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Grazing interactions between Oxyrrhis marina and Synechococcus strains grown in single nitrogen sources

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Grazing Interactions between *Oxyrrhis marina* and *Synechococcus* Strains Grown in Single Nitrogen Sources

By

Virginia Selz

Accepted in Partial Completion
Of the Requirements for the Degree
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MASTER'S THESIS

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Grazing Interactions between *Oxyrrhis marina* and *Synechococcus* Strains Grown in Single Nitrogen Sources

A Thesis
Presented to
The Faculty of
Western Washington University

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

By
Virginia Selz
June, 2010

ABSTRACT

The goal of this study was to assess the interaction between abiotic and biotic factors on diverse *Synechococcus* strains isolated from the coastal California Current (CC9311, CC9605, CC9902) and the oceanic Sargasso Sea (WH8102 and mutants: JMS40 and SIO7B). Previous research has demonstrated that abiotic factors, such as nutrient source or concentration, can alter cellular structure and chemistry. These cell characteristics in turn influence biotic factors such as predation by protozoan grazers. *Synechococcus* strains isolated from coastal and open ocean waters were grown to nitrogen (N) depletion in N-reduced medium. After reaching stationary phase, strains were transferred to media containing nitrate, ammonium, urea, proline, alanine, glycine, or glutamine to assess the growth rates for each strain on these individual N sources. Compared to growth rates prior to N-limited stationary phase, all strains increased their growth rate in the single N source media. *Synechococcus* strains appear to have diverse abilities to grow on a broad range of N sources; however, the pattern of N use was not related to coastal or oligotrophic clade association. The majority of strains showed maximal growth on glycine, rather than on nitrate, ammonium, or urea. However, coastal strain CC9902 and mutants of the Sargasso Sea strain WH8102 either did not grow on or were actively inhibited by several amino acids. Further analysis of cell size, shape, and carbon:nitrogen (C:N) ratios of N source-grown coastal strain CC9311 and oceanic strain WH8102 demonstrated that cell physiological and morphological characteristics, in addition to growth rates, varied among N sources within a strain, as well as between strains. Coastal strain CC9311 and oceanic strain WH8102 were used in 30-minute grazing experiments with the heterotrophic dinoflagellate *Oxyrrhis marina*. Overall, grazing on coastal strain CC9311 was consistently higher than grazing on open ocean strain WH8102. However, within each strain grazing behavior also varied depending on N sources for strain growth. Physiological and morphological analysis of prey, in concert with grazing experiments, suggested that N source alters prey morphology and physiology, and the predator *O. marina* responds to these cell alterations. While many characteristics such as C and N content, cell size, and cell shape were inter-related, grazing on coastal strain CC9311 was

strongly linked to cell shape (highest on more rounded cells) and C and N content (higher on cells with higher nutrient content). In contrast to coastal strain CC9311, few clear relationships could be discerned between ocean strain WH8102 N source-grown cell characteristics and the feeding behavior of the heterotrophic dinoflagellate, *O. marina*. While previous work has shown that *O. marina* readily eats coastal strain CC9311, this study showed *O. marina* grazing rate is also affected by prey growth condition, reflected in the physiology and morphology of the cell. Further studies expanding the breadth of protozoan predators and *Synechococcus* strains would aid in the understanding of the microzooplankton's role in top-down control of *Synechococcus* populations under different nutrient regimes and in more general issues of how resource use might affect predation.

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INTRODUCTION

Microbial Loop

The picophytoplankton community, responsible for 50-70% of total production in the global oceans, is largely comprised of 0.2-2 μm-sized cyanobacteria and picoeukaryotic cells. Because of their widespread distribution and often high abundance, picophytoplankton play important roles in the oceanic food web (Garrison, 2005). Understanding the abiotic and biotic factors that control their distribution and abundance enhances knowledge of their roles in energy transfer and biogeochemical cycles.

As photoautotrophs, picophytoplankton need light and nutrients to fuel primary production. The majority of picophytoplankton require nitrogen (N) and phosphorous (P) in a 16:1 molar ratio, as well as a suite of other nutrients at trace concentrations including iron, nickel, cobalt, and copper. Light and nutrient availability vary on unpredictable timescales in the pelagic environment. While light availability in the pelagic environment is controlled by environmental factors such as season, cloud cover, or particle concentrations in the water column, nutrients are chemically and biologically reactive, leading to temporal or spatial variation on very small scales. Nitrogen is of particular interest because it exists in many different forms, both organic [urea, amino acids, and other forms of dissolved organic N (DON)] and inorganic (nitrate, nitrite, ammonium). In addition, N is often limiting for growth (Zehr et al., 2002; Moore et al., 2008). The proportional distribution of N forms also varies between coastal versus oceanic sites. More productive coastal areas receive nutrients from terrestrial sources as well as from coastal upwelling experiencing an increase in concentration and variety of N source compared to the open ocean. Open ocean environments are generally oligotrophic and are dominated by recycled or regenerated N sources, such as ammonium. Understanding picophytoplankton's ability to access different N pools is important to elucidate the impact particular N sources have on picophytoplankton production in diverse environments.

While picophytoplankton must acquire nutrients, they must also avoid grazers and viruses to survive. Grazers (microzooplankton) and viruses constitute biotic controls on the picophytoplankton and their activity enhances the marine food web by recycling nutrients and relaying energy to larger zooplankton (Suttle, 2007). The methods used by marine viruses and grazers to select picoplankton (or, alternatively, that picoplankton use to avoid capture) are hypothesized to relate to the prey's size, motility, digestibility, and cell surface properties (Shannon et al., 2007). While marine viruses recognize prey cell surface sites and subsequently adhere to and diffuse into the cell (Xu, 1997), a grazer's recognition of the prey cell surface is less understood. A confounding factor in understanding predator-prey recognition is the diversity and magnitude of feeding mechanisms that exist within the microzooplankton and, one grazer may have several different feeding mechanisms. Furthermore, feeding mechanism is not the only factor that governs feeding selectivity. For example, two heterotrophic nano-flagellates (HNF) with different feeding mechanisms and phylogeny grew on similar strains out of 37 *Synechococcus* surveyed (Zwirglmaier, 2009). Therefore, it is necessary to understand multiple factors that govern selective feeding of grazers in order to predict the magnitude of top-down control for the different members of the picophytoplankton community.

Because abiotic and biotic factors influence a cell's ability to survive, understanding how nutrient source affects cell properties and how those may induce or inhibit grazing is a relevant ecological question. Autotrophs grown in different nutrient regimes can have vastly different nutritional qualities, represented in their carbon: nitrogen (C:N) ratio (Malzhan et al., 2010). Nutritional quality of prey is hypothesized to influence grazing preference. For instance, the grazer *Ochromonas danica* (a heterotrophic flagellate) ingested *Pseudomonas fluorescens* cells with lower C:N:P ratios (high quality) at higher rates than cells with higher C:N:P ratios (low quality) (Shannon et al., 2007). However, less is known about how information regarding the nutritional status of a prey cell is reflected by the prey's cell surface and if any cell surface modification attracts or deters grazers. Diverse prey types have been observed to produce "stress-specific proteins in specific

membrane or cell fractions" in the presence of different N source and concentrations (Wilhelm, 1995, Zinovieva, 1997), providing evidence that cells are altering structures that are potentially recognizable to a grazer. In one specific case, it was demonstrated that photoautotrophic *Isochrysis galbana* cells grown in N-deplete medium produced more mannose at their cell surface then N-replete cells (Martel, 2009). This is of particular interest to the heterotrophic dinoflagellate grazer, *Oxyrrhis marina*, which uses mannose-binding lectins to recognize its prey (Wootton et al., 2007). Because *O. marina* has been well studied and is known to be responsive to prey cell surface structures, it is an excellent grazer to use in the present study to elucidate interactions between nitrogen source demand and grazing pressure on picophytoplankton.

Synechococcus

A Model Genus of Picophytoplankton

Within the phylum cyanobacteria, marine *Synechococcus* strains fall into the genus-level taxon, Marine Cluster 5.1 (A). Cluster 5.1 (A) has been divided into 10 clades using the 16S rRNA genes of isolates; additional clades are discovered as sampling efforts expand. Each clade contains genetically distinct but closely related *Synechococcus* strains. Within a clade, groups of strains may be defined as ecotypes: strains that have similar physiological characteristics of ecological importance (Ahlgren, 2006). For example, strains may be "chromatically adaptive," such that the pigment ratios phycourobilin (PUB) to phycoerythrobilin (PEB) vary with light environment. Strains may also have unique metal binding capabilities, or different N and P requirements (Moore et al 2002; Rocap 2002; Dupont et al., 2008). Much knowledge exists on the environmental variables that govern *Synechococcus* distribution and abundance in the ocean's photic zone. However, a large portion of the variation in distribution and abundance remains unexplained. This is potentially related to biotic factors, such as the role of selective grazing and its interaction with environmental conditions. Many

different phylotypes that fall into one or multiple clades are found within a given *Synechococcus* community (Schattenhofer, 2009). Therefore, within natural communities there is the potential for a wide range of abilities to utilize nutrients and evade predation; at the same time, there is the potential for grazers to select *Synechococcus* populations based on properties unique to a given strain. Furthermore, the potential exists for a strain to experience varying levels of susceptibility to grazing in fluctuating nutrient regimes.

Nitrogen Uptake and Metabolism: Laboratory and Field Studies

Genomic analysis suggests that *Synechococcus* N metabolism encompasses the ability to utilize several different inorganic and organic N sources. *Synechococcus* acquire N using membrane transport proteins that can be universally distributed among *Synechococcus* strains or specific to a *Synechococcus* isolate; they then assimilate N through shared metabolic pathways for a given N source (Table 2) (Muro-Pastor et al., 2005; Scanlan et al., 2009). Interestingly, when comparing N uptake and metabolism among strains, the diversity of genes related to urea metabolism appears greater than that of genes related to nitrate or ammonium metabolism (Collier, 1999). Little is known of amino acid metabolism, but all *Synechococcus* strains have the genetic potential to take up acidic and neutral amino acids from the environment (Scanlan et al., 2009).

