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Comparing growth rates of natural phytoplankton communities to laboratory culturing experiments

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NONTECHNICAL SUMMARY

With the rise of atmospheric carbon dioxide, understanding the various facets of the carbon cycles through global systems is critical. In temperate coastal systems, the dominant phytoplankton group, diatoms, play a key role in the carbon cycle by sequestering carbon from the atmosphere through photosynthesis and transporting that carbon into deep ocean sediments as they die or are consumed and excreted. Many scientists turn to laboratory culturing experiments to make predictions about the responses of phytoplankton, like diatoms, to changes in their environment. But do these laboratory studies serve as realistic proxies for environmental responses? This study compared growth rates of phytoplankton communities from Trevor Channel within Barkley Sound, British Columbia using field and laboratory experiments. Field experiments were comprised of in situ community growth rates measured using diffusion chambers, in addition to classic shipboard incubations measuring the community growth rate in response to added nutrients. Field samples were used to isolate the dominant diatom species into a mixed-species culture for laboratory experiments. An analogous culture using laboratory isolates—of the same genera, obtained from a culture collection—was created, and the two cultures were kept in a semi-continuous batch culture experiment tracking the growth rates in response to increased nutrients. Comparisons of the growth rates among the in situ, shipboard, and laboratory experiments indicated how growth rates differ between natural phytoplankton assemblages and laboratory cultures. In the field, growth rates were calculated using changes in the concentration of the photosynthetic pigment chlorophyll *a*, a standard proxy for cell densities. Growth rates were significantly higher in the incubations than in the diffusion chambers, and in turn, the laboratory cultures had significantly higher growth rates than the incubations. Light levels, and not nutrient concentrations, appear to have influenced growth rates in the diffusion chamber and incubation experiments. In the laboratory experiment, growth rates did not differ between laboratory and environmental strains or between high and low nutrients. Higher temperatures in the laboratory incubations may have induced growth rates that were higher than in the field experiments. The results indicate that above a minimum threshold concentration of nutrients, other parameters like light exposure and temperature have a greater influence on growth. Furthermore, similarities between environmental and laboratory isolate growth rates in culture implies laboratory cultures can be treated as reasonable proxies of environmental communities.

ABSTRACT

In a world facing ever-increasing atmospheric carbon dioxide, understanding the various facets of the carbon cycle is critical. In temperate coastal systems, diatoms play a key role in the carbon cycle by contributing to the biological pump that sequesters carbon into deep ocean sediments. Many scientists use culturing experiments to make predictions about the responses of phytoplankton to environmental

changes. But do these highly controlled laboratory studies serve as realistic proxies for environmental responses? This study investigated growth rates of phytoplankton communities from Trevor Channel within Barkley Sound, British Columbia using field and laboratory experiments. Field experiments were two-fold: 1) in situ baseline community growth rates measured using diffusion chambers and 2) shipboard incubations that measured the community growth rate in treatments without added nitrate and nitrate added to 50 μmol L^{-1} concentrations. Representatives of two of the most dominant diatom genera in the field samples, *Thalassiosira* and *Skeletonema*, were isolated in laboratory experiments. Analogous cultures of *Thalassiosira* and *Skeletonema* were created from isolates that have been kept in laboratory conditions for many generations. The two culture types were kept in a semi-continuous batch culture experiment tracking the growth rates between 50 μmol L^{-1} nitrate and 100 μmol L^{-1} nitrate concentrations. Growth rates differed significantly between the three experiments by 0.1-0.2 day^{-1}. No significant differences in growth rates between the control and high nitrate treatments in the incubation imply the systems were not nitrate limited. The similar growth rates in the laboratory experiments imply that diatom isolates maintained in culture for generations accurately reflect the physiology of more recent field isolates.

In the face of increasing levels of atmospheric carbon dioxide, understanding the role of phytoplankton within global biogeochemical cycles has become increasingly important. Phytoplankton are primary producers, which means they photosynthesize and use carbon dioxide to make simple sugars, and as a result of that process release oxygen. These photosynthetic organisms form the base of the food chain and feed the zooplankton that are, in turn, consumed by higher trophic level organisms like fish. One of the most important functions of marine phytoplankton in geochemical cycling concerns the biological pump. Phytoplankton incorporate atmospheric carbon into their cells, and then either die and sink to the sea floor or are eaten by other organisms, removing that atmospheric carbon from the ocean-atmosphere system. While phytoplankton communities around the world have diverse and variable assemblages, the most prevalent group in temperate, coastal systems are the diatoms (Taylor and Haigh 1996, Forbes et al. 1990).

Diatoms are the most diverse group of phytoplankton, found either as solitary cells or in chains, and can be as small as a few micrometers or as large as a few millimeters (Armbrust 2009). Diatoms arose in the Triassic period (Sorhannus 2007) as the result of a union between a cyanobacterium and a eukaryotic heterotroph (Yoon et al. 2004). Now, diatoms occur in oceans around the world and are primary contributors to oceanic primary productivity. A study by Moriceau et al. (2009) asserted that diatoms alone contribute as much as 40% of the organic matter exported to depth. An earlier study by Nelson et al. (1995) estimated 35-75% of the total primary production in coastal systems was attributable to diatoms. Diatoms grow best in relatively nutrient-rich waters, which tend to occur in coastal areas in temperate and polar latitudes, where offshore upwelling and terrestrial runoff create waters higher in nutrients than tropical or open ocean oligotrophic regimes (Thomas et al. 2004). Diatom growth rates are dictated by the interplay between biotic and abiotic controls like ambient temperatures (Montagnes et al. 2003), irradiance levels, nutrient concentrations (Goldman et al. 1979), and predation by zooplanktonic grazers, heterotrophic bacteria, and viruses (Calbet and Landry 2004, Tijdens et al. 2008).

