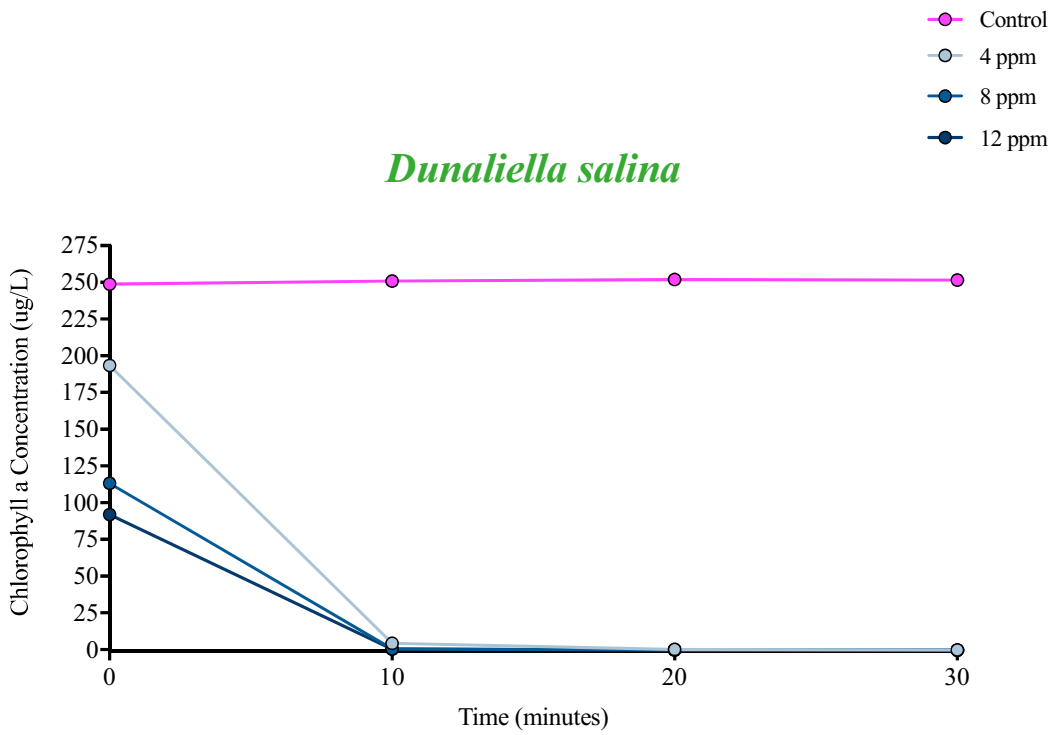

Figure 19. *Dunaliella salina* chlorophyll *a* concentrations exposed to different concentrations of bleach and increasing exposure times.

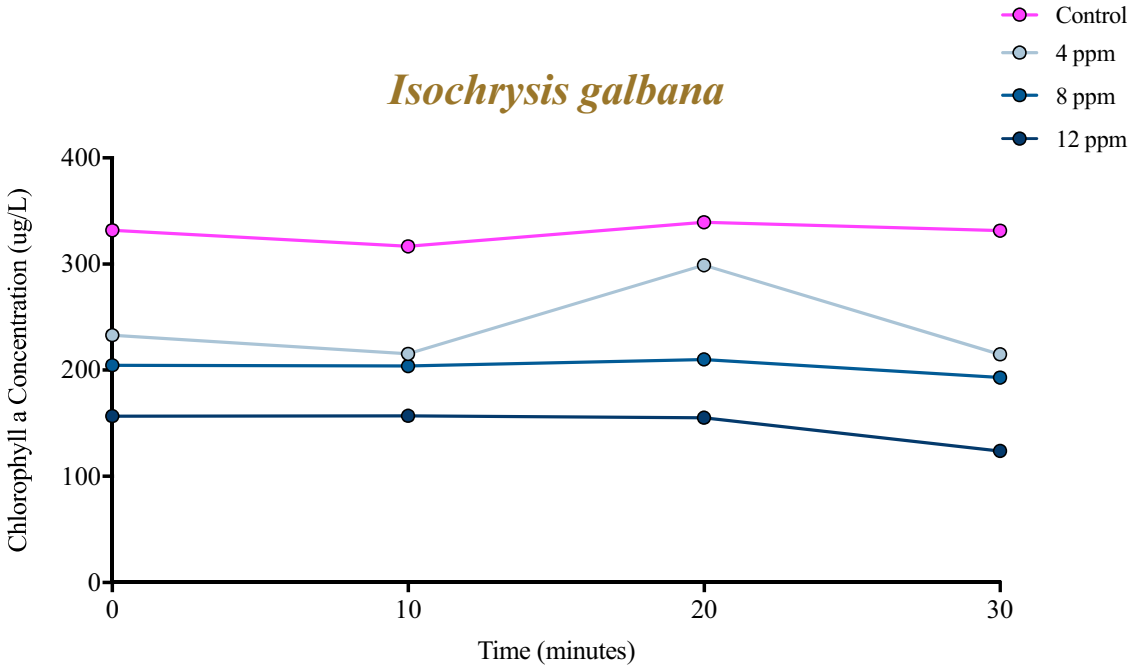

Figure 20. *Isochrysis galbana* chlorophyll *a* concentrations exposed to different concentrations of bleach and increasing exposure times.

Heat

A microbial community sample taken from Moss Landing Harbor was treated with constant heat at 55º C for two hours with ATP samples collected every 15 minutes. *(Fig. 21)* ATP concentrations decreased drastically with a 600-fold decrease after 30 minutes and an overall gradual decline in ATP values to near zero values after 2 hours. Four phytoplankton cultures, *Thalassiosira weissflogii*, *Amphidinium carterae*, *Dunaliella salina* and *Isochrysis galbana* were treated with constant heat at 50º C and were plotted over time. The same experiment in phytoplankton cultures had large ATP reductions in 30 minutes, with roughly 30-600 fold declines in ATP concentraitons. *Thalassiosira weissflogii (Fig. 22)* ATP per cell concentrations decreased 21-fold from control sample after 15 minutes of heat followed by a gradual decline. *Amphidinium carterae (Fig. 23)* ATP per cell concentrations decreased 68-fold from control sample after 15 minutes of heat followed by a gradual decline. *Dunaliella salina (Fig. 24)* ATP per cell concentrations decreased 38-fold from control sample after 15 minutes of heat followed by a gradual decline. *Isochrysis galbana (Fig. 25)* ATP per cell concentrations decreased 33-fold from control sample after 15 minutes of heat followed by a gradual decline. After 120 minutes, microbial sample had a 687-fold decrease from the control while *Thalassiosira weissflogii* had an 82-fold decrease, *Amphidinium carterae* had a 1241-fold decrease, *Dunaliella salina* had a 118-fold decrease and *Isochrysis galbana* had a 480-fold decrease.