Despite the widespread genetic potential of strains to use an array of N sources, field and laboratory observations have shown that not all strains are able to grow on all N sources, that N source affects growth rate, and that the nature of this effect is strain-dependent (Moore et al. 2002). Studies have confirmed nitrate and ammonium as the preferred N sources for most strains. For instance, estimated field nitrate and ammonium uptake rates of *Synechococcus* exceeded rates of urea and amino acid uptake, even though urea and amino acid ambient concentrations were highest (Glibert 2004; Warwick, 2009). *Synechococcus* field populations have also been observed at bloom

concentrations after only picomolar nitrate fluctuations, indicating that *Synechococcus* are successful competitors for this N source at low concentrations (Glover, 2007). Exceptions abound, as nitrate and/or ammonium had a negative effect on individual *Synechococcus* strains in laboratory growth studies (Moore et al., 2002). Clearly, *Synechococcus* communities have the potential to preferentially use many different forms of N, but how is strain-specific N utilization related to predation by a grazer such as *O. marina,* if at all? Given the broad metabolic potential of *Synechococcus*, there is much to be explored concerning how N affects cellular processes and how these impact *Synechococcus*-grazer interactions.

Microzooplankton Grazing On *Synechococcus*

While estimated grazing rates are variable, grazers are responsible for removing a large fraction (45-117%) of *Synechococcus* production daily in all major ocean basins and coastal areas (Putland, 2000). Potential consumers of *Synechococcus* (Prymnesiophyceae, Dictochophyceae, Bolidomonas, and Dinoflagellates, Raphidophytes, Heterotrophic nanoflagellates (HNF)) comprise many different feeding types, indicating *Synechococcus* is subject to diverse selective pressures (Jeong et al., 2010; Friaz-Lopez, 2008; Agawin et al., 2004). In contrast, exceptions exist where data indicate a lack of grazing pressure on *Synechococcus*. For example, laboratory studies show small heterotrophic flagellates (3-5 μm) either did not graze or did not grow on *Synechococcus* (Guillou et al., 2001) and entire microzooplankton communities in the field has been observed not to graze *Synechococcus* (Berninger et al., 2005). The diverse species of protozoan grazers co-existing with equally diverse *Synechococcus* populations may explain the discrepancies among data sets, suggesting that predator-prey relationships could account for some of the unexplained variance in the phylogenetic biogeography of *Synechococcus* (Zwirlgmaier, 2008). However, grazing experiments using 37 different strains of *Synechococcus* belonging to clades with differing biogeographies (I, II, III, IV) found no link between *Synechococcus* phylogeny and grazer growth, suggesting that a high

level of diversity in predator-prey interactions exists even among strains in a given clade (Zwirglmaier, 2009). While grazing is an important aspect of *Synechococcus* ecology, physical factors such as nutrients, light, and temperature are critical to this primary producer's success. Because research demonstrates *Synechococcus* strains utilize N and evade predation differently, linking these two areas together provides a way to understand how these abiotic and biotic factors interact. I hypothesize that *Synechococcus* strains will vary in their growth response to N sources. In addition, grazing susceptibility for a given *Synechococcus* strain will vary dependent upon N source for growth.

Experimental Approach

To explore these interactions, *Synechoccocus* strains with diverse physiological adaptations to contrasting environmental conditions were grown on various N regimes (described below), then fed to the dinoflagellate grazer *O. marina*. Characteristics likely to affect feeding rates (size, shape, nutrient content) were characterized for each strain in each N regime. *Synechococcus* strains isolated from the California Current included CC9311, a coastal strain dominant prior to the spring bloom (hereafter coastal-spring bloom), CC9902, a coastal strain dominant for most of the year (hereafter coastal-dominant), and CC9605, a coastal strain associated with oligotrophic conditions (hereafter coastal-oligotrophic). An oceanic strain WH8102 (hereafter ocean-oligotrophic) isolated from the Sargasso Sea, as well as its two mutants JMS40 and SIO7B, were also included in the study (Table 1). Mutant SIO7B lacks the SwmA protein, a 130-kDa glycoprotein associated with the S-layer on the outer cell surface (Fig. 1, Brahamsha, 1996; McCarren et al., 2005). JMS40 lacks the SwmB protein, a 1.12-megadalton protein that is distributed sporadically around the outside of the cell (McCarren et al., 2009). Expression of SwmA and SwmB occurs independently, as SIO7B expresses SwmB and JMS40 expresses SwmA; however, both proteins are necessary for motility (McCarren et al., 2005). In addition to variations in cell surface proteins as represented by the ocean-oligotrophic strain

Table 1. Isolation, date, source location, and features of *Synechococcus* strains used in this study.

Designated name	Strain	Date Isolated	Location Collected	Features
Coastal-oligotrophic	CC9605	1993	California Current	Clade II
Coastal-dominant	CC9902	1999	California Current	Clade IV
Coastal-spring bloom	CC9311	1993	California Current	Clade I
Ocean-oligotrophic	WH8102	1981	Tropical Atlantic Ocean	Clade III, motile
	JMS40		Mutant Strain	Mutant strain of WH8102, Lacks SwmB[1]
	SIO7B		Mutant Strain	Mutant strain of WH8102, Lacks SwmA[2]

[1]Swm B is a protein associated with the cell surface that is required for motility
[2]Swm A is a protein associated with the cell surface and more specifically the S-layer, associated with motility.

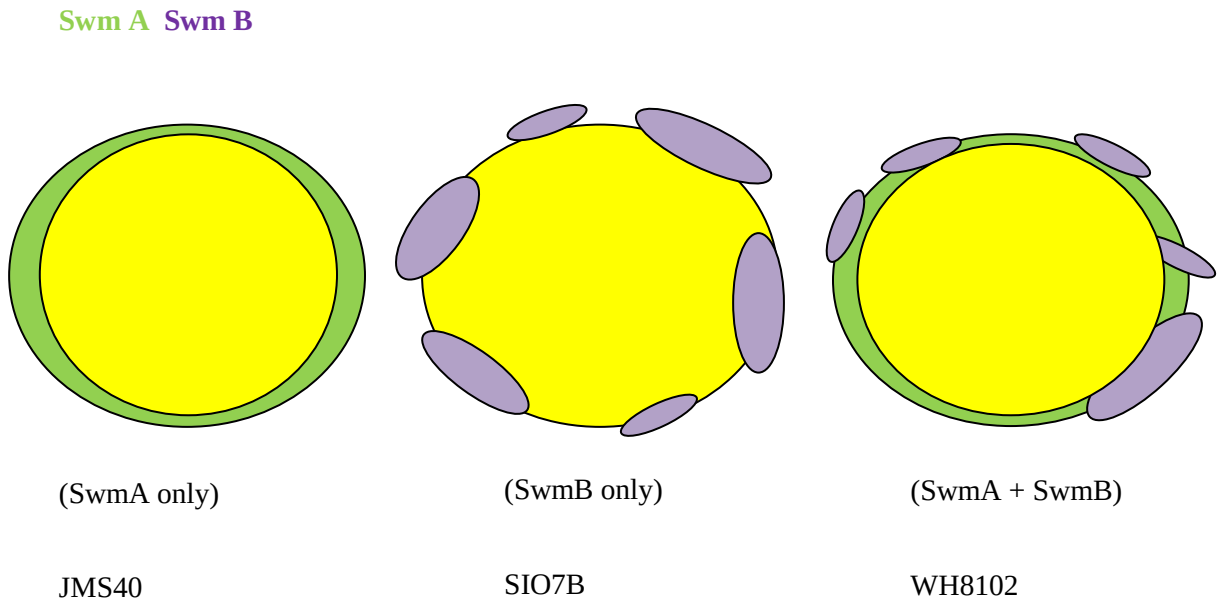

Figure 1. Conceptual drawing of cell surface proteins on *Synechococcus* WH8102 and mutants JMS40 and SIO7B based on (McCarren et al., 2005, 2009).

(WH8102) and its mutants, many other factors that alter the cell surface or nutritional quality vary among clades, including: N physiology (Algrhen et al., 2006), sensing and response system types, oxidative stress tolerance (Stuart et al., 2009), iron utilization genes (Scanlan et al., 2009; Palenik et al., 2006; Palenik et al., 2003) and lipopolysaccharide (LPS) structure (Synder, 2009). In addition, genes associated with membrane transport proteins for all strains included in my study have been catalogued (Paulsen et al. 2010). Therefore, the predicted substrate and function of many *Synechococcus* membrane transport proteins is accessible. This information may be related to strain-specific responses to N sources or to strain-specific grazing responses, as *O. marina* has been shown to respond to cell surface properties.

Certainly, the ability of these *Synechococcus* strains to acquire light and nutrients through their various uptake mechanisms and metabolic processes is critical to their success. However, how do these strategies affect the rate at which they get eaten by a grazing microzooplankter? The coalescence of biotic and abiotic effects leads to the following questions and hypotheses:

1. **What effect does N source have on the growth response of a given *Synechococcus* strain?**

   H_1: I hypothesize that N sources will have significantly different effects on growth response among and between strains.

Because the *Synechococcus* strains in my study have diverse temporal and spatial distributions, it is likely that they are exposed to a diverse array of N sources and concentrations. Therefore it is plausible that they would possess many different adaptations to be better suited to grow on different N sources. For example, the coastal-dominant strain (CC9902) might be expected to grow on a wide range of N sources because it is dominant throughout the year and persists over many N source fluctuations. Whereas the coastal-spring bloom strain (CC9311) might be expected to grow on only certain N sources that spike in concentration prior to the spring bloom. In contrast, the coastal-oligotrophic and ocean-oligotrophic strains (CC9605 and WH8102) might be expected to

grow on the widest range of N sources, because they may be adapted to using any N source available given their low N environment.

To determine how diverse *Synechococcus* strains associated with coastal (CC9311 and CC9902) and oligotrophic (CC9605, WH8102, and mutants JMS40 and SIO7B) conditions respond to various N sources, N-deplete *Synechococcus* strains were added to low-nutrient media containing only a single N source. Nitrogen sources tested included nitrate, urea, ammonium, alanine, glutamine, glycine, and proline. The amino acids were chosen based on the reported substrates for the catalogued genes encoding for membrane transport proteins. Interestingly, the various strains contain genes that code for membrane transport proteins that have different structure and function, but require similar amino acids as substrates (Table 2). The diversity of responses to the array of N sources was analyzed based on growth rate, cell size, and cell elemental (C and N) composition.