To understand the effect of bottom-up controls on diatom growth, this study compared phytoplankton growth rates in three experiments: in situ diffusion chambers, shipboard incubations, and laboratory cultures. The field component took place 26 January through 2 February 2013 in Trevor Channel within Barkley Sound, Canada and was conducted aboard the *R/V Thomas G. Thompson* through the University of Washington's School of Oceanography. Laboratory experiments continued through May 2013 in the Armbrust Lab of the Center for Environmental Genomics at the University of Washington.

Barkley Sound is an embayed, fjord-type estuary on the southwestern coast of Vancouver Island, British Columbia, Canada (Fig. 1). Trevor Channel is the body of water in the southeastern

section of Barkley Sound, bordered to the south by the coastline of Vancouver Island and to the north by the Broken Islands Group (Fig. 1). A shift in prevailing winds during the summer produces coastal upwelling of nutrient-rich deep waters (Harris et al. 2009). However, this upwelling only occurs for a few months of the year, and the surface waters of the sound are typically nitrate depleted, but have adequate concentrations of phosphate and silica (Taylor and Haigh 1996). Previous studies by Lutz, Moreno, and Rombeau in 2010 assessed phytoplankton community structure and primary productivity in Trevor Channel. The diatom genera most prevalent in March 2010 were *Skeletonema* and *Thalassiosira*, with *Cylindrotheca*, *Asterionellopsis*, *Pseudo-nitzchia*, *Chaetoceros*, and *Thalassionema* present but less abundant (Rombeau 2010). To date, no studies have compared differences in growth rates between environmental and laboratory conditions in this location.

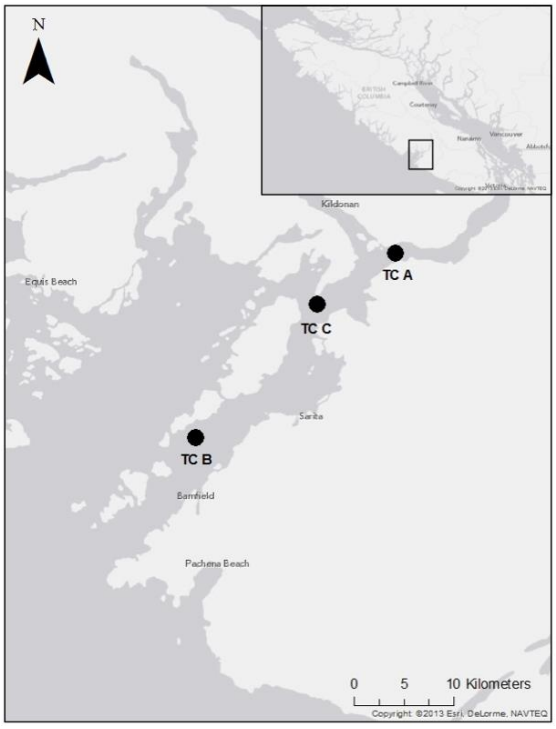

Figure 1. Map of Barkley Sound showing the study locations in Trevor Channel.

The lower cost and relative ease, convenience, accessibility, and controllability of laboratory experiments—compared to in situ growth experiments—have made laboratory studies standard practice in phycology and oceanography (Garcés and Masó 2001). However, predictions of the responses of whole phytoplankton communities to changes in the environment are often made based off controlled and inherently artificial laboratory experiments. A paper by Furnas (1990) stipulated that laboratory measured growth rates varied insignificantly compared to in situ growth rates, but later contended that there is little corroboration between mathematical community growth models based on laboratory experiments and rates measured in situ. To address this issue, complimentary field and laboratory growth experiments were implemented in this study.

Chlorophyll *a* and fluorescence analysis were used to determine and compare growth rates for the in situ, incubation, and culturing experiments. Chlorophyll *a* and fluorescence increase and decrease proportionally to cell numbers, and as such provide a measure of net growth that takes into account cell death mortality (Garcés and Masó 2001).

Due to higher temperatures and more stable conditions in the laboratory, the laboratory experiments were expected to yield higher growth rates than the community field experiments. The results of this study can be used in future studies of productivity in Trevor Channel, and the methods could be applied in other regions to perform similar calibrations between the laboratory and the field.

METHODS

Both field and laboratory experiments were conducted to compare the growth rate response between the natural phytoplankton assemblage and laboratory-acclimated test cultures. In the field, baseline community growth was measured using in situ diffusion chambers at three sites in Trevor Channel (Fig. 1): TC A (48°N 58.77', 124°W 57.61'), TC B (48°N 52.15', 125°W 08.68'), and TC C (48°N 56.80', 125°W 01.91'). Shipboard incubations were also used to assess community growth rate in response to added nitrate. The study occupied three stations close to previous study sites that had also examined phytoplankton community structure (Lutz 2010, Moreno 2010, and Rombeau 2010).

The stations were designed to be close to the 2010 study locations, but outside of the shipping lanes in order to safely conduct the diffusion chamber experiments.