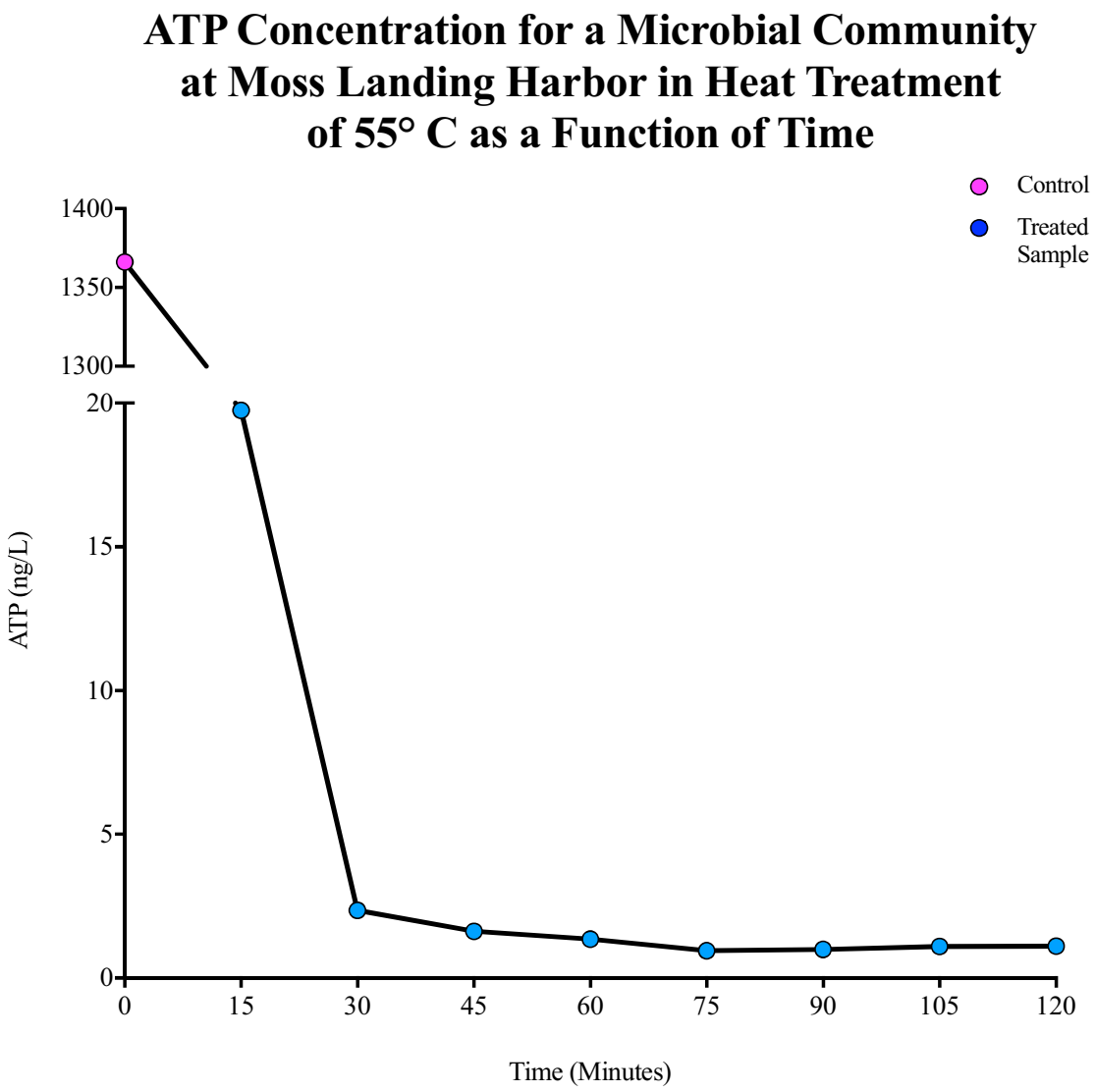

Figure 21. ATP concentration (ng/L) of a microbial community at Moss Landing, CA harbor after heat treatment of 55º C for various time periods (minutes)

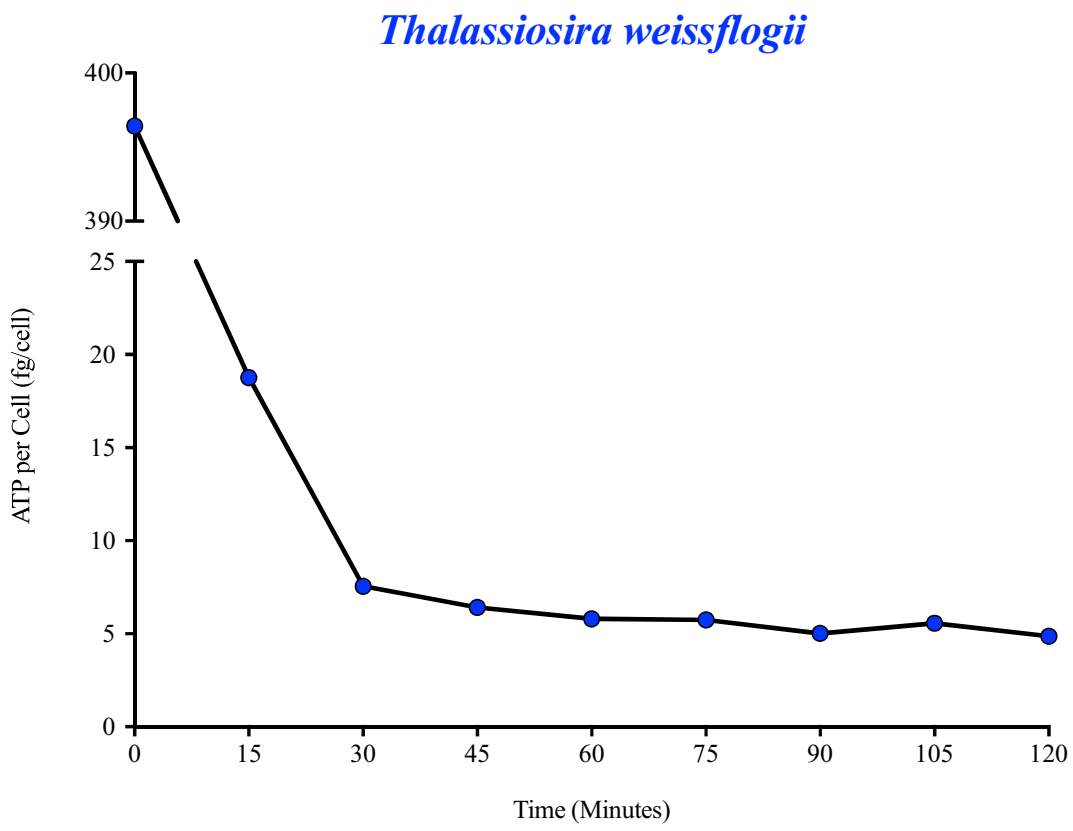

Figure 22. ATP concentration (ng/L) of *Thalassiosira weissflogii* under heat treatment of 50º C over time (minutes)