2. **Does the N source for *Synechococcus* growth affect the grazing response of *O. marina* to a given strain?**

   H_1: Growth-supporting N sources of *Synechococcus* will affect the grazing response of *O. marina* on both WH8102 and CC9311.

   H_2: Similar growth-supporting N sources of *Synechococcus* will affect the grazing response of *O. marina*.

   H_3: The effect the N-source grown *Synechococcus* has on O. marina grazing rates will be strain-dependent.

Because N source affects the cellular morphology and physiology of cells, this in turn has the potential to affect grazers. It is probable that strains used in the grazing experiments, the coastal-spring bloom and ocean-oligotrophic strains (WH8102 and CC9311), will experience changes in size, shape, or nutritional content. These factors are hypothesized to affect grazing of *O. marina*. If the two strains respond similarly to the N sources, or have similar characteristics that attracts or deters the grazer, *O. marina*, the interaction between N source and grazer may be primarily dependent on N

Table 2. Families or superfamilies of membrane transport proteins encoded by genes with their hypothesized amino acid substrates including alanine, glutamine, glycine, and proline for coastal (CC9311, CC9605, CC9902) and ocean (WH8102) *Synechococcus* strains. Strains lacking genes for membrane transport proteins with specified substrates are designated by X.

Designated name	Strain	Alanine	Glutamine	Glycine	Proline
Coastal-spring bloom	CC9311	AGCS 2°[1]	ABC ATP-dependent [2]	BCCT 2°[3]	MFS °[4]
Coastal-oligotrophic	CC9605	AGCS 2°	X	BCCT 2°	SSS 2°[5]
Coastal-dominant	CC9902	AGCS 2°	X	BCCT 2°	X
Ocean-oligotrophic	WH8102	AGCS 2°	X	BCCT 2° ABC ATP-dependent	ABC ATP-dependent

[1]AGCS: Membrane transport proteins in the "alanine or glycine:cation symporter family transport alanine and/or glycine in symport with Na^+ or H^+ and are generally 445-542 amino acyl residues in length, possessing 8-12 putative transmembrane α-helical spanners (Paulsen et al., 2010)."

[2]ABC: Membrane transport proteins in the "ATP-binding cassette superfamily" represents uptake, as well as efflux, transport. "ATP dependent represents a membrane transport protein that uses ATP hydrolysis without protein phosphorylation to energize transport (Paulsen et al., 2010)."

[3]BCCT: Membrane transport proteins in the "Betaine/Carnitine/Choline Transporter family" are "found in gram-negative and gram-positive bacteria and archaea. They all transport molecules with a quaternary ammonium group [$R\text{-}N^+(CH_3)_3$] and vary in length between 481 and 706 amino acyl residues with 12 putative transmembrane α-helical spanners (TMSs). Transport is pmf-driven or smf-driven proton or sodium ion symport, respectively, or else by substrate:substrate antiport. Some of these permeases exhibit osmosensory and osmoregulatory properties inherent to their polypeptide chains (Paulsen et al., 2010)."

[4]MFS: Membrane transport proteins in the "Major Facilitator Superfamily" **"**catalyze uniport, solute:cation (H^+ or Na^+) symport and/or solute:H^+ or solute:solute antiport. Most are of 400-600 amino acyl residues in length and possess either 12, 14 or 24 putative transmembrane α-helical spanners. exhibit specificity for sugars, polyols, drugs, neurotransmitters, Krebs cycle metabolites, phosphorylated glycolytic intermediates, amino acids, peptides, osmolites, siderophores (efflux), iron-siderophores (uptake), nucleosides, organic anions, inorganic anions, etc. They are found ubiquitously in all three kingdoms of living organisms (Paulsen et al., 2010)."

[5]SSS: Membrane transport proteins in the "Solute:Sodium Symporter family" "catalyze solute:Na^+ symport. The solutes transported may be sugars, amino acids, organo cations such as choline, nucleosides, inositols, vitamins, urea or anions, depending on the system. Members of the SSS family have been identified in bacteria, archaea and animals, and all functionally well-characterized members normally catalyze solute uptake via Na^+ symport. Proteins of the SSS vary in size from about 400 residues to about 700 residues and probably possess thirteen to fifteen putative transmembrane helical spanners (TMSs) (Paulsen et al., 2010)."

source and independent of strain. However, if the two strains respond differently to N sources and possess variable traits that deter or attract *O. marina*, the N source-grazer interaction may be primarily dependent on strain and secondarily influenced by N source.

Because the cellular response of *Synechococcus* to N treatments has the potential to cue or deter grazers, strains growing on different N sources potentially have different predation risks. To examine strain and N source-specific predation rates, grazing experiments were conducted using the heterotrophic dinoflagellate *O. marina,* which is approximately 20-30 µm in length and feeds through phagocytosis on a wide range of prey sizes (Hansen, 1996). This design allowed me to explore how the grazing rate of *O. marina* changes among N sources for a given *Synechococcus* strain, and whether *Synechococcus* cell size, C:N ratio, and/or C and N content were related to *O. marina* grazing rate variation.

METHODS

Synechococcus Culture Maintenance

Synechococcus strains (Table 1), obtained from B. Brahamsha at Scripps Institute of Oceanography, were maintained in culture at 22˚C, continuous light (15-20 µEin $m^{-2-1}sec^{-1}$). Cultures were transferred to new SN medium (nutrient additions of 9 X 10^{-3} M $NaNO_3$, 9.9 X 10^{-5} M K_2HPO_4, 1.5 x10^{-5} M Na_2EDTA H_20, 1 X 10^{-4} M Na_2CO_3, 7.38 x 10^{-7} M Vitamin B_{12}, and trace metal solution*:* 3.25 x 10^{-5} M Citric Acid, 6g $1L^{-1}$ Ferric ammonium citrate, 7.08 X 10^{-6} M $MnCl_2$, 1.61 x 10^{-6} M Na_2MoO_4, 7.72 x 10^{-7}M $ZnSO_4$, 8.59 x 10^{-8} M $Co(NO_3)_2$) approximately every two weeks (Anderson, 2005). Using aseptic technique under a laminar flow hood, *Synechococcus* were added to SN medium at a ratio of 1 ml (approximately 10^6 *Synechococcus* cells) to 50 ml SN media. The genetic integrity of mutants was maintained by adding Kanamycin (20 µg ml^{-1} final concentration), an antibiotic to which mutant strains SI07B and JMS40 are resistant.

Creating SN/5 N-reduced medium to generate N-deplete *Synechococcus* cultures

Before assessing growth in single source N media (1NS SN/5 medium), it was necessary to empty the cellular N reserves of *Synechococcus* cultures. To accomplish this, *Synechococcus* were cultured to N-limited stationary phase in SN/5 medium, with $NaNO_3$ further reduced to 30 µM (SN/5 N-reduced). The physiological response of *Synechococcus* strains driven to N-limitation were characterized using growth rate and elemental analysis (see methods below).

N-depleted autoclaved seawater (-N ASW): In order to create N-reduced medium and to invoke N limitation among strains, seawater containing no N was generated. Seawater was collected from East Sound, Orcas Island, WA on 5/16/09 and 6/18/09 and incubated for approximately 2 weeks. High light levels combined with the natural nutrient concentrations induced a phytoplankton

bloom that reduced N concentrations. Nitrate plus nitrite analysis confirmed that N reached minimal levels of 0 and 2 µM NO_3 for 5/16/09 and 6/18/09, respectively. After the organic matter from the bloom settled to the bottom, the seawater only was siphoned into a clean acid-washed carboy and further filtered (0.2 µm) into 1 L bottles (750 ml seawater per bottle). To complete the 75% ASW base for SN/5 growth medium, ultrapure water (Nanopure) was added (250 ml per bottle) to achieve 75% seawater, and the mixture was then autoclaved.

SN/5 medium (SN/5, N-reduced)**:** To achieve SN/5 N-reduced medium, SN medium molar concentrations were added to –N ASW at 1/5 their specified concentrations except N ($NaNO_3$), which was further reduced to reach a final concentration of 30 µM (yielding a 3:2 N:P ratio). This ensured that *Synechococcus* growth was N-limited. Medium nutrients were added to –N ASW using sterile filtration technique.

Growth Rate Analysis

To transfer *Synechococcus* into different media, 1ml *Synechococcus* stock culture was transferred to three 60 ml borosilicate glass culture tubes each containing 30 ml SN/5 N-reduced medium. Mutant strains were transferred similarly with the special addition of kanamycin. Culture conditions were maintained as described above.

Cell density and growth measurements: Cell density was estimated using a 10-AU fluorometer (Turner Designs) to measure *in vivo* fluorescence in each 60 ml tube every day between approximately 1000 and 1300 hrs. Growth rates were estimated from the slope of the natural log-transformed fluorescence values versus time (d) for time periods exhibiting exponential growth for each replicate. Growth rates were then analyzed using one-way ANOVA, with pairwise comparisons made using Tukey HSD (SPSS v.17, 2008). The onset of stationary phase was defined as the time when the slope decreased substantially, as estimated from growth curve analysis (Fig 2).

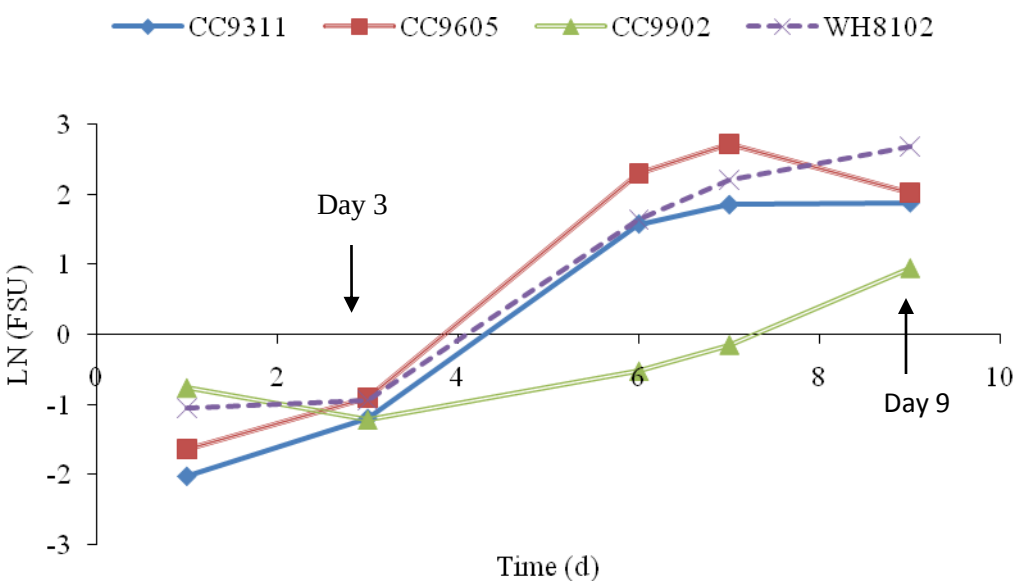

Figure 2. Growth curves based on natural log-transformed fluorescence (FSU) of *Synechococcus* strains grown in SN/5 N-reduced medium. Arrows denote days cell counts were made to determine fluorescence yield (FSU per [cells ml^{-1}]).