Basic environmental properties including temperature, salinity, chlorophyll *a*, and nutrients were measured at each station using a Seabird 911Plus CTD. The CTD casts were also used to collect water at 5 m for the diffusion chambers, incubation experiments, initial chlorophyll *a* measurements, and nutrient samples. Water for the diffusion chamber and incubation experiments was drained from the Niskin bottles on the CTD apparatus into a 10 L carboy—a hose with 53 µm Nitex mesh on the outflow end was used during draining to remove larger heterotrophs like zooplankton while retaining the larger diatom species.

Additional water samples were collected at 60 m, 30 m, 25 m, 20 m, 15 m, 10 m and the surface for higher resolution nutrient profiles. Nutrients were analyzed at the University of Oceanography Marine Chemistry Laboratory with a Technicon AAII system following the World Ocean Circulation Experiment method (Gordon et al. 1993). Nutrient samples were prepared by filtering 50 mL through a 0.20 µm syringe filter and freezing the samples at -20°C for storage. To measure chlorophyll *a* concentrations, 136 mL of sea water were filtered through a 0.7 µm pore size Whatman GF/F filter, according to an adapted version of the Mantoura and Llewllyn (1983) method combined with the fluorometric method developed by Lorenzen (1966) using a Turner Designs 700 fluorometer.

The equations used to calculate growth rates were:

Equation 1.

$$[\text{Chl } a] = \frac{\frac{F_0}{F_{a\,max}} \Big/ \frac{F_0}{F_{a\,max} - 1} \times \mathrm{K}_x \times (F_0 - F_a)}{\text{Volume of sample filtered (in L)}}$$

Where [Chl a] is the amount of chlorophyll a in the sample in µg L^{-1}; $F_0/F_{a\ max}$ was 2.11131 and is the ratio between the initial and final chlorophyll reading on the fluorometer for a sample with only chlorophyll a and no phaeopigments; and K_x was 0.1011524 and is a calibration coefficient specific to the fluorometer.

Equation 2.

$$\mu = \frac{\ln([\text{Chl } a]_f) - \ln([\text{Chl } a]_0)}{t_f - t_0}$$

Where µ= specific growth rate per day (day^{-1}); $[\text{Chl a}]_0$ is the initial chlorophyll a value and $[\text{Chl a}]_f$ is the final chlorophyll a value; and $t_f - t_0$ is the time spent in situ or in the incubations.

Net tows were also collected at each station, and used to isolate two of the predominant diatom genera, *Thalassiosira* and *Skeletonema,* for comparative laboratory experiments.

Diffusion Chambers

Diffusion chambers are a modified form of in situ bottle incubations (Furnas 1991, Fig. 2) that allow chemical contact with the surrounding water but exclude larger grazers. Diffusion chambers allow for baseline community growth rate measurements with minimized predation, and thus mortality. In this experiment, 500 mL clear polycarbonate wide-mouth jars with the lid cut out and replaced with 20 µm Nitex mesh were moored at a depth of 5 m at each of the stations—to provide consistency and continuity with the 2010 studies in the absence of an observed chlorophyll maximum (Moreno 2010, Rombeau 2010). Each station had three diffusion chambers as well as a fourth control jar filled with 53 µm pre-filtered water samples but retaining the original non-permeable lid. Pre-filtered water from the storage carboy was divided into the corresponding diffusion chambers and the chamber mooring deployed over the side of the work vessel the *Welander*. After being in place for 107 hours (4.5 days) at TC A, 130 hours (5.4 days) at TC B, and 137 hours (5.7 days) at TC C, the diffusion chambers were collected and the contents filtered for chlorophyll *a* analysis. One sample was collected from each diffusion chamber while three subsamples were collected from the control jar for statistical analysis. Water collection and chamber deployment for station TC A were postponed because small boat operations were limited to day light hours, resulting in a shorter deployment time for these samples. Comparing initial and final chlorophyll *a* levels gave a community growth rate after grazing pressure was reduced for the diffusion chamber experiment. An Onset

Computing UA-002-64 HOBO data logger pendant was attached to the suspension apparatus to measure in situ light levels. The diffusion chambers were expected to exhibit growth rates comparable to the shipboard incubation control bottles (see below).

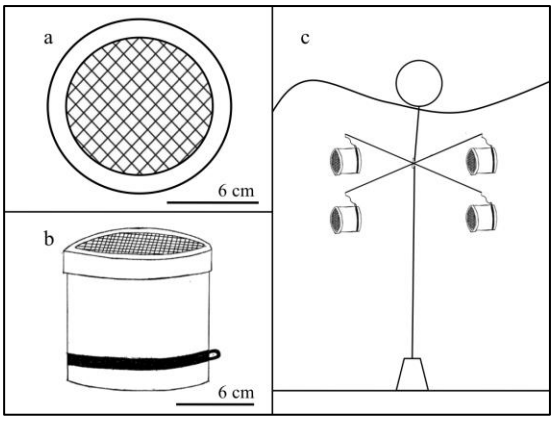

Figure 2. Schematic of diffusion chamber design: a) top view of a chamber; b) side view of a chamber (cross-hatching represents 20 μm mesh, dark band is the hose-clamp attachment); and c) mooring set up (not to scale) with buoy, anchor, and suspension apparatus at 5 m.