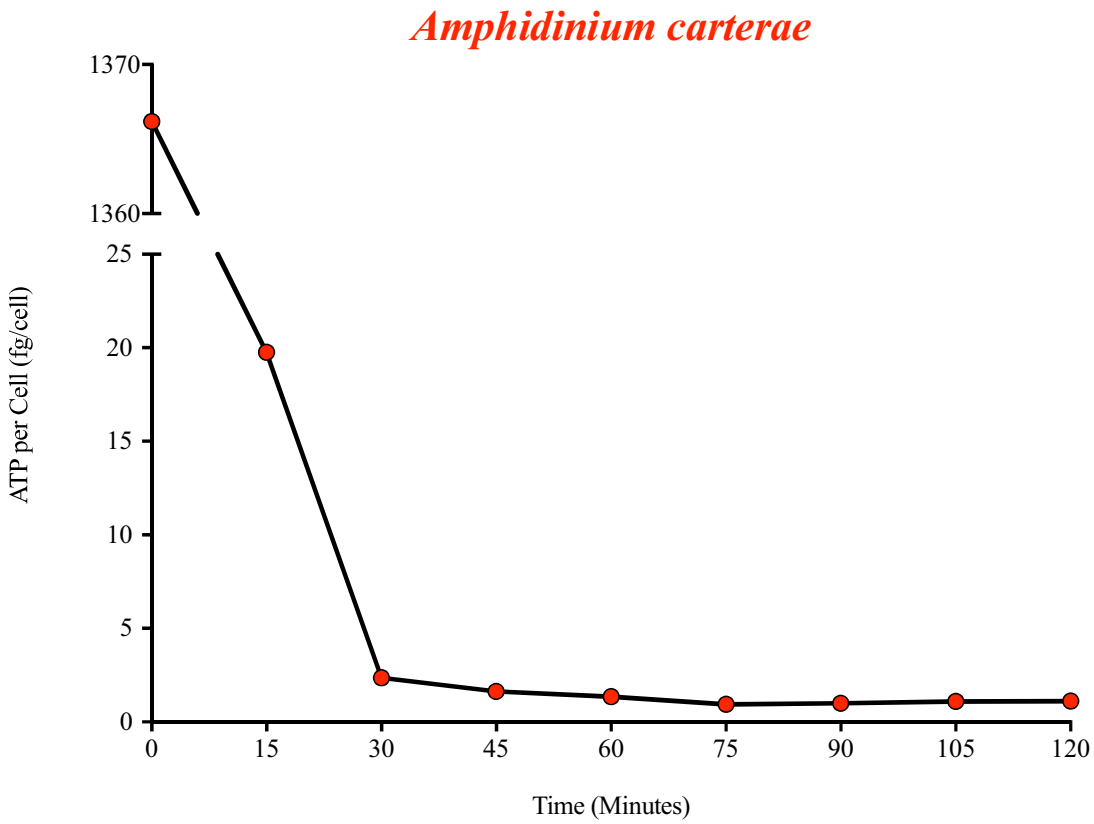

Figure 23. ATP concentration (ng/L) of *Amphidinium carterae* under heat treatment of 50º C over time (minutes)

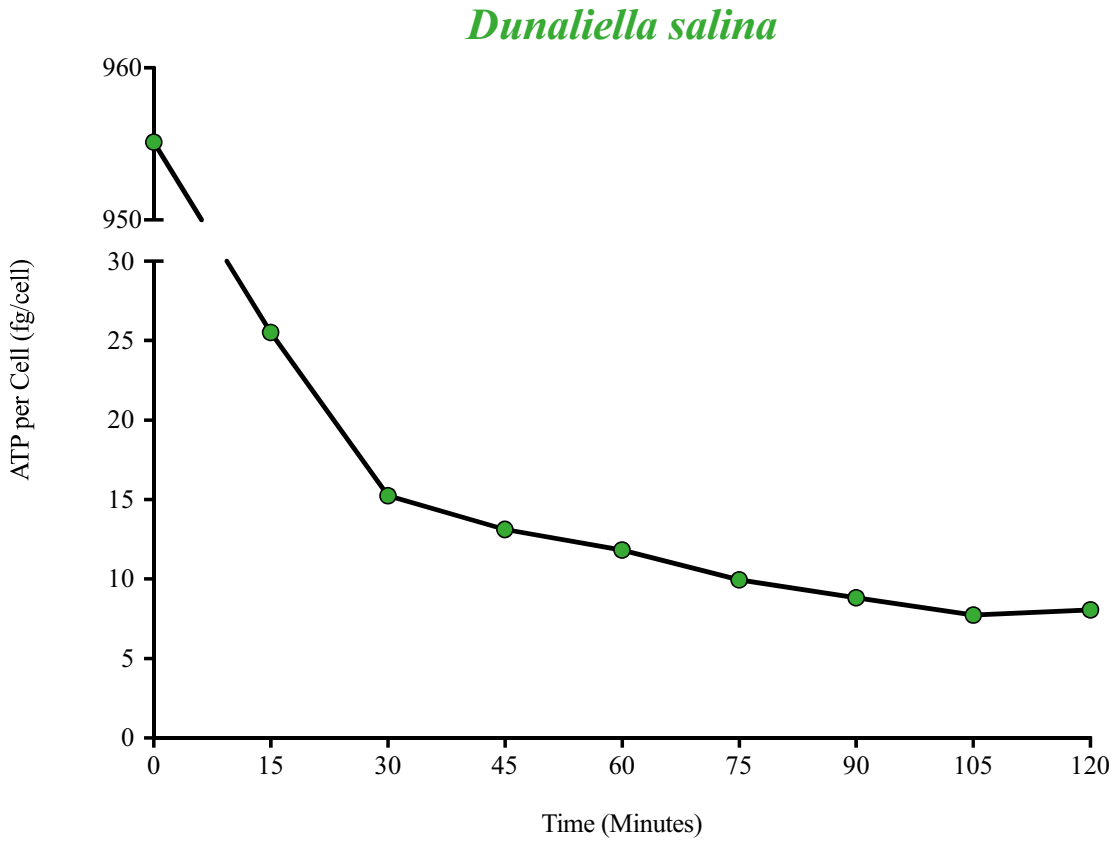

Figure 24. ATP concentration (ng/L) of *Dunaliella salina* under heat treatment of 50º C over time (minutes)

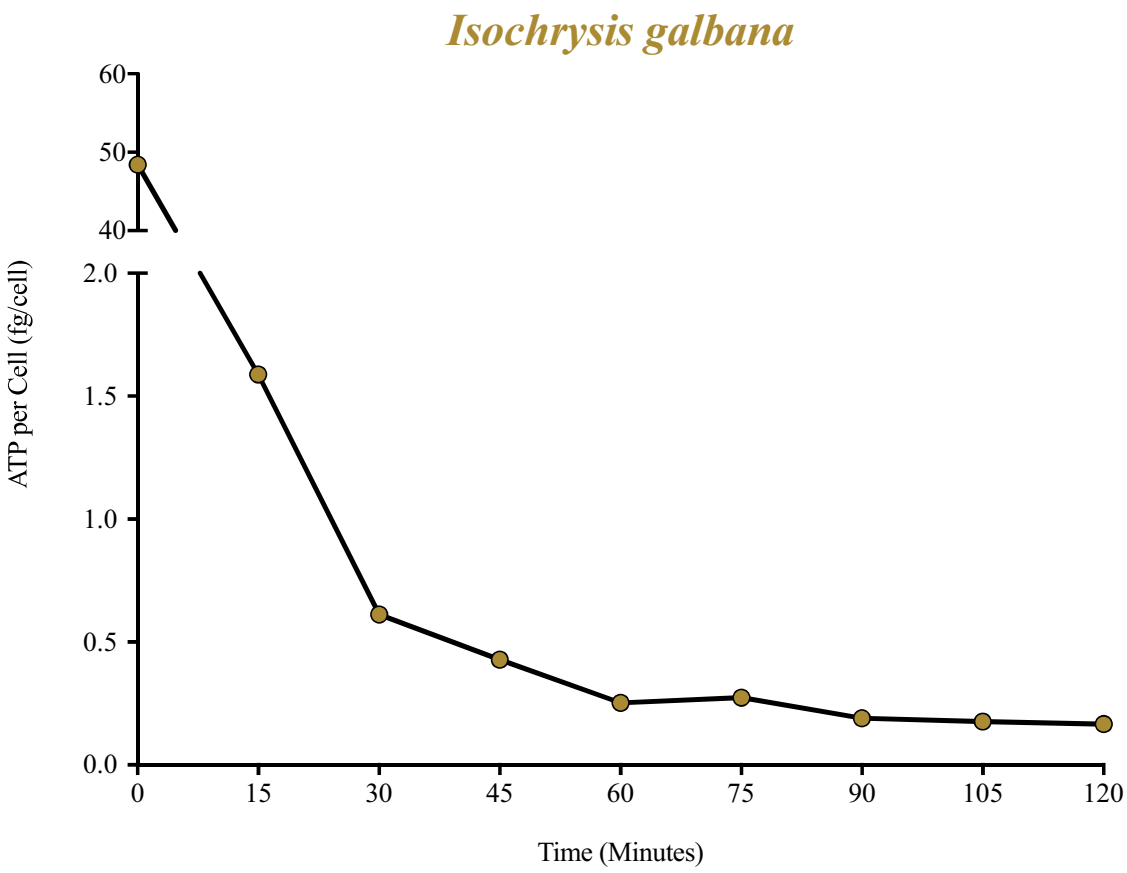

Figure 25. ATP concentration (ng/L) of *Isochrysis galbana* under heat treatment of 50º C over time (minutes)