Samples (2-4 ml) were taken during exponential phase for CHN analysis (see below).

Fluorescence Yield: In using *in vivo* fluorescence to estimate growth rates, I assumed that fluorescence bore a consistent relationship to cell concentration throughout the growth experiment. To confirm this, fluorescence yield (a measure of the amount of fluorescence per *Synechococcus* cell) was estimated on two widely separated days during the experiment. Cell counts were made using a BD Facscalibur flow cytometer and epifluorescence microscopy (see below). Both flow cytometer and epifluorescence microscopy cell concentration data were used to estimate fluorescence yield. Fluorescence yield (a proxy for the amount of fluorescence per *Synechococcus* cell) was calculated by dividing in *vivo* fluorescence by cell concentration (cells ml^{-1}) to establish the consistency of strain fluorescence over time (Figs. 2, 3).

Single Nitrogen Source Experiments

To assess *Synechococcus* growth response to different N sources, N-depleted cultures were transferred to single source N SN/5 media (1NS SN/5, N concentration 30 μM). The N treatments included nitrate, ammonium, urea, proline, alanine, glutamine, and glycine. Responses of *Synechococcus* strains were assessed based on growth rate, cell size, and cell C and N content. While nitrate, ammonium, and urea are known to be responsible for the majority of oceanic primary production, the presence of genes encoding for amino acid transporters suggests these may serve as complementary N sources. Based on the genetic potential of coastal (CC9311, CC9605, CC9902) and oceanic (WH8102) *Synechococcus* strains to express membrane transport proteins with specific amino acid substrates, alanine, proline, glycine, and glutamine were chosen (Table 2). All strains had genes encoding for membrane transport proteins for all chosen amino acids except glutamine. Encoded membrane transport proteins specific to proline belonged to various protein families among *Synechococcus* strains. Glycine has frequently been used in other N response studies and therefore was included.

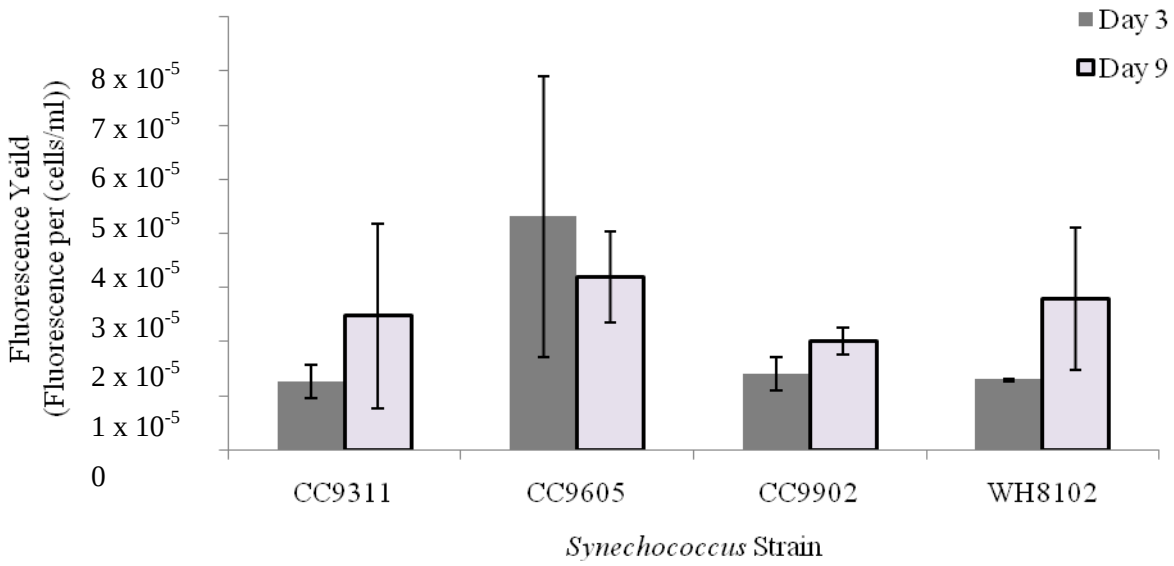

Figure 3. For all *Synechococcus* strains, fluorescence yield (fluorescence per (cells ml^{-1})) did not significantly differ between days 3 and 9 when grown in SN/5 N-reduced medium (n=2, +/- 1 SE).

Nitrogen Treatment Stock Solutions (1NS SN/5 medium): In order to create six different 1NS SN/5 media, separate N stock solutions were made by adding 99.1 mg ammonium [$(NH_4)_2SO_4$], 90.1 mg urea [$(NH_4)_2CO$], 127.5 mg nitrate ($NaNO_3$), 175 mg glycine betaine, 172.6 mg proline, 89.1 mg alanine, or 146.1 mg glutamine separately to 100 ml ultrapure H_20. The addition of 0.1 ml of a given N stock solution to SN/5 containing no nitrogen yielded 1NS SN/5 medium containing 30 μM N.

Nitrogen source experiments: After reaching stationary phase in the SN/5 N-reduced medium, 1 ml of N-depleted *Synechococcus* culture was added to 29 ml of 1NS SN/5 medium (nitrate, urea, ammonium, glutamine, glycine betaine, L-proline, or alanine) in four 60 ml tubes per N source. An additional control no-nitrogen (No N) treatment consisted of SN/5 without any added N and provided a reference point for any possible *Synechococcus* growth in the absence of supplemental N. *Synechococcus* growth rates on the various N sources were estimated using *in vivo* fluorescence and growth rate analysis. In addition, for each strain, growth rates were normalized to the maximum observed rate to yield percent of maximum growth rate. Samples were taken during exponential growth phase for cell counts, elemental analysis (C and N), cell size analysis, and grazing experiments.

Grazing Experiments

Short-term grazing experiments were conducted to assess how N source of *Synechococcus* affects the rates of *O. marina* predation for ocean-oligotrophic *Synechococcus* strain WH8102, from the Sargasso Sea, and coastal-spring bloom *Synechococcus* strain CC9311, from the California Current, to the heterotrophic dinoflagellate *O. marina*. To estimate grazing rates, samples were taken at 10, 20, and 30 min after the introduction of *O. marina*.

Grazer preparation

Oxyrrhis marina stock cultures were continuously maintained on a diet of microalgae (*Isochrysis galbana, Pyrenomonas salina, Emiliana huxleyi,* and *Dunaliella tertiolecta)* in dim light at 15°C and transferred 1x per week to autoclaved filtered SW with replenished food. Because the food vacuole method (described below) requires grazers to completely empty their food vacuoles prior to the grazing experiment, *O. marina* were fed a *Dunaliella tertiolecta* -only diet one week prior to the experiment. *Oxyrrhis marina* feeds efficiently on *D. tertiolecta* and can remove the majority of food cells from the culture in approximately the one week preceding the experiment. *Oxyrrhis marina* were not fed again before the experiment and this allowed them to ingest essentially all prey cells in the stock culture and empty their food vacuoles.

Oxyrrhis marina were enumerated the day of the experiment using the drop count method: *O. marina* cultures were mixed and then a subsample was poured into a Petri dish. Drops (5 or 2 μL) containing *O. marina* were pipetted onto a glass plate; the number of *O. marina* cells in approximately 10 drops was counted using a dissecting microscope. Counts were converted to *O. marina* per mL and averaged to obtain an estimate of *O. marina* cell concentration in the stock culture.

***Synechococcus* Preparation**

Because the ocean-oligotrophic strain (WH8102) and the coastal-spring bloom strain (CC9311) grew on a wide range of N sources, they were chosen for use in grazing experiments. Quadruplicate *Synechococcus* cultures for each N source were combined the day of the grazing experiment to reduce the time it would take to filter and count *Synechococcus.* Because several *Synechococcus* strains form clumps of cells (Jude Apple and Suzanne Strom personal

communication), which introduce bias into estimation of grazing rates, it was necessary to screen out clumped cells immediately before use of *Synechococcus* for grazing experiments. Cultures were filtered using syringe or vacuum filtration through a 3 µm pore-size polycarbonate filter to remove clumped *Synechococcus* cells. *Synechococcus* cell concentrations in the filtrate were enumerated using epifluorescence microscopy (see below).

The California Current coastal-spring bloom strain (CC9311) grown to exponential phase in SN media was used as a control treatment in all grazing experiments (hereafter referred to as control CC9311). Prior experiments have repeatedly shown that *O. marina* reliably grazes on control CC9311.

Grazing Experiments

Incubation bottles (in quadruplicate for each treatment) contained 40 ml total volume. The *O. marina* concentration was 400 cells ml^{-1} and that of *Synechococcus* was 10^6 cells ml^{-1}. The volume of ASW was calculated by subtracting *O. marina* and *Synechococcus* addition volumes from the total (40 ml) desired incubation volume. ASW was added to 125 ml polycarbonate bottles, followed by *O. marina* volumes for each treatment. At time 0 (T_0) *Synechococcus* volumes were added in ordered 30 s intervals. At 10, 20, and 30 min after *Synechococcus* addition, 10 ml aliquots from each incubation bottle were poured into vials containing 10% glutaraldehyde (final concentration 0.5%) and 10% 4',6-diamidino-2-phenylindole solution (DAPI, final concentration 0.5%). Time intervals were chosen based on previous experimentation, to encompass a linear increase in the number of ingested *Synechococcus* per *O. marina* over the 30 min time interval. Fixed samples were stored in a dark freezer (11-24 hrs, -20°C), before making slides.