Shipboard Incubations

Shipboard incubations are common practice for measuring community growth rates in response to changes in the environment (Liu et al. 1995, Hurst and Bruland 2007)—for example light limitation, nutrient addition or limitation, etc. This study conducted a nutrient addition experiment. Pre-filtered water samples (see above) from each station were partitioned into 500 mL polycarbonate bottles (Fig. 3). Each station had triplicate controls—no added nutrients—and triplicate test bottles with sodium nitrate added at a final concentration of 50 μmol L^{-1}. The bottles were placed in an acrylic water-bath tub with water pulled from the water-intake pump on the *R/V Thompson* that draws in surface water and kept the bottles at the same temperature as the sound. To simulate the appropriate light levels, the water bath was under a mesh cover that allowed 50% light penetration (Fig. 3). Light levels were also measured by a HOBO H21-001 Data Logger Weather Station from Onset Computing, equipped with the S-LIA-M003 PAR (photosynthetically-active radiation) sensor, placed next to the incubation tank. An initial chlorophyll *a* sample was taken at time zero, and then each sample was measured for chlorophyll *a* concentrations after an incubation period of 72 hours (3 days) for TC A samples, 83 hours (3.5 days) for TC B samples, and 88 hours (3.7 days) for TC C samples. Growth rates were calculated by comparing the initial and final chlorophyll *a* measurements to one another (Equations 1 and 2). Time spent in the water bath varied because of the delayed sampling of station TC A (see above). Because nitrate was being added at the elevated concentrations typical of summer conditions (Taylor and Haigh 1996, Harris et al. 2009), the nitrate-added bottles were expected to exhibit higher growth rates than the control bottles.

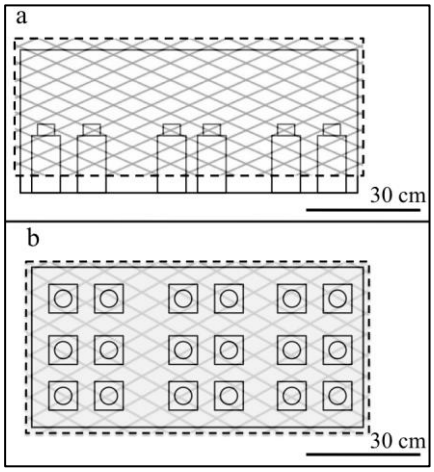

Figure 3. Schematic of shipboard incubation experiment: a) side view of incubation tank with bottles and b) top view of incubation tank with bottles depicting triplicate controls and nitrate additions for the three stations. Cross-hatching represents the light-blocking mesh. Flow-through hose connection not depicted.

Laboratory Experiments

The final aspect of the project was a semi-continuous batch culture experiment (Baird et al. 2001) comparing wild and laboratory-derived mixed-genera diatom cultures. This component of the study was conducted in the Center for Environment Genomics within the University of Washington School of Oceanography in collaboration with the Armbrust laboratory group. Net tows with 20 μm mesh were collected following diffusion chamber deployment at a depth of 5 m for 5 minutes at 0.5-1 knots at the

three stations. The samples collected were be used to establish Trevor Channel representative isolates for the laboratory experiments. A standard dilution technique (Berges et al. 2001) was employed to isolate five of the most predominant diatom species into culture flasks filled with f/2 media (Guillard and Ryther 1962, Guillard 1975) and 0.2 µm filtered seawater collected from Trevor Channel (see below). The two surviving isolates, *Thalassiosira* and *Skeletonema*, were then used for laboratory experiments.

The two field isolates as well as analogous laboratory strains of the same genera were kept separately in glass culture tubes filled with 25 mL of modified f/2 culture media. The laboratory *Thalassiosira* isolate originated from the CCMP strain # 3367 and the laboratory *Skeletonema* isolate from the CCMP strain # 780. The f/2 media was made using seawater collected in Trevor Channel, with added nutrient concentrations of 50 µmol L^{-1} sodium nitrate, 4.1 nmol L^{-1} sodium phosphate, and 12.0 nmol L^{-1} silicic acid. The nitrate concentration was scaled to a 1:18th proportion of the original f/2 recipe to match the incubation concentration. The other nutrient concentrations, trace metals, and vitamins were scaled to 1:9th of the original f/2 recipe to ensure adequate concentrations of all other nutrients. The cultures were kept at 13°C on a 12 hour light-dark cycle at light levels that were a best approximation of light levels experienced in the shipboard incubations and were measured using Onset Computing UA-002-64 HOBO data loggers. Light levels were achieved by wrapping the tube racks in light-blocking films. Growth was monitored four times a week for six weeks using a Turner Designs model AU-10 fluorometer, to measure relative fluorescence units (RFU), and generate growth rate curves by taking the natural log of RFU (Fig. 4).

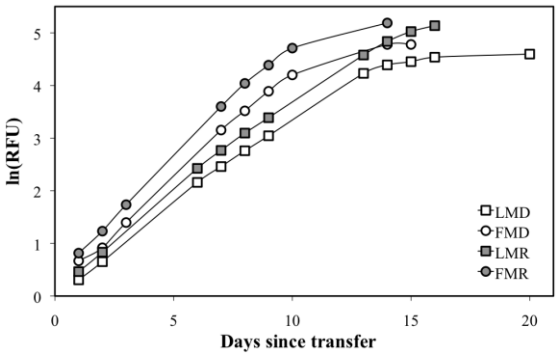

Figure 4. Example continuous growth curve. LMD and FMD refer to the laboratory and field-sourced cultures, respectively, in depleted nitrate f/2 media. LMR and FMR refer to the laboratory and field-sourced cultures, respectively, in replete nitrate f/2 media.