II. FIELD RESULTS

ATP samples were taken from surface waters approximately every 4 hours on a transect line from Honolulu, Hawaii to Seattle, Washington from June 4th to 13th, 2017 *(Fig. 26)*. ATP concentrations fluctuated across different oceanic regions, starting from Hawaiian oligotrophic waters transitioning into mesotrophic, California current and then into coastal waters. Areas with high nutrients and total biomass produces more variability in ATP concentration, as shown in figure 26, when approaching the California current and coastal waters near Washington and the Puget Sound, in contrast to the relatively low ATP variability in oligotrophic water as seen near Hawaii and the open Pacific. Diel variability of ATP *(Fig. 27)* was analyzed by from eliminating eutrophic ATP concentrations where spatial patchiness masked the subtle changes in ATP observed in more uniform oligotrophic waters. Midnight samples *(Fig. 27)* are represented by dark bars along with the red circles while noon samples are represented by yellow bars and yellow circles. Overall, low ATP values typically seen at noon and high ATP values were typically seen at midnight. ATP concentrations were compared with chlorophyll *a* concentrations along the transect line to show the positive correlation between the two biomass estimates acorss the North Pacific Ocean *(Fig. 28)*. When calculating the ratio between ATP and chlorophyll *a* the correlation coefficient was reasonably high (0.9025) as expected *(Fig. 29)*. The data from the ratio of ATP to chlorophyll *a* was further dissected into various water masses *(Fig. 30)*. The results had fluctuating correlation coefficients, with oligotrophic and mesotrophic waters having the lowest correlation coefficients and California current and coastal waters having the highest correlation coefficients. Thus, when gross changes in total living planktonic biomass are relatively large, as in the coastal environment, ATP and chlorophyll *a* correlate reasonably well. In oligotrophic water, where biomass was low, the relation between ATP and chlorophyll *a* was relatively poor, even though diel fluctuations in ATP up to 2-fold per day were observed often in this environment *(Fig. 27)*. Total living biomass (as indicated by ATP as a proxy) seemed to fluctuate independently of photoautotrophic biomass indicated by chlorophyll *a*. It could be possible that the photoautotrophic increased in ATP from noon to midnight represented both autotrophic production and incorporation of producer

biomass into microzooplankton grazer biomass, followed by night time degradation of ATP by metabolism of the same heterotrophic consumers.

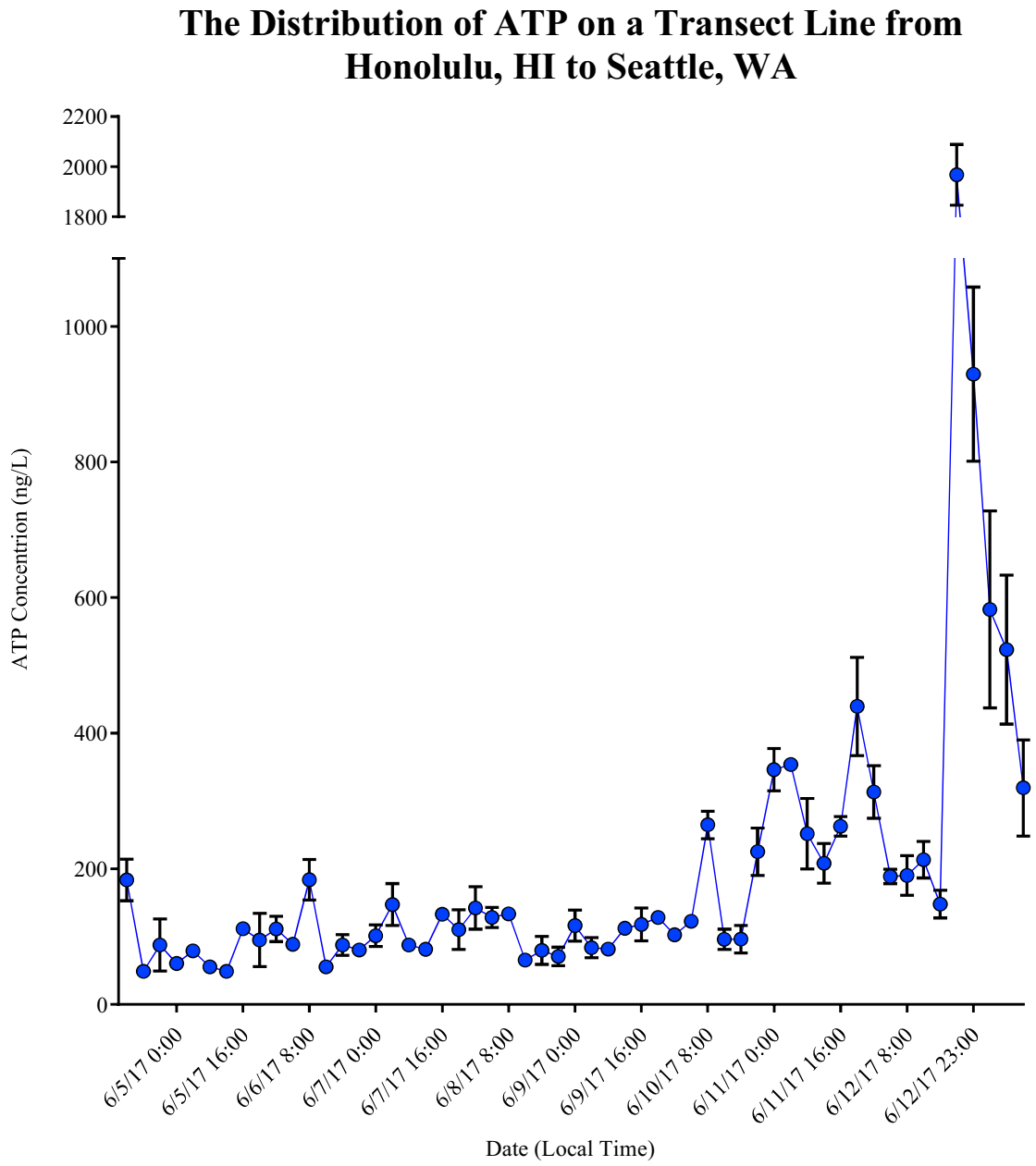

Figure 26. ATP concentration (ng/L) in triplicates at each sampling location on transect line from Honolulu, HI to Seattle, WA.