Slide preparation and grazing rate determination: Fixed samples were vacuum filtered through a 5 µm pore size cellulose backing and 3 µm pore size polycarbonate filter. Polycarbonate

filters were placed on slides, covered with low fluorescence immersion oil, and immediately stored in a freezer (-20°C). Grazing was determined by the food vacuole method which quantifies the amount of ingested *Synechococcus* cells within a grazer's food vacuole using an epifluorescent microscope and blue light illumination. The blue light induces a yellow-orange fluorescence in the *Synechococcus* cells; the number of cells inside the food vacuoles of individual *O. marina* can then be counted. The number of ingested *Synechococcus* cells in 100 *O. marina* cells per filter was counted under 1000x magnification. Out of the 100 *O. marina* counted, a fraction contained no *Synechococcus* cells and were recorded as zero *Synechococcus* ingested. *Oxyrrhis marina* with empty food vacuoles were excluded from analyses that were based on the feeding fraction of the population, as described below.

Grazing Data Analysis

Two grazing rates were calculated for each incubation bottle in grazing experiments involving the ocean-oligotrophic strain (WH8102) and the coastal-spring bloom strain (CC9311). Grazing rates were calculated separately for 0-10 and 10-30 min intervals due to an obvious rate change at 10 min in most data sets. An overall 0-30 min grazing rate was not calculated due to a change in the slope of the relationship between 0-10 and 10-30 min (Fig. 4).

Within each time interval, grazing rates were further calculated in two ways: 1) average number of ingested *Synechococcus* cells per *O. marina,* and 2) average number of *Synechococcus* cells per feeding fraction only of *O. marina (*i.e. just the portion of the *O. marina* population containing ingested *Synechococcus*). For each replicate incubation bottle, the slope of the relationship between *Synechococcus* per *O. marina* and time yielded the grazing rate: ingested *Synechococcus* per *O. marina* per min or feeding *O. marina* per min. For each treatment, replicate rate estimates were averaged to yield a mean grazing rate (n=4).

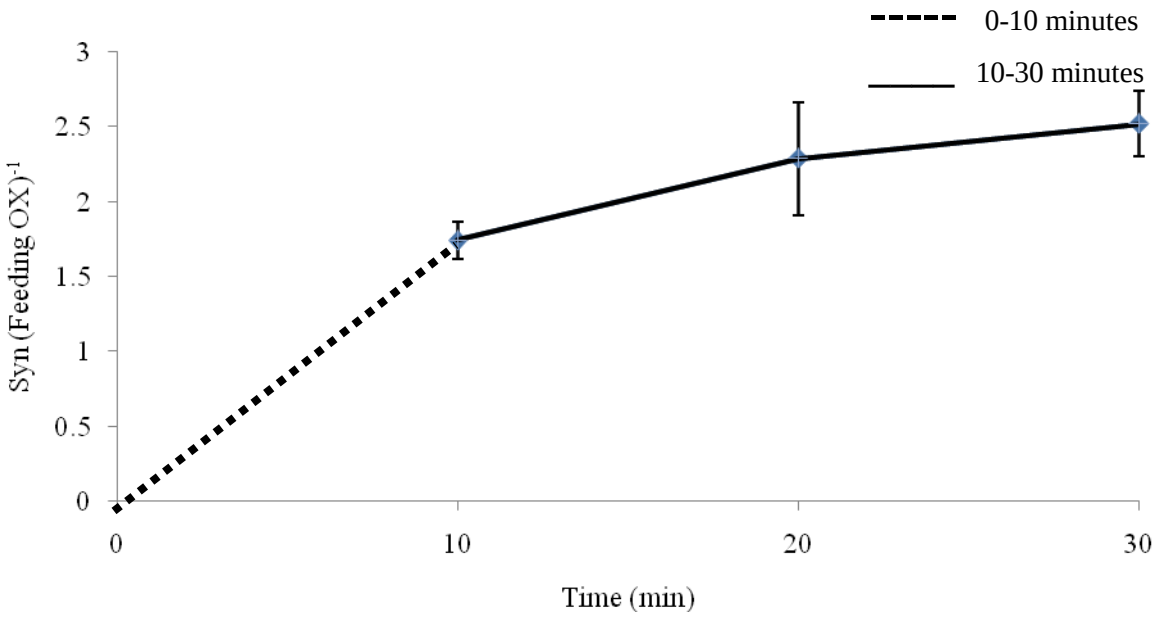

Figure 4. Example of urea-grown CC9311 (coastal-spring bloom strain) data from which feeding rates were calculated. Data plotted are the average number of ingested *Synechococcus* cells in the feeding portion of the *O. marina* (OX) population (Syn[Feeding OX]$^{-1}$) over time. The slope of the relationship yields a feeding rate estimate (Syn [Feeding OX]$^{-1}$ min^{-1}). Note the substantial change in slope between 0-10 and 10-30 min (n=4, +/- 1 SE).

One-way ANOVAs were used to analyze the two grazing rates (i.e. for total *O. marina* and feeding *O. marina*) for time intervals 0-10 and 10-30 min for the ocean-oligotrophic strain (WH8102) and the coastal-spring bloom strain (CC9311) (using SPSS v.17, 2008, where significance was accepted at $\alpha<0.05$). If ANOVA results indicated a significant treatment effect, post-hoc Tukey's HSD was utilized to differentiate among N treatments for each strain. This family-wise (vs. pairwise) comparison test was chosen to minimize Type I error.

Percentage of *O. marina* feeding*:* To calculate the percent of the *O. marina* population feeding, the number of *O. marina* containing ingested *Synechococcus* was divided by the total number of *O. marina* examined (100). The average percent of *O. marina* actively feeding was calculated at each time point and for each N treatment within the two prey strains including the coastal spring bloom (CC9311) and ocean-oligotrophic (WH8102) strains.

The percentage of the *O. marina* population feeding among N source treatments was analyzed differently for the ocean-oligotrophic (WH8102) and coastal-spring bloom (CC9311) strains. The slope of the relationship between the fraction of grazers feeding on the coastal-spring bloom strain (CC9311) and time between 10 and 30 min was zero for all N treatments, indicating there was no change in fraction feeding over time after the first 10 min. Therefore, the fraction of grazers feeding at 10, 20, and 30 min within each replicate were considered subsamples, while the mean of these time points represented one experimental unit for each replicate. Replicates of N treatments were averaged to yield a single mean fraction feeding for each N treatment (n=4). Averages for each N treatment were analyzed using one-way ANOVA and post-hoc Tukey's HSD.

In contrast to the coastal-spring bloom strain (CC9311), the fraction of grazers feeding on the ocean-oligotrophic strain (WH8102) changed over time and required a separate statistical method. Analysis of variance on repeated measures (ANOVAR) was used to explore how time and N source

affected the fraction of the *O. marina* population feeding. ANOVAR results violated the assumption of sphericity (Mauchly's W, p=0.017) which is similar to violation of the homogeneity of variance assumption in ANOVA. The more conservative Huynfeldt analysis met the stipulation that p>0.7 (p=0.947). Post-hoc comparisons, Tukey's HSD and Fischer's LSD, were used to compare the within- and between-subject effects. Fisher's LSD was used for pairwise comparisons of grazing rates during 10, 20, and 30 min across all N source treatments. Because the number of comparisons increased from three (time points) to five (N sources), Tukey's HSD was used to compare grazing of the ocean-oligotrophic strain (WH8102) grown in different N sources across all times. The time*N source interaction was graphically illustrated to provide a further interpretation of post-hoc comparisons (see Fig. 9 in results)

Characterizing Physiological and Morphological Properties of *Synechococcus* Cultures

Cell Enumeration

Synechococcus cell counts were made using a BD Facscalibur flow cytometer and epifluorescence microscopy. Epifluorescent microscopy was also used to estimate cell concentrations for CHN analysis and grazing experiments, and to assess contaminating bacteria concentrations. In order to generate slides that would yield accurate cell counts, cultures were diluted at least 1: 20 (50 µL *Synechococcus*, 950 µL ASW) in vials, fixed with glutaraldehyde (final concentration 0.5%), and filtered on a 0.65 µm pore-size cellulose backing filter and a 0.22 µm pore-size polycarbonate filter. Depending upon concentration, *Synechococcus* were enumerated under either 400x or 1000x magnification, with 5-8 Whipple plots counted per slide. In addition to the above methods, samples for enumeration of contaminating bacteria were stained with DAPI and counted under UV light at 1000x magnification. *Synechococcus* cells were clearly distinguishable from thread-like heterotrophic bacteria because, unlike heterotrophic bacteria, *Synechococcus* fluoresce yellow under blue light

excitation. Toggling between blue and UV light provided a check to confirm cells counted were heterotrophic bacterial cells.

Synechococcus concentrations were also enumerated on the flow cytometer using low flow (17 uL per s) for 10 s. Primary detectors were set on sidescatter using E02 voltage settings that determine the event detection threshold. Events were assumed to be synonymous to cells and were quantified based on the FL-3 fluorescence parameters, using a 488nm blue laser that excites red fluorescence of passing *Synechococcus* cells (Becton-Dickinson Co, 2008).

CHN analysis

Samples for determination of particulate C and N were collected from SN/5 N-reduced and 1NS SN/5 experiments during exponential growth stage, as determined from growth curves. Approximately 5-10 ml of culture were syringe-filtered through precombusted 13 mm GF/F Whatman glass fiber filters in duplicate or triplicate, depending on sample volume. A sample was also taken for cell enumeration by flow cytometry (for fluorescence yield only) or epifluorescence microscopy. Filters were transferred to tin foil boats and dried in a 50°C oven for 24 hrs. Tin foil boats were then carefully wrapped around the filters and placed in a dessicator for 1-2 months until analysis using the CE Elantech elemental analyzer. The standard curve was generated by using standards made the day of analysis which included: atropine (0.433 and 1.347 mg) and aspartic acid (0.184, 0.431, 1.297, 1.662, and 2.331 mg). Batches of CHN samples were analyzed in June and October 2009. During October, the CE Elantech Elemental analyzer recorded C and N content, but did not recognize the recorded values as C content or calculate grams C. Therefore, C content had to be defined manually for each sample using the area under the carbon peak. Carbon weights were recalculated using the parameters of the best fit line generated by standards.