Triplicate cultures of each diatom strain were allowed to acclimate in the 50 µmol L^{-1} f/2 media for a period of 46-60 days until analysis of covariance (ANCOVA) of the growth rates showed F-distribution values equal to 0.0 or greater than 0.05, both among triplicate tubes and between successive generations. These F-distribution values indicate no significant difference between the growth curves being compared. An acclimation period was necessary to prevent stress in both the field and laboratory cultures, and to establish predictable growth rates.

Once acclimation was achieved, the individual diatom strains were combined into mixed-genera cultures to mimic a simple phytoplankton community. These mixed cultures were designated field-mixed and laboratory-mixed, depending on the source isolates. Controls strains were kept in the same media as during the acclimation period, designated as f/2 "depleted" media with 50 µmol L^{-1} nitrate concentrations. Experimental strains were cultured in filtered seawater f/2 media that doubled the nitrate concentration to 100 µmol L^{-1}, but maintained the concentrations of the other nutrients, designated as f/2 "replete" media. Nitrate levels were intentionally higher than would be expected in the natural environment to exaggerate physiological responses. Each strain—laboratory-mixed depleted, field-mixed depleted, laboratory-mixed replete, and field-mixed replete—was maintained in triplicate. Differences in growth rates between

the field-sourced and laboratory-sourced cultures could illustrate differences in adaptability to changes in nitrate concentrations. The replete nitrate conditions were expected to produce higher growth rates in both the laboratory and field mixed cultures. The laboratory cultures were expected to have a lower growth rate in the low nitrate condition compared to the field-sourced cultures because, historically, the laboratory isolates have been maintained at very high nitrate conditions (882 µmol L^{-1}).

The growth rates from the laboratory cultures, diffusion chambers, and incubation experiments were compared using the average of the triplicate measurements with standard errors to determine if the three methods exhibit significantly different growth rates.

RESULTS

Environmental Data

Water temperatures in Trevor Channel across all stations and depths remained consistent around 9-10°C. Salinity was similarly homogenous at 31 psu. Nutrient profiles showed similar values across all stations and depths (Fig. 5), with a total average nitrate concentration of 14.94 ± 2.84 µmol L^{-1} (mean ± standard deviation), a total phosphate concentration of 1.30 ± 0.20 µmol L^{-1}, and a total silicic acid concentration of 30.84 ± 3.47 µmol L^{-1}, resulting in a ratio of approximately 31 Si : 15 N : 1 P.

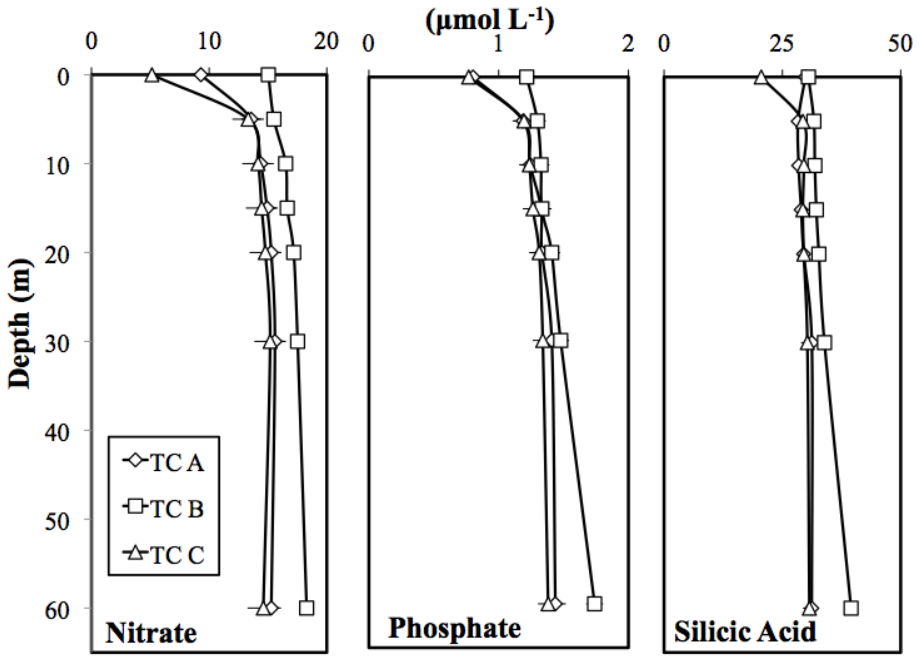

Figure 5. Depth profiles of nutrients, from left: nitrate, phosphate, and silicic acid. All values are in µmol L^{-1}.

Light levels in the incubation were 101.28 ± 64.03 µmol quanta, or converted to 7100 ± 4500 lm m^{-2} (mean ± standard deviation over 5 days). Malfunctions with the HOBO data loggers attached to the diffusion chambers prevented irradiance levels from being measured. Light levels in the laboratory incubations were unable to match those experienced in the incubation, and were more than an order of magnitude lower at 82.6 ± 4.3 lm m^{-2} (mean ± standard deviation).