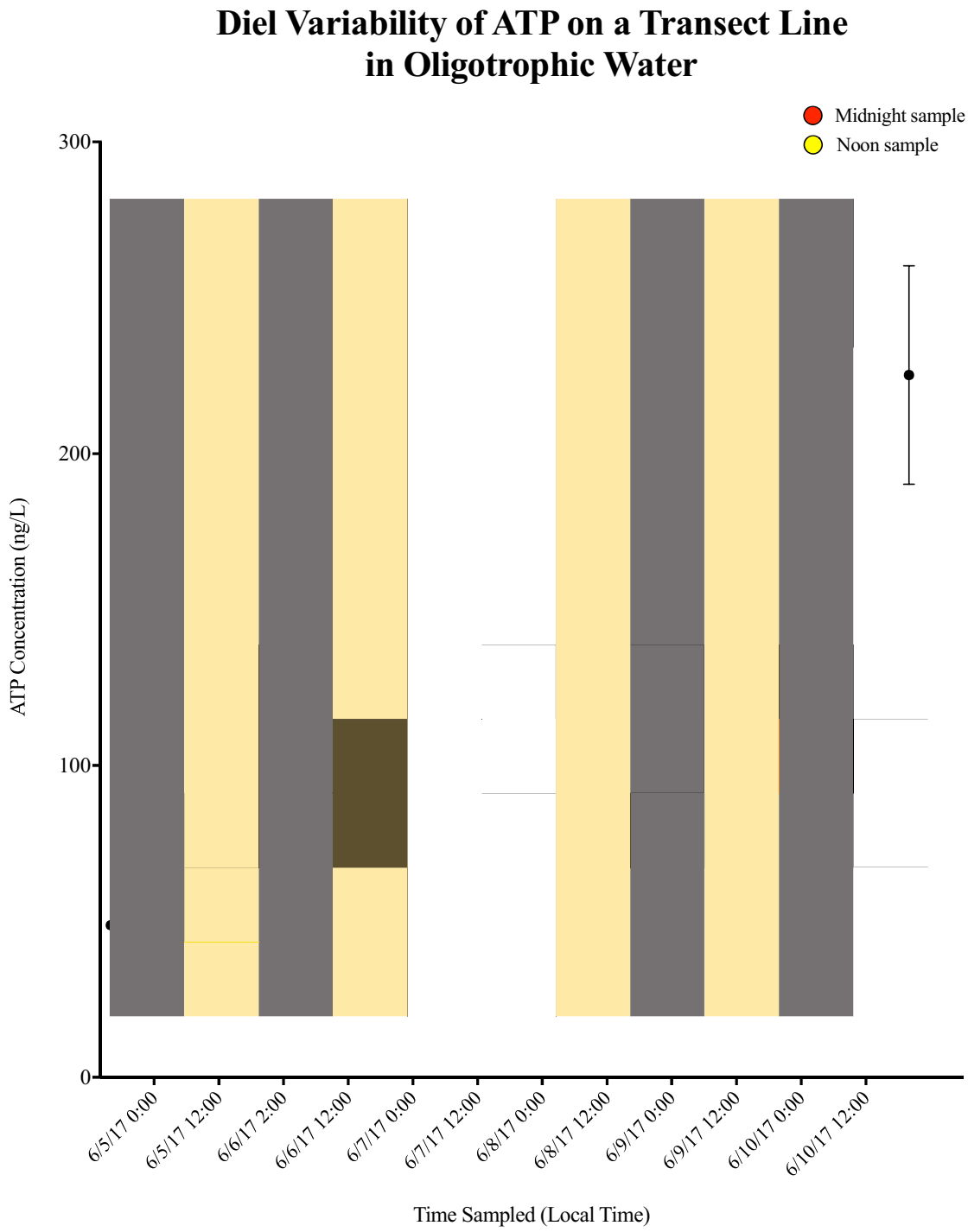

Figure 27. Diel variability of average ATP concentration (ng/L) on a transect line from Honolulu, HI to Seattle, WA. Dark bars (and red filled symbols) represent local midnight and yellow bars (and yellow filled symbols) represent local noon sampling events.

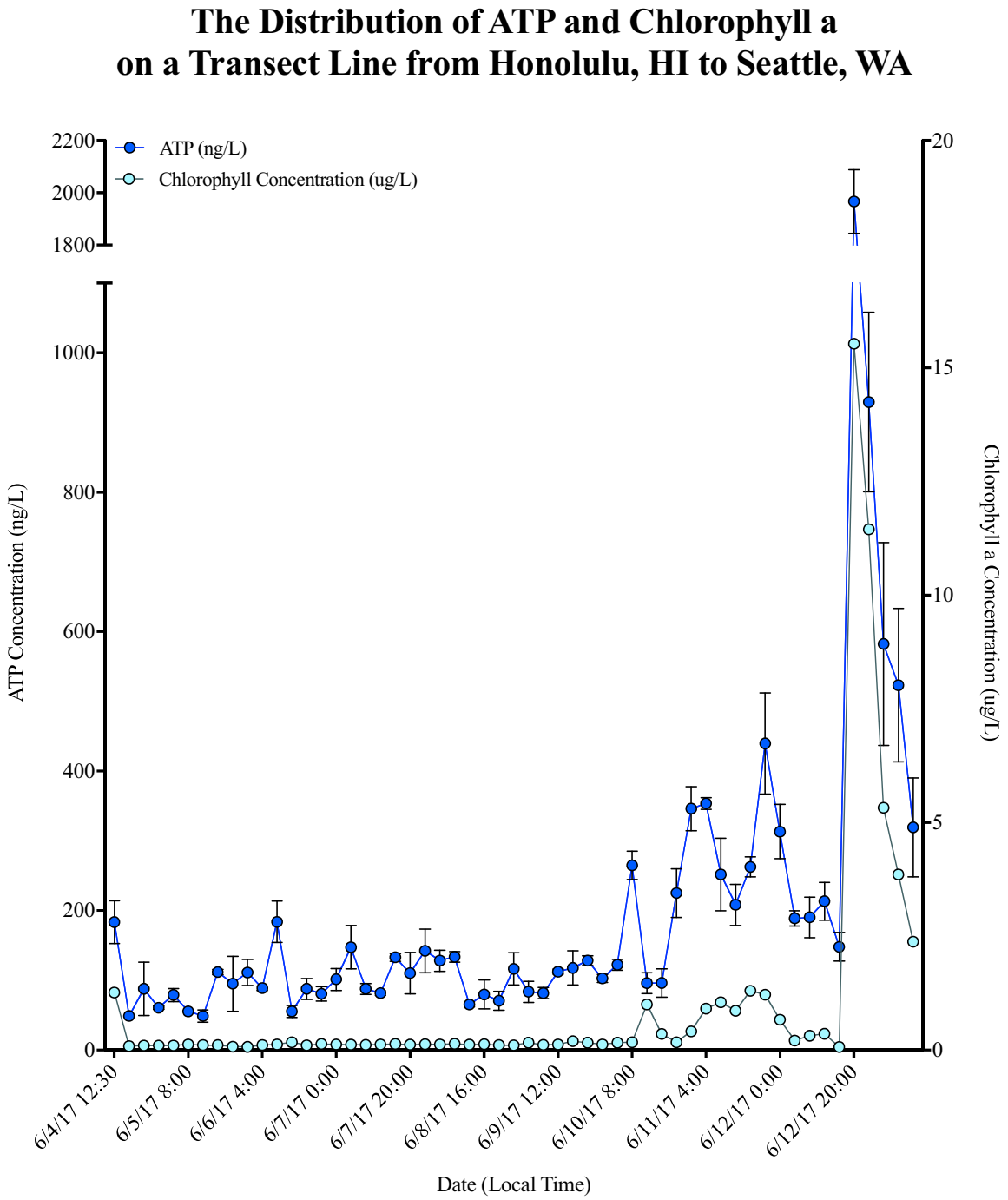

Figure 28. ATP concentrations (ng/L) displayed on the left y-axis and chlorophyll *a* concentrations (µg/L) displayed on the right y-axis over the transect line.