Cell Size Analysis

Using methods similar to those for the epifluorescence microscopy cell enumeration technique, slides were made using samples of *Synechococcus* culture from each N-treatment. Ocean-oligotrophic strain (WH8102) samples were collected in late August, the day of the grazing experiments. Coastal-spring bloom strain (CC9311) samples were regrown through all N treatments and collected during exponential phase in late October, several months after the grazing experiment. Using a tower-mounted Photometrics Coolsnap camera, photos were taken under 1000x magnification on a Leica epifluorescent microscope and captured using Photometrics RS Image software. Software program Image Pro plus (MediaCybernetics) was used to measure the length (l) and width (w) in μm for 100 cells from each N treatment. Cell volume (V, μm^3) was calculated using the formula for an oblate spheroid , where:

$$V = \frac{4}{3}\pi ab^2 \text{ and } a = \frac{1}{2}l, b = \frac{1}{2}w.$$

Cell sizes were statistically compared using one-way ANOVA and post-hoc Tukey's HSD (SPSS v.17, 2008)

Related Studies: Correlation and Regression Analysis

After measuring the growth rate, cell dimensions, and C and N content of coastal strain CC9311 and ocean strain WH8102, statistical analyses were performed to understand how these variables were related to one another. For variables including *Synechococcus* morphological and physiological characteristics and *O. marina* grazing rates, linear regressions were performed (Excel, Microsoft Office 2007) when there was reason to assume that one variable was explanatory of another variable. Outliers were assessed by performing several linear regressions, in which questionable outliers, as well as other random data points, were excluded. The series of regressions quantified the

impact potential outliers had on the r^2 value. Linear regressions for both data sets (including and excluding outliers) are shown on figures. Outliers were excluded from the linear regression analysis and termed lurking variables, or an unknown third variable that skewed the relationship of the two variables in question for a given treatment. These data were not completely discarded but rather were discussed, as important information lies in the possibility of unknown but influential variables.

Correlation analyses were performed when there was reason to expect variables to be related and no reason to expect causality or an explanatory relationship between the two. Pearson correlations were performed (SPSS v. 17, 2008).

RESULTS

Synechococcus Growth in SN/5 N-reduced Medium

Synechococcus N metabolism is intricately linked to cellular processes that can affect fluorescence, such as pigmentation and photosynthesis. Therefore, fluorescence per unit cell concentration (cells ml^{-1}) was compared at two widely separated time points to confirm it as an accurate proxy for strain growth (Figs. 1, 2). The fluorescence yields for day three, representing early exponential growth phase, and day nine, representing early stationary phase, were comparable within strains. Similar N usage studies have found agreement between growth rates measured by FSU and flow cytometry (Algrhen et al., 2006).

Contaminating heterotrophic bacteria were considered as a possible confounding factor in single N source experiments because of their potential ability to alter the concentration and form of N available to *Synechococcus.* Contaminating bacteria comprised less than 12% of the cells in stock *Synechococcus* cultures; therefore, it was assumed that any background heterotrophic bacteria had a minimal effect on *Synechococcus* N usage (Table 3).

Several *Synechococcus* strains grown on SN/5 N-reduced medium had growth rates that were significantly higher than those of other strains (ANOVA $F=7.8$, d.f.=18, $p=0.001$, Table 4). The growth rates of the coastal-spring bloom strain (CC9311), 0.48 d^{-1}, and the coastal-oligotrophic strain (CC9605), 0.52 d^{-1}, were significantly greater than those of coastal-dominant strain (CC9902), 0.30 d^{-1}, and the ocean-oligotrophic strain (WH8102), 0.33 d^{-1} (Tukey's HSD). Growth rates of mutant strains (JMS40 and SIO7B) were not significantly different and fell between the higher coastal-spring bloom and coastal-oligotrophic (CC9311, CC9605) group and the lesser coastal-dominant and ocean-oligotrophic (CC9902, WH8102) group. These rates overall are hereafter referred to as the pre-add rates (i.e. rates determined before transfer to single N source treatments); they represent cells that had not reached N limitation. The C:N ratios of all strains (SIO7B data not determined) did not differ amongst each other and were comparable to ratios measured in prior

Table 3. Percent of contaminating bacteria in stock *Synechococcus* cultures

Designated name	Strain	*Synechococcus* Concentration (cells ml $^{-1}$)	Bacteria Concentration (cells ml $^{-1}$)	Percent Contamination
Coastal-spring bloom	CC9311	2.27×10^{8}	7.69×10^{6}	3
Ocean-oligotrophic	WH8102	3.82×10^{8}	2.05×10^{7}	5
Coastal-dominant	CC9902	1.28×10^{8}	7.31×10^{6}	6
Coastal-oligotrophic	CC9605	4.53×10^{7}	4.79×10^{6}	11

Table 4. *Synechococcus* strain mean growth rates (d^{-1}, n=3) and molar C:N ratios in SN/5 N-reduced medium. Growth rates differed among strains (ANOVA ($p<0.001$)). Treatments with shared letters (A or B) did not differ significantly (Tukey's HSD, $p<0.05$) . SD=1 standard deviation, nd=not determined.

Designated name	*Synechococus* Strain	Growth Rate +/- SD (d^{-1})	C:N +/- SD
Coastal-spring bloom	CC9311	0.48 +/- 0.03 [a]	3.6 +/- 0.7
Coastal-oligotrophic	CC9605	0.52 +/- 0.01 [a]	4.3 +/- 2.2
Mutant	JMS40	0.41 +/- 0.14 [ab]	3.3 +/- 0.2
Mutant	SIO7B	0.42 +/- 0.01 [ab]	nd
Coastal-dominant	CC9902	0.30 +/- 0.02 [b]	2.6 +/- 0.2
Ocean-oligotrophic	WH8102	0.33 +/- 0.02 [b]	3.5 +/- 0.5

studies, ranging from 2.6-4.3 (Table 4). The slowest growing coastal-dominant strain (CC9902) displayed the lowest C:N ratio while the fastest growing coastal-oligotrophic strain (CC9605) displayed the highest C:N ratio.

Synechococcus Growth on Different N Sources

Growth varied among N treatments within each *Synechococcus* strain. Growth was attributed to a single N source if growth in that N treatment was significantly greater than growth in the No-N control. In contrast, N sources were considered inhibitory when growth in N treatment was significantly less than that in the No-N control. Single N source growth rates represent an N-limited cell's response to N-replete conditions. In addition to comparisons among N sources, these N-deplete to N-replete rates were also compared to the pre-add growth rate. The latter is representative of continuous exposure to N-replete conditions.

Ocean-oligotrophic Strain WH8102 and Mutants

Ocean-oligotrophic strain (WH8102) exhibited growth (0.45-0.57 d^{-1}) in 6 of the 7 N source treatments, including all but glutamine (ANOVA, $F=15.1$, d.f.= 26, $p<0.001$, Tukey's HSD, Fig. 5 & 6, Table 5). Within N sources, nitrate and glycine supported the highest growth rates at 0.55 and 0.57 d^{-1}. These were significantly greater than rates in glutamine and the pre-add rate. When considering growth rates in N sources that supported growth, all except alanine-supported rates were significantly greater than the pre-add rate.

Mutant JMS40 exhibited a broader range of growth rates, 0.03 d^{-1} to 0.51 d^{-1} than the ocean-oligotrophic strain (WH8102); however, growth could not be unequivocally attributed to any single N source (ANOVA, $F=11.85$, d.f.=18, $p<0.001$, Tukey's HSD, Fig. 5 & 6, Table 5). Nonetheless, data suggest that nitrate, ammonium, and urea supported rates approximately 50% higher than No-N

control, proline, and glutamine treatments and 100% higher than amino acids alanine and glycine. Unlike glycine-supported maximal growth of the ocean-oligotrophic strain (WH8102), data suggest that mutant JMS40 showed maximal growth in urea and minimal growth in glycine. While not significantly different from the No-N control, data suggest some N sources enhanced mutant JMS40 growth relative to others. Nitrate and ammonium may have increased growth rates by approximately 50% compared to urea, amino acids proline and alanine, and the No-N control. Interestingly, mutant SIO7B contrasted with mutant JMS40 in urea treatment and grew at a reduced growth rate, relative to the maximum, rather than a rate comparable to the maximum.

While there were no N sources that supported growth rates for JMS40 that significantly differed from those in the No-N control, growth in two amino acids was significantly lower than growth in other N sources. Growth rates in nitrate, urea, ammonium and pre-add treatments (0.48-0.51 d^{-1}) were significantly greater than in amino acid treatments alanine and glycine (0.03-0.04 d^{-1}). From these patterns it can be inferred that no growth occurred in alanine and glycine treatments. These amino acids may have been inhibitory, given that rates were much lower than those in the No-N control.

Similar to mutant JMS40 and in contrast to the wild type ocean-oligotrophic strain (WH8102), mutant SIO7B experienced a wide range of growth rates from -0.41 d^{-1} to 0.62 d^{-1}. While growth could not be unequivocally attributed to N source treatments, several amino acids significantly inhibited growth (ANOVA, F=13, d.f.=22, p=0.001, Tukey's HSD, Fig. 5 & 6 , Table. 5). Data showed the amino acids glutamine and glycine inhibited mutant SIO7B growth. Growth rates in nitrate, ammonium, and urea treatments, No-N control, and pre-add (0.23 to 0.62 d^{-1}) were significantly greater than in glutamine and glycine (-0.29 d^{-1} to -0.41 d^{-1}), which actually supported negative rates (mortality). While glycine inhibited SIO7B growth and reduced JMS40 growth, it supported maximal ocean-oligotrophic strain (WH8102) growth. Interestingly, growth could not be unequivocally attributed to glutamine for ocean-oligotrophic strain (WH8102) or mutants JMS40 and

SIO7B; however, glutamine inhibited only mutant SIO7B and did not inhibit either ocean-oligotrophic strain (WH8102) or mutant JMS40.