Diffusion Chambers

Growth rates at station TC A were not significantly different between the control

chamber and the experimental chambers (Fig. 6), with a control growth rate of -0.08 ± 0.01 day^{-1} (mean ± standard error) and growth rates in the chambers of -0.09 ± 0.03 day^{-1}. TC B experienced growth rates in the control chamber of 0.08 ± 0.01 day^{-1}, and 0.10 ± 0.03 day^{-1} in the experimental chambers, which were not significantly different (Fig. 6). Growth rates at TC C were significantly different, with the controls growing at 0.02 ± 0.00 day^{-1} and the experimental chambers growing at 0.13 ± 0.02 day^{-1}; however, only two samples from the control chamber were analyzed for chlorophyll due to water availability issues. Growth rates averaged across all of Trevor Channel were not significantly different between the controls and the experimental chambers, with 0.00 ± 0.03 day^{-1} for the controls and 0.05 ± 0.04 day^{-1} for the experimental chambers.

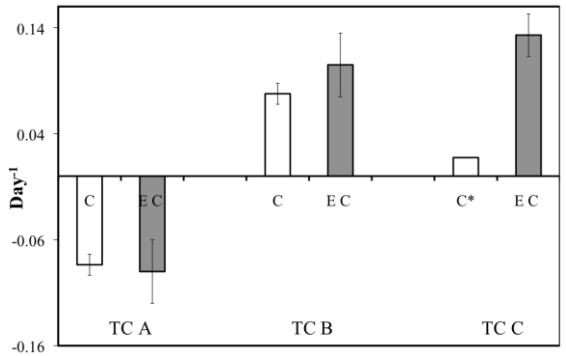

Figure 6. Histogram showing growth per day in the diffusion chambers at the three study sites. 'C' stands for 'Control' and 'E C' stands for 'Experimental Chamber'. The '' indicates where only two samples were processed because of limited water availability.*

Shipboard Incubations

Growth rates were highest for TC A incubated bottles; there were no significant differences between the control bottles, 0.23 ±0.01 day^{-1} (mean ± standard error), and the nitrate added bottles, 0.22 ± 0.01 day^{-1} (Fig. 7). Similarly, there was no significant difference between the control bottles for TC B and TC C (0.13 ± 0.01 day^{-1} and 0.12 ± 0.01 day^{-1}, respectively), and the nitrate added bottles (0.13 ± 0.01 day^{-1} and 0.12 ± 0.00 day^{-1}, respectively). Growth rates from stations TC B and TC C were not significantly different from each other, but were significantly different from the TC A growth rates. Incubation growth rates averaged for all of Trevor Channel showed no significant difference between treatments with controls experiencing growth of 0.16 ± 0.02 day^{-1} and nitrate added bottles growing at 0.16 ± 0.02 day^{-1}.

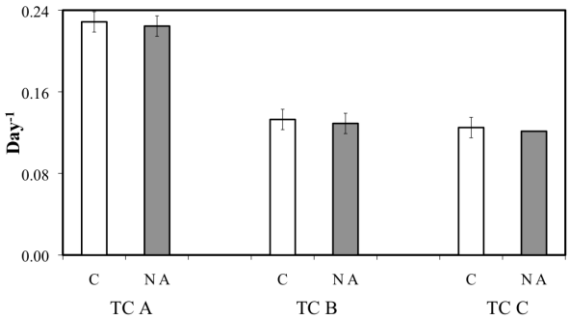

Figure 7. Histogram showing growth per day in the incubation experiment for the three study sites. 'C' stands for 'Control' and 'N A' stands for 'Nitrate Added'.

Laboratory Experiments

Growth rates in the control media (added nitrate concentration of 50 μmol L^{-1}) were not significantly different between the laboratory-sourced isolates—0.36 ± 0.06 day^{-1} (average ± standard deviation)—and the Trevor Channel-sourced isolates—0.34 ± 0.06 day^{-1} (Fig. 8). For the high nitrate media (100 μmol L^{-1}), growth rates were not significantly different between the laboratory isolate-sourced cultures—0.36 ± 0.06 day^{-1}—and the Trevor Channel isolate-sourced cultures—0.34 ± 0.06 day^{-1} (Fig. 8). Growth rates were also not significantly different between the control and high nitrate treatments for both the laboratory-sourced cultures and the field-sourced cultures.

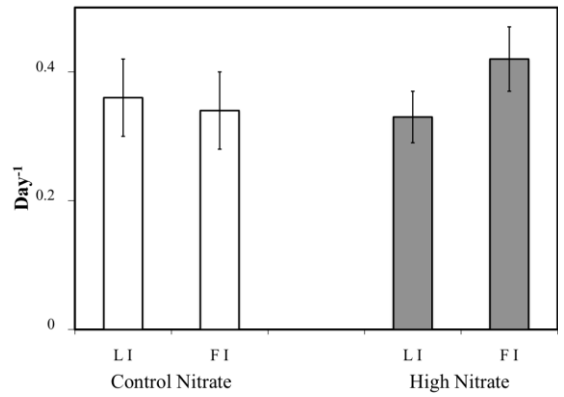

Figure 8. Histogram showing average growth per day in the laboratory culturing experiment. 'L I' stands for 'Laboratory Isolates' and 'F I' stands for 'Field Isolates'.