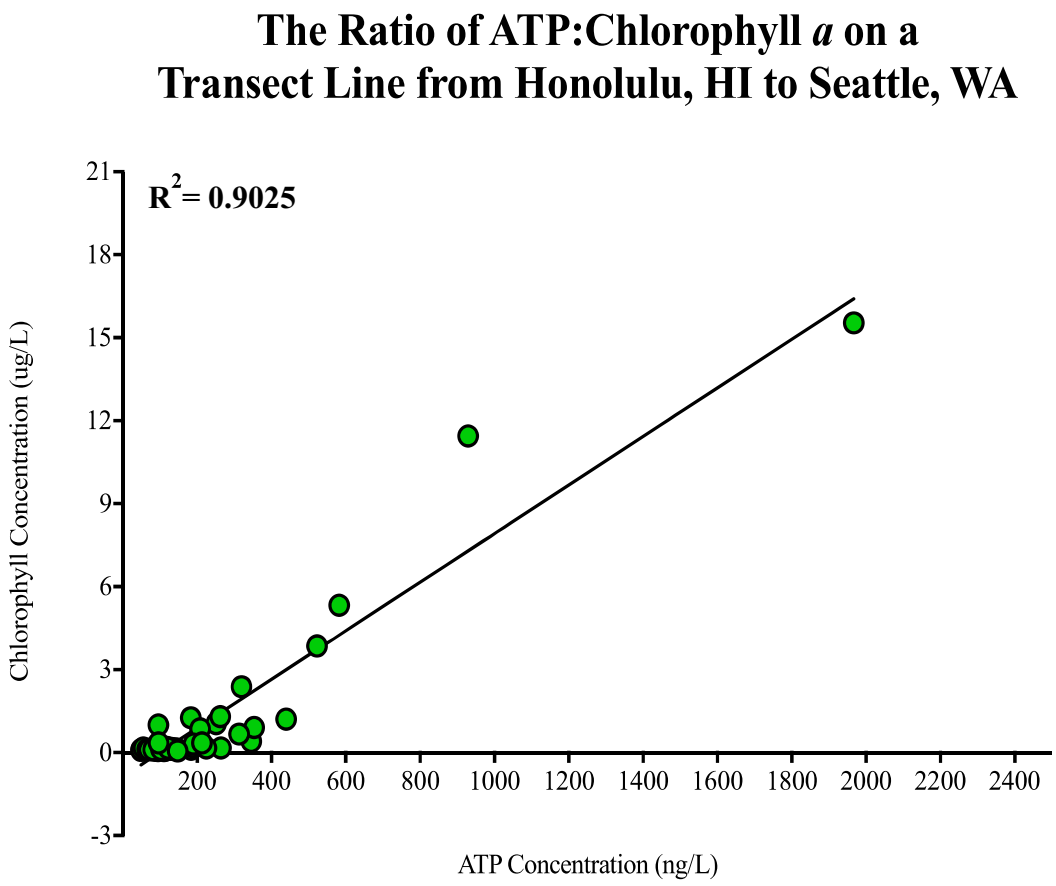

Figure 29. Ratio of ATP concentrations (ng/L) to chlorophyll *a* (µg/L) on a transect line form Honolulu, HI to Seattle, WA.

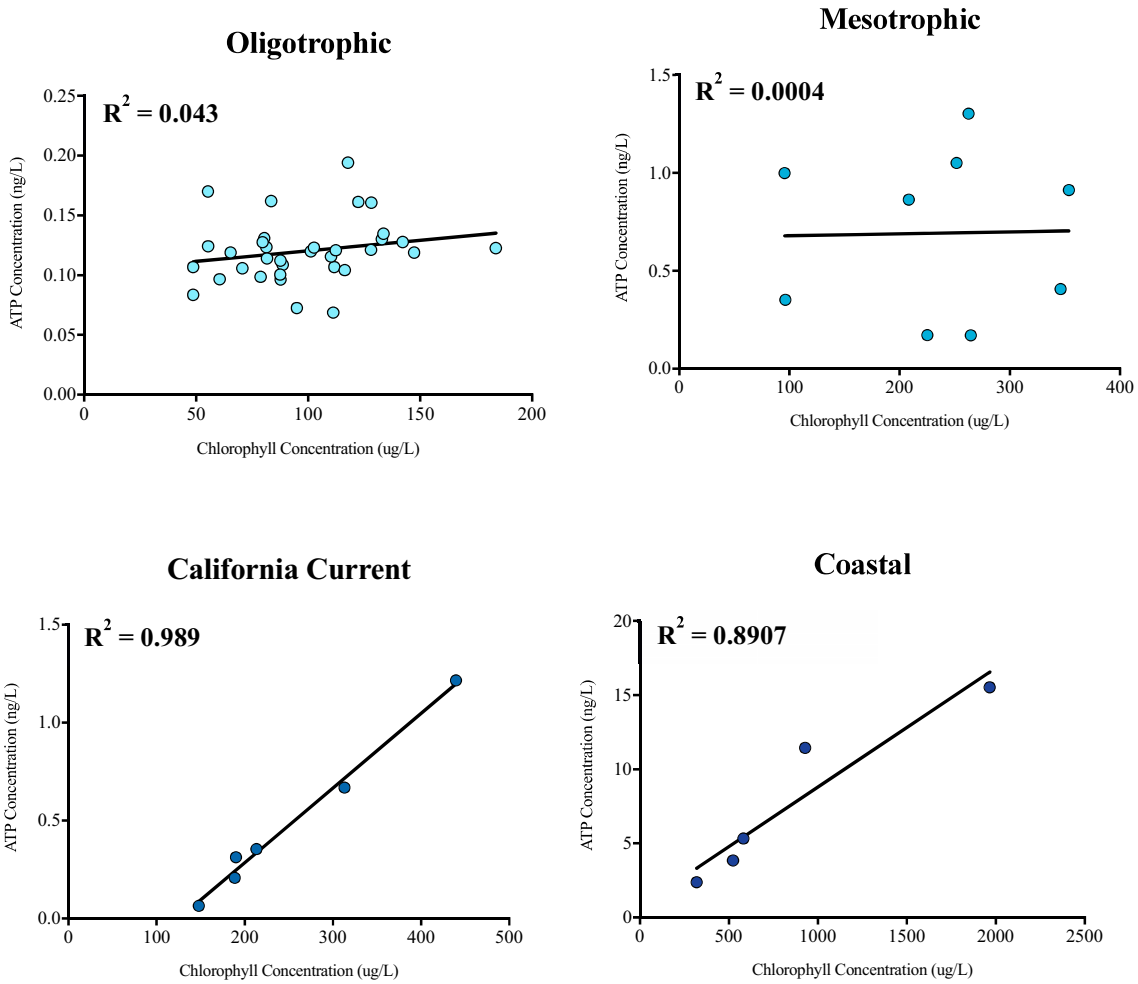

Figure 30. Ratio of ATP concentrations (ng/L) to chlorophyll *a* (µg/L) for four different water masses on a transect line form Honolulu, HI to Seattle, WA.

In addition to measuring ATP and chlorophyll *a*, bulk FDA was measured along the transect line as an additional method of measuring cell-specific phytoplankton viability *(Fig. 31)*. Trends in ATP concentrations and chlorophyll *a* was also observed in bulk FDA data. Higher concentrations of biomass dominated coastal regions, which was to be expected in upwelling or coastal regions that have higher nutrient concentrations. This can also be seen in Figure 32, displaying the concentration of total nitrogen and phosphate along the transect line. Lower phosphate and nitrogen concentrations in clear, oligotrophic waters were seen with a sharp increase in concentrations of both nutrients towards eutrophic, coastal waters *(Fig. 32)*.