Coastal Strains: CC9902, CC9311, CC9605

Coastal *Synechococcus* strains had elevated growth rates compared to ocean-oligotrophic strain (WH8102) and its mutants. In addition, single N source-supported growth rates of coastal strains were often much higher than pre-add rates and these elevated rates occurred on a broader set of N sources compared to ocean-oligotrophic strain (WH8102) and mutants. Interestingly, the coastal-spring bloom (CC9311) and coastal-oligotrophic (CC9605) strains displayed maximum growth on glycine, similar to ocean-oligotrophic strain (WH8102). The coastal-dominant (CC9902) and coastal-oligotrophic (CC9605) strains experienced reduced growth in specific amino acid treatments, similar to mutants JMS40 and SIO7B (Fig. 5 & 6).

The coastal-oligotrophic strain (CC9605) grew on all N sources at rates ranging from 0.45 d^{-1} to 1.26 d^{-1}, while mortality (-0.75 d^{-1}) was seen in the No-N control (ANOVA, F=17, d.f.=23, p=0.001, Tukey's HSD, Fig. 5 & 6, Table 6); however, growth rates did not significantly differ among N source treatments. Despite variance, data suggest that nitrate, ammonium, urea, glutamine and glycine enhanced growth more than alanine and proline. Maximum growth rate of the coastal-oligotrophic strain (CC9605) occurred in the glycine treatment (1.26 d^{-1}). Growth rates supported by most N sources were greater than the pre-add rate. Growth rates in N source treatments glutamine, glycine, ammonium, nitrate, and urea ranged from 0.99 d^{-1} to 1.26 d^{-1} and were approximately double the pre-add growth rate of 0.52 d^{-1}. Growth rates in amino acid treatments alanine and proline were similar to the pre-add growth rate.

Similar to the coastal-oligotrophic strain (CC9605), the coastal-spring bloom strain (CC9311) grew in all N source treatments (0.61 d^{-1} to 1.11 d^{-1}) and growth rates reached a maximum in glycine.

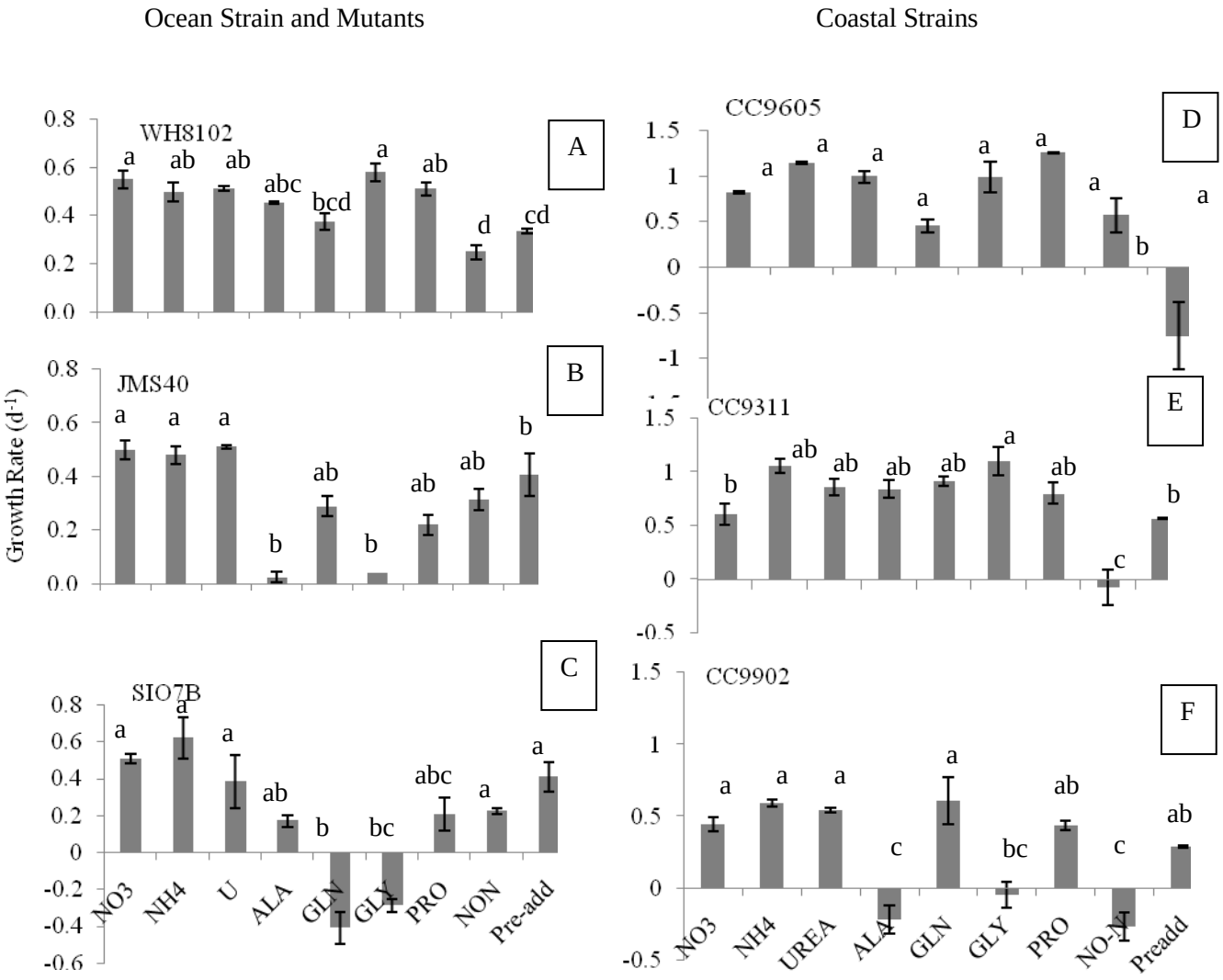

Figure 5. Mean growth rate (A-F, n=3,4 +/- 1 SE) for ocean (A-C) and coastal (E-F) *Synechococcus* strains grown in single N sources. Abbreviated N sources appear as nitrate (NO3), ammonium (NH4), urea (UREA), alanine (ALA), glutamine (GLN), glycine (GLY), proline (PRO) and No-N control (NON).

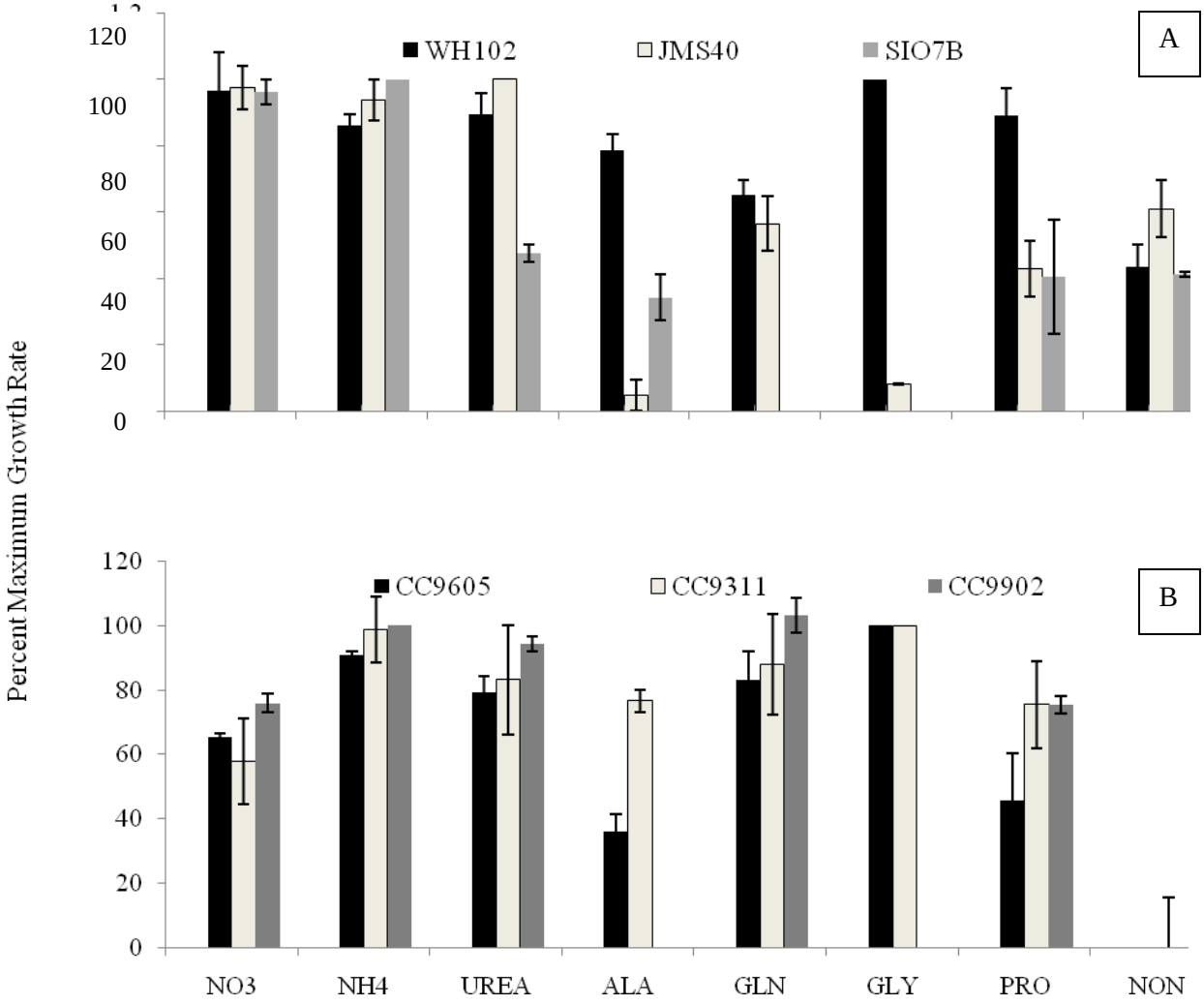

Figure 6. Growth rates normalized to percent of the maximum growth rate for ocean strain and mutants (A: JMS40, SIO7B, and WH8102) and coastal strains (B: CC9605, CC9311, CC9902) grown in single N source treatments(G,H). Zeros denote no or negative growth (n=3, +/- 1 SE).