DISCUSSION

The Stability of the Water Column

Environmental parameters appear to be seasonally stable when compared to results from the Moreno and Rombeau studies conducted in March 2010. Observed temperature values were around 9°C in both this and the 2010 studies. The silica to nitrate to phosphate ratios (Si:N:P) were similar between the two years at approximately 31Si:15N:1P. While nitrogen and phosphorous were close to the Redfield ratio (16N:1P), silica values in both years were twice the Redfield ratio of 15Si:16N:1P (Lutz 2010). Silica values in Trevor Channel could be higher than the Redfield ratio because of high rainfall leading to high levels of runoff, or rocks in the surrounding watershed could be high in silica. The Redfield ratio is a stoichiometric minimum ratio of nutrients required for optimum growth (Goldman et al. 1979). Chlorophyll *a* concentrations from 5 m were also similar between studies, with Moreno and Rombeau measuring 0.37-1.74 μg L^{-1}, compared to 2013 levels ranging between 0.35-1.16 μg L^{-1}.

Water column properties are determined by physical processes like currents, tidal flushing, and freshwater runoff. These properties, especially nutrient concentrations and temperature, in turn affect phytoplankton growth as bottom-up controls. The similarities in conditions between years and seasons—evinced by similar values in temperature, nutrients, and chlorophyll *a* concentrations—indicate a relatively stable environment that is not nutrient limited.

Diffusion Chambers

The diffusion chambers measured intrinsic community growth rates in the surface waters. The growth rates in the diffusion chambers showed variability both between locations and treatments (Fig. 6). Negative growth rates in the TC A chambers signify net mortality, potentially caused by contamination with grazers, stress, or another unknown factor. Alternatively, these low growth rates could have resulted from procedural error if the samples were not properly shaken before chlorophyll *a* analysis. An environmental cause for low growth rates at station TC A is unlikely because environmental properties were similar at all three station—as seen in temperature and salinity values as well as nutrient levels (Fig. 5)—but stations TC B and TC C both exhibited positive growth (Fig. 6), indicating net community growth.

Control and experimental growth rates were not significantly different at TC B, indicating growth in the control chamber was not affected by nutrient exchange with the environment. Growth rates between TC B and TC C experimental chambers were not significantly different, implying the phytoplankton communities were similar. The growth rates were significantly different between the TC C control and experimental chambers, but, again, this could have been caused by contamination, procedural error, or grazing by microzooplankton or bacteria (which would have been too small to be filtered out). When all the stations were averaged together, the growth rates between the control and experimental chambers were not significantly different (Fig. 9). The similarities in growth rates in conjunction with observations from the phytoplankton net tow further indicated regional environmental homogeneity in the phytoplankton community. (Fig. 9)

Shipboard Incubations

Shipboard incubations were designed to yield community growth rates in response to a change in environmental conditions—in this study, the change was increasing nitrate concentrations. Across all the samples, there was no significant

difference in growth rates between the control and nitrate added bottles (Fig. 7), even though 50 μmol L^{-1}—more than twofold ambient levels of 14.94 ± 2.84 μmol L^{-1}—was added to emulate summertime nitrate levels in the treatment bottles (Taylor and Haigh 1996). Furthermore, when growth rates were averaged across all three stations, there was no significant difference between the control and nitrated added treatments (Fig. 9). Therefore, nitrate was not a limiting nutrient in Trevor Channel during the study period. Other factors like light availability or limitation by other nutrients may have dictated the growth patterns (Kruskopf and Flynn 2006). However, the silica ratios described earlier were in excess of Redfield ratio values and nitrogen and phosphorous were at Redfield ratio concentrations, indicating nutrient limitation was not contributing to differences in growth rates. The significantly higher growth rates in the TC A bottles could have multiple causes, for instance differences in handling the samples during chlorophyll *a* analysis or sampling during different phases in the growth cycles.

Comparing In Situ and Shipboard Experiments

Growth rates were significantly higher in the incubation treatments than in the diffusion chambers (Fig. 9). Since increased nitrate did not influence growth rates, some other physical parameter, such as light, may have accounted for the differences. Without the data logger data or useable PAR data from the CTD casts—the CTD packet did not have a functioning PAR sensor—direct evidence cannot support whether or not light levels were comparable between the incubations and the diffusion chamber deployment depths. However, personal observation indicated light penetration at 5 m depth was likely less than the 50% assumed for the incubation set up, which could have resulted in the measured higher growth rates in the incubations.

Laboratory Experiments

Observations during diatom species isolation from net tow samples indicated similar community structures as those observed by Moreno in 2010. *Skeletonema* and *Thalassiosira* species predominated in both years and were the only successful isolation attempts. *Cylindrotheca* was only a minor component of the 2010 community assemblage, but observations indicated higher abundances in this study. Unfortunately, the *Cylindrotheca* isolates did not survive the transition from the field into the laboratory. *Chaetoceros*, *Thalassionema,* and *Asterionellopsis* species were also observed in both studies, while *Pseudo-nitzchia* was absent from net tow samples, despite comprising a significant portion of the 2010 diatom community. Time limitations prevented quantifying the species abundance, but the general community structure appears to have remained consistent between the two studies.

Growth rates in the laboratory conditions were significantly higher than in the field studies (Fig. 9). With field nutrient levels already at Redfield ratios (see above), the nutrient-replete conditions of the laboratory media should not have produced the elevated growth rates. The temperature in the laboratory incubator, however, at 13 °C, was 4°C higher than in the field. Using Q_{10} parameters for various *Skeletonema* and *Thalassiosira* species from Montagnes et al. 2003, the elevated growth rates observed in the laboratory conditions could be accounted for by temperature-dependent enzyme activity. While the length of the photoperiod—16:8 hours light:dark in the laboratory compared to approximately 8:16 hours light:dark in the field—could also have contributed to increased growth, daytime irradiance in the field was two orders of magnitude higher than in the laboratory.