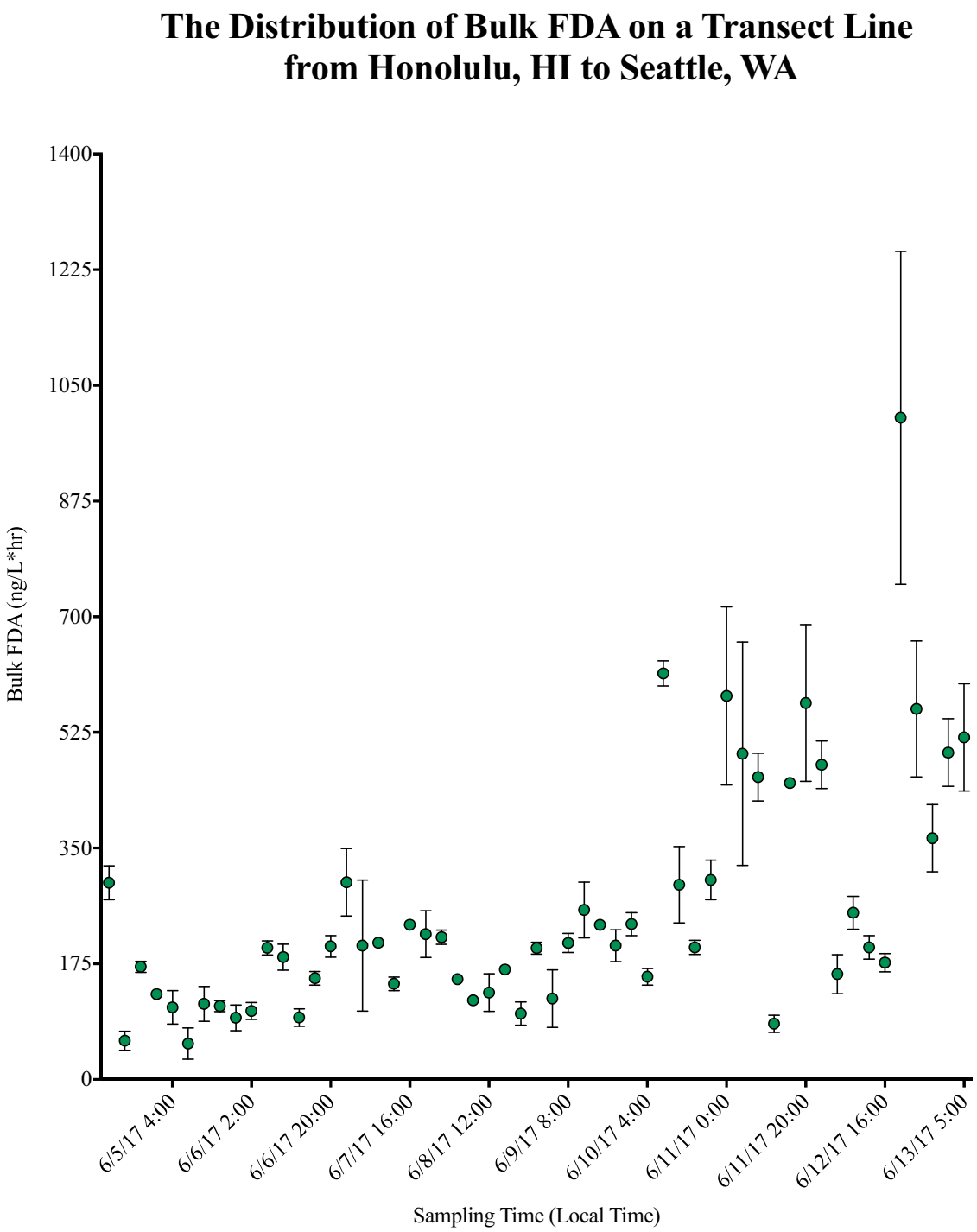

Figure 31. Bulk FDA concentration (ng/L*hr) shown in triplicates at each sampling location on transect line from Honolulu, HI to Seattle, WA.

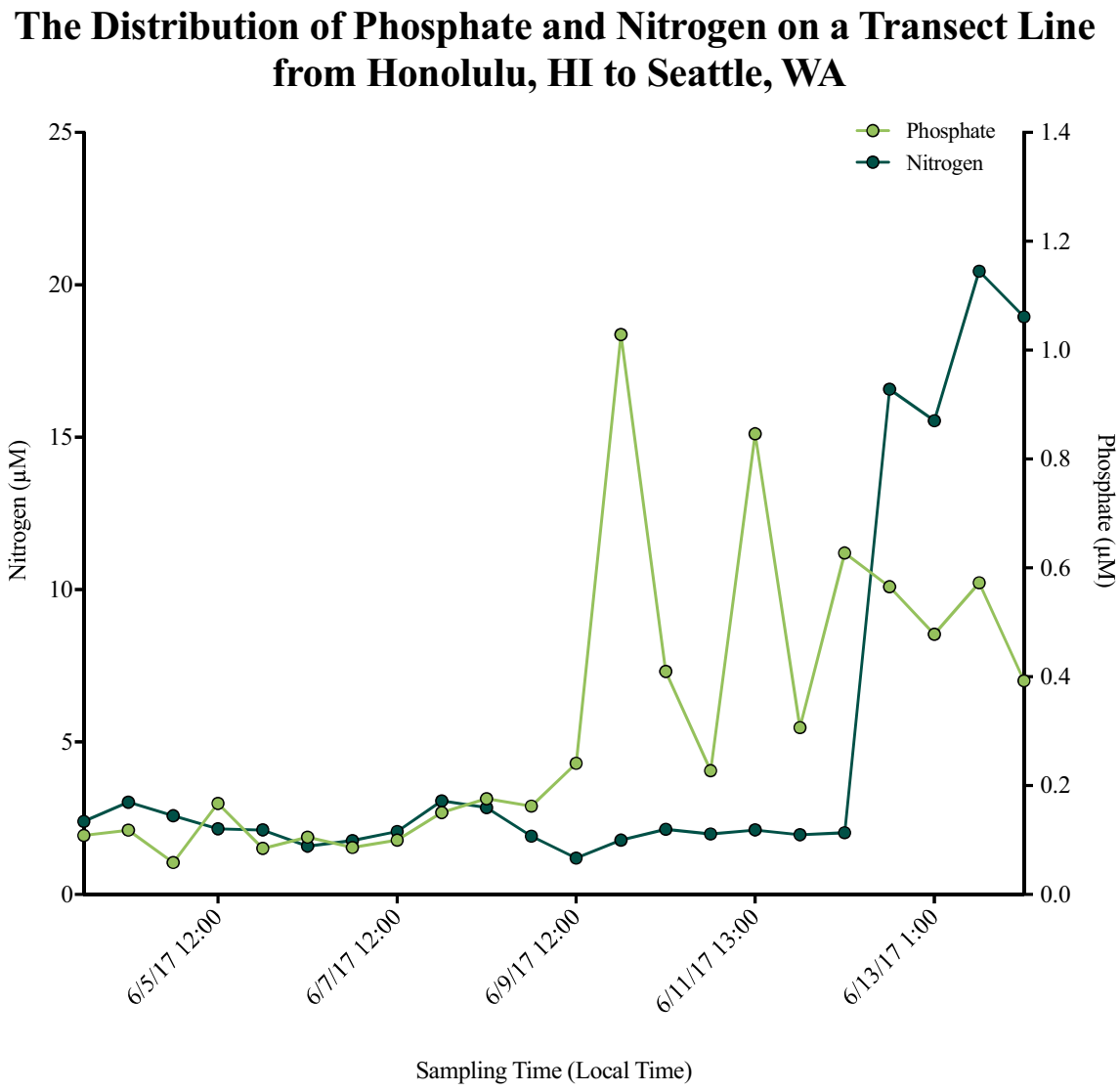

Figure 32. Distribution of total nitrogen (µM) on the left y-axis and phosphate (µM) on the right y-axis and sampling time (local time) displayed on the x-axis over the transect line.

DISCUSSION

In order to effectively use ATP as an accurate proxy of biomass in biological oceanography, the assumptions made by Holm-Hansen and Booth (1966) must remain true under all oceanic conditions, including the conclusion that dead cells rapidly lose ATP. Based on the research that was presented in this study, ATP was demonstrated as an appropriate method of quantifying microbial biomass undergoing necrosis. The results suggested that species, time, type of disinfection method, concentration and dosages influence ATP and should be considered when using ATP as a representation of biomass.

Quantifying ATP in Phytoplankton Cultures

Three different methods of achieving cell death; glutaraldehyde, chlorination and heat, all yielded comparable yet different results. Glutaraldehyde was not as effective in terminating ATP signals in microbial and phytoplankton samples. Although an overall decreasing trend in ATP concentration was observed in these experiments, as shown in Figures 8-12, ATP concentrations did not reach near zero. This can be explained by the characteristics of how glutaraldehyde interactions with the cell. Glutaraldehyde has the ability to effectively act as a cell preserver by cross-linking proteins and therefore maintaining cell membrane and form (Migneault *et al.*, 2004). For this reason, glutaraldehyde is often used when analyzing samples in microscopy. Glutaraldehyde did not break down microbial cells in this study but rather retained ATP and preserved cells possibly explaining the reduced significance of glutaraldehyde treatments compared to chlorination and heat treatments.

The effects of chlorination on ATP content varied by species and concentration. *Thalassiosira weissflogii*, *Amphidinium carterae* and *Dunaliella salina* all experienced significant decreases in ATP concentrations in both 6 ppm and 12 ppm concentrations for both ATP and chlorophyll *a* as seen in Figures 13-15 and 17-19. *Isochrysis galbana* had different results from the three other species and showed that the ATP per cell in treated samples was not significantly different from the control for both 6 ppm and 12 ppm treatments. Chlorination acts as an oxidizing agent when introduced in a water sample

and disinfects by breaking down cell membranes which ultimately destroys the cells (Werschkun *et al.*, 2014). Bleach acts as a disinfectant when used in appropriate dose levels relative to biomass standing crops. Therefore, samples that have higher biomass must be accounted for by adding additional bleach into the sample.

Results from the heat experiment suggested that this method was best for extensively reducing ATP signals in both microbial communities and phytoplankton samples as well as driving the ATP signal to near zero. Even for *Isochrysis galbana* samples, where cell counts were near 8-fold higher than the average of *Thalassiosira weissflogii*, *Amphidinium carterae* and *Dunaliella salina*, a 33-fold decrease was observed after 15 minutes of heat and ATP per cell reached near zero after 30 minutes *(Fig. 25)*. Heat was appropriate for deminishing high concentrations of biomass because heat energy was applied continuously and was not consumed by biomass as would be seen in chlorination techniques. As a result, heat worked as the best method of driving ATP signals to near zero in as little as 30 minutes for both microbial and phytoplankton samples. Chlorination was the second best method of achieving cell death, with the caveat that biomass needs to be considered when using this method. Glutaraldehyde works better as a preservative of cells rather than destroying cells and therefore was least effective in both achieving cell death and driving ATP signals to near zero.

Detecting ATP Diel Variability in Oligotrophic Waters

The results from the field experiments yielded interesting outcomes. It is clear from all figures that the sampling water drastically changed when navigating from clear, warm oligotrophic waters of Hawaii to cold, nutrient-rich waters of Washington. As seen in figure 27 and 28, ATP and chlorophyll *a*, both proxies of total living biomass and autotrophic biomass respectively, start to increase and vary together when sampling locations change from oligotrophic water to eutrophic regions.

The goal of the field study was to determine if diel variation of ATP could be observed in oligotrophic environments where picoplankton primarily dominates *(Fig. 27)*. The results showed that ATP concentration is highest at midnight and lowest at noon. This suggests that either diel cycle fluctuations of higher biomass at night or a

potential cycle of production and consumption (degradation) of living biomass operates on a daily basis and is tracked by ATP levels in-situ. Previous research in the subtropical Pacific gyre (Ribalet et. al. 2015), in a similar sampling location compared to the transect line studied here from Honolulu to Seattle, detected similar findings: a day and night pattern was observed in morality rates, production rates and cell abundances of *Prochlorococcus*. It was shown that the primary dominant marine cyanobacteria had cell production and morality rates that were tightly synchronized to the day/night cycle across the subtropical Pacific Ocean with essentially no cell mortality observed during daylight hours (Ribalet *et al.*, 2015). In addition, the data showed *Prochlorococcus* cell division was impacted by temperature, in warm waters (>19° C) cell numbers were tightly coupled to day/night cycle and a net increase in abundance during the day and a net decrease at night. Therefore, regardless of grazing or viral lysis, the day/night cycle restricts *Procholorococcus* mortality, either directly or indirectly, and allows abundance to recover each day and maintain a stable population in open oceans (Ribalet *et al.*, 2015). This finding is consistent with the observation reported here that ATP concentrations in the Pacific sub-tropical gyres were highest in concentration at midnight and lowest at noon.

As previously mentioned, chlorophyll *a* is often used a method to measure phytoplankton biomass in oceanography. Therefore, in order to observe how well ATP measurements correlate with chlorophyll *a* measurements, the ratio between the two measurements were compared as shown in Figure 29. The correlation coefficient between the two methods was 0.9025, implying that the two methods were highly correlated when measuring water samples on the transect line. Furthermore, when dissecting the data into four different water masses; oligotrophic, mesotrophic, California current and coastal waters, the correlation coefficient varied. Waters that had higher biomass, California current and coastal waters, had a higher correlation coefficient then waters with lower biomass, oligotrophic and mesotrophic. This is likely due to the large changes in total living biomass and chlorophyll biomass that were observed in the coastal regions of this study. Overall, the field results show that ATP seems to be a good proxy of biomass, especially in regions with higher abundances of micro-organisms.

CONCLUSION

Through a series of field and laboratory experiments and observations, ATP has been shown to quickly dissipate in composite water samples after cell death. From a ballast water treatment perspective, heat was shown to be the most effective for lab and field collected phytoplankton sterilization, quickly driving ATP signals to near zero in approximately 30 minutes. Whereas chlorination and glutaraldehyde were also effective in lowering ATP signals, these two treatments must be considered with the following caveats. Chlorination is most effective when cell numbers or biomass are approximately known in a sample and if addition bleach may be added throughout the experiment to compensate for the bleach degraded upon oxidation of organic matter. As a preservative, glutaraldehyde was least effective in both achieving cell death and degrading ATP. Diel variability was detected in oligotrophic regions implying that grazing pressures may balance autotrophic growth in these regions, leading to little new production. ATP signals were shown to be highest at midnight when both grazers and phytoplankton were present in the surface waters, rather than noon representing the net accumulation of new living biomass through the diel cycle.

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