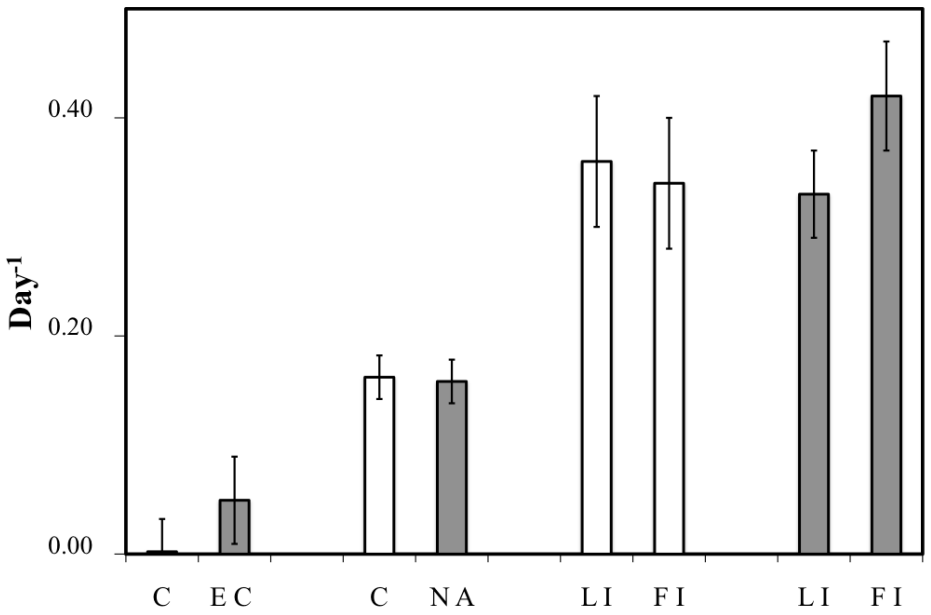

Figure 9. Histogram showing from left: average diffusion chamber growth rates for Trevor Channel, average incubation growth rates for Trevor Channel, control nitrate concentration growth rates for the laboratory experiments, and high nitrate concentration growth rates for the laboratory experiments. Acronyms are the same as in previous figures. Empty bars represent control conditions.

While nitrate levels of 100 μmol L-1 would be unreasonable to find in natural, coastal settings, comparing growth rates in the two nitrate conditions would speak to the adaptability of the phytoplankton communities. However, growth rates both between the 50 μmol L^{-1} control treatments and the 100 μmol L^{-1} experimental treatments were not significantly different for either the laboratory or environmental cultures (Fig. 8), signifying increases in nitrate above Redfield ratios do not increase growth rates. However, varying nitrate concentrations above the Redfield ratio value could affect diatoms' nitrogen uptake and storage processes (Lomas and Gilbert 2000). With the observed similarities in growth rates between the environmentally-sourced strains and the laboratory-sourced strains, it would appear laboratory isolates retain the basic growth rates they would have in the environment. Furthermore, the similarities in growth rates imply that laboratory-cultured species can serve as reasonable proxies for environmental communities so long as physical factors like temperature and light are taken into account.

Limitations and Further Studies

While this study serves as proof of concept for approaching growth rate studies from an integrated environmental and laboratory perspective, there were inherent limitations in implementation. For one, quantifying the community structure and conducting size-fractionated chlorophyll *a* analysis would have confirmed the assumption that diatoms were the dominant members of the phytoplankton community. Also, staggering CTD casts and water collection would have allowed for identification of the chlorophyll *a* maximum depth prior to water collection and diffusion chamber deployment. Furthermore, photosynthetically-active radiation levels could have been used to better simulate environmental light levels in the shipboard incubations.

CONCLUSIONS

This study compared laboratory growth rates to growth rates in environmental phytoplankton communities using a comparison of three experimental techniques: in situ diffusion chambers for baseline community growth rates with minimized grazing pressure; shipboard incubations for community growth rates in response to increased concentrations of an essential nutrient, nitrogen; and finally, laboratory cultures of mixed-species isolates to compare laboratory-cultured strains and newly-isolated environmental strains. Differences in growth rates were not influenced by the nitrate concentrations. Additionally, no differences were found in the growth of the new environmental isolates compared to the older laboratory strains. Furthermore, the differences observed between the laboratory and incubation experiments were reasonably accounted for by a relationship between increased temperature and increased growth rates.

While this study serves as a template for future studies investigating differences in phytoplankton community growth rates in different conditions, future studies could better resolve the role of nutrient concentrations, light levels, and temperature in altering growth rates. A priori studies could be conducted throughout the year to assess temporal variations in nutrient, temperature, and salinity values to better characterize the region. Additionally, studies of this nature could be repeated at different times of year to address potential seasonal variation in growth rates. Future studies could also manipulate other nutrients, light levels, and grazing pressure to identify other controls on growth. To that end, future studies could incorporate artificial seawater media to better control the concentrations and sources of nutrients. Finally, incorporating more members of the phytoplankton community would better approximate natural communities by simulating more complex intraspecific interactions.

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