Table 5. *Synechococcus* ocean-oligotrophic strain (WH8102) and mutants (JMS40 and SIO7B) mean growth rates (d^{-1}, n=3) in SN/5 medium with various single N sources. Growth rates differed among N sources (ANOVA). Treatments with shared letters (A or B) did not differ significantly (Tukey's HSD, $p<0.05$). SD=1 standard deviation.

N source	WH8102 Growth Rate +/- SD	JMS40 Growth Rate +/- SD	SIO7B Growth Rate +/- SD
Glycine	0.57 +/- 0.07[a]	0.04 +/- 0.00[b]	-0.29 +/- 0.05[bc]
Nitrate	0.55 +/- 0.06[a]	0.49 +/- 0.06[a]	0.51 +/- 0.05[a]
Proline	0.51+/- 0.05[ab]	0.22 +/- 0.07[ab]	0.21 +/- 0.13[abc]
Urea	0.51+/- 0.02[ab]	0.51 +/- 0.01[a]	0.38 +/- 0.25[a]
Ammonium	0.49 +/- 0.07[ab]	0.48 +/- 0.06[a]	0.62 +/- 0.20[a]
Alanine	0.45 +/- 0.01[abc]	0.03 +/- 0.04[b]	0.17 +/- 0.05[ab]
Glutamine	0.37+/- 0.06[bcd]	0.29 +/- 0.07[ab]	-0.41 +/- 0.20[b]
Pre-add[1]	0.34 +/- 0.02[cd]	0.41 +/- 0.14[a]	0.42 +/- 0.02[a]
No-N Control[2]	0.25 +/- 0.05[d]	0.31 +/- 0.07[ab]	0.23 +/- 0.03[a]

[1]Pre-add growth rate was estimated from the cultured strain grown in SN/5 Reduced N (Table 4) during exponential phase before strain was transferred to single N source media.

[2] No-N control was composed of SN/5 medium with no added N. *Synechococcus* strains were considered to grow on single N sources if growth rates were significantly greater than those in the on No-N Control.

Table 6. Coastal *Synechococcus* strains mean growth rates (d^{-1}, n=3) in SN/5 medium with various single N sources. Growth rates differed among N sources (ANOVA ($p<0.001$)). Treatments with shared letters (A or B) did not differ significantly (Tukey's HSD, $p<0.05$). SD=1 standard deviation.

	Coastal-oligotrophic	Coastal-spring bloom	Coastal-dominant
N source	CC9605 Growth Rate +/- SD	CC9311 Growth Rate +/- SD	CC9902 Growth Rate +/- SD
Glycine	1.26 +/- 0.01 [a]	1.11 +/- 0.27 [a]	-0.05 +/- 0.16 [bc]
Ammonium	1.14+/- 0.02 [a]	1.06 +/- 0.14 [ab]	0.59 +/- 0.04 [a]
Urea	0.99 +/- 0.11 [a]	0.86 +/- 0.16 [ab]	0.54 +/- 0.03 [a]
Glutamine	0.99 +/- 0.23 [a]	0.92 +/- 0.10 [ab]	0.61+/- 0.23 [a]
Nitrate	0.82 +/- 0.02 [a]	0.61 +/- 0.21 [b]	0.45 +/- 0.13 [a]
Proline	0.57 +/- 0.32 [a]	0.80 +/- 0.20 [ab]	0.43 +/- 0.05 [ab]
Pre-add[1]	0.52 +/- 0.01 [a]	0.57 +/- 0.01 [b]	0.29 +/- 0.02 [ab]
Alanine	0.45 +/- 0.12 [a]	0.84 +/- 0.17 [ab]	-0.22 +/- 0.17 [c]
No-N Control[2]	-0.75+/- 0.64 [b]	-0.07 +/- 0.33 [c]	-0.22 +/- 0.17 [c]

1,2 as in Table 5

Growth in glycine was significantly greater than growth in nitrate (ANOVA, F= 12.3, d.f.=31, $p<0.001$ Tukey's HSD, Fig. 5 & 6, Table 6). Excluding nitrate, growth rates for all other treatments were within 20% of the glycine growth rate maximum. Interestingly, while the coastal-oligrotrophic (CC9605) and the coastal-spring bloom strains (CC9311) both grew 50% faster on ammonium and glycine compared to the pre-add rate, the coastal-oligotrophic strain (CC9605) also grew at elevated rates on urea and glutamine.

The coastal-dominant strain (CC9902) exhibited a wide range of growth rates (-0.22 d^{-1} to 0.61 d^{-1}) in N source treatments and experienced cell mortality in some treatments similar to that observed for mutant JMS40 (Fig. 5, Table 6). Growth occurred in nitrate, ammonium, urea, proline, and glutamine (ANOVA, F= 11.5, d.f.=15, p=0.001, Tukey's HSD). Similar to the coastal-oligotrophic strain (CC9605), glutamine and ammonium supported growth rates of the coastal-dominant strain (CC9902) that were 50% higher than the pre-add rate. However, the coastal-dominant strain (CC9902) showed growth on fewer N sources than that of the coastal-oligotrophic (CC9605) or coastal-spring bloom (CC9311) strains. No growth occurred in alanine and glycine treatments and the No-N control, and all of these treatments experienced cell losses. Furthermore, all N-supported growth rates that resulted in growth were either significantly greater than rates supported by both glycine and alanine or by alanine alone. While SIO7B experienced reduced grown on amino acids, growth was not inhibited like that of JMS40.

Characterization of N Source-grown Ocean and Coastal *Synechococcus* Strains

Ocean-oligotrophic *Synechococcus* Strain *(*WH8102)

Overall, there were few differences among N source-grown ocean-oligotrophic (WH8102) cells. While urea-grown ocean-oligotrophic strain (WH8102) were significantly larger compared to other N source-grown cells, there were no significant physiological differences (C and N content (CN) and C:N ratio) among N source-grown cells.

Cell volumes of ocean-oligotrophic strain (WH8102) cells grown in different N treatments ranged from a minimum of 0.73 μm^3 in nitrate to a maximum of 1.59 μm^3 in urea. Control CC9311 cell volume was intermediate at 0.86 μm^3 (Table 7). Urea-grown ocean-oligotrophic strain (WH8102) cells had significantly larger volumes than both cells grown in other N treatments and control CC9311 cells (ANOVA $F=12$, d.f.=482, $p<0.001$, Tukey's HSD).

Urea-grown ocean-oligotrophic strain (WH8102) cells were the longest and widest (1.57 x 1.26 μm) compared to nitrate-grown cells, which were the shortest and narrowest (1.29 x 0.96 μm). Nitrate-grown and ammonium-grown ocean-oligotrophic strain (WH8102) cells were significantly shorter than urea-grown ocean-oligotrophic strain (WH8102) and control strain CC9311 cells (ANOVA Length: $F=5.3$, d.f.=410, $p<0.001$; Width: $F=15.4$, d.f.=410, $p<0.001$, Tukey's HSD). Ocean-oligotrophic strain (WH8102) cells grown in nitrate, ammonium and proline, and control strain CC9311 were significantly narrower than urea-grown ocean-oligotrophic strain (WH8102) cells. Interestingly, control CC9311 cell length was similar to urea-grown ocean-oligotrophic strain (WH8102) cell length; however, control CC9311 cell width was similar to the ocean-oligotrophic strain (WH8102) nitrate-grown cell width, suggesting the control strain was more elongated than its ocean-oligotrophic counterpart (WH8102). Excluding proline, ocean-oligotrophic strain (WH8102) cells grown in N sources had similar CN with a narrow range of C:N ratios. Average C:N molar ratios of ocean-oligotrophic strain (WH8102) cells grown in N source treatments ranged from 7.6 to 9.9 compared to a ratio of 4.2 for the control CC9311 (Table 7).

Due to the narrow range of the majority of measured cell characteristics, there were no discernable relationships between any of the parameters discussed above that would provide further insight into the N response of the ocean-oligotrophic strain (WH8102).

Table 7. Ocean-oligotrophic *Synechococcus* strain (WH8102) mean (+/- SD) cell dimensions (n>100, ANOVA $p<0.05$, Tukey's HSD, $p<0.05$), cell carbon and nitrogen content (f mol^{-1} n=2,3) and C:N ratio (molar, n=2,3). Cell volume, length, and width with shared letters (a,b) indicate no significant difference. Among N sources, carbon, nitrogen, and C:N ratio did not significantly differ (ANOVA ($p>0.05$)). SD=1 standard deviation.

N Source	Cell Volume (μm^3)	Cell Length (μm)	Cell Width (μm)	Carbon (fmol $cell^{-1}$)	Nitrogen (fmol $cell^{-1}$)	C:N Ratio (molar)
Nitrate	0.73 +/- 0.63[a]	1.29 +/- 0.37[a]	0.96 +/- 0.26[a]	8.56 +/- 4.16	1.13 +/- 0.55	7.6 +/- 1.0
Ammonium	0.83 +/- 0.45 [a]	1.33 +/- 0.36 [a]	1.05 +/- 0.21 [a]	8.95 +/- 4.32	0.90 +/- 0.55	9.9 +/- 1.1
Urea	1.59 +/- 1.72 [b]	1.57 +/- 0.45 [b]	1.26 +/- 0.34 [b]	7.94 +/-1.91	1.01 +/- 0.64	7.8 +/- 1.4
Proline	0.94 +/- 0.62 [a]	1.37 +/- 0.31 [ab]	1.08 +/- 0.25 [a]	25.68 +/- 0.17	3.19 +/- 2.39	8.1 +/- 0.6
Control, CC9311	0.86 +/- 0.65 [a]	1.52 +/-0.70 [b]	0.99 +/-0.26 [a]	8.96 +/- 0.83	2.16 +/- 1.77	4.2 +/- 1.9

Coastal-spring Bloom *Synechococcus* Strain (CC9311)

The coastal-spring bloom *Synechococcus* strain (CC9311) cells varied to a broader degree in cell characteristics than the ocean-oligotrophic strain (WH8102). While N source-grown coastal-spring bloom (CC9311) cell volumes only differed slightly, length and width significantly varied among N sources. Coastal-spring bloom strain (CC9311) CN and C:N ratios were distributed over a broader range than the ocean-oligotrophic strain (WH8102) cells; however, these were not significant among N sources.

Cells grown in nitrate, glutamine, and glycine had volumes of approximately 1.03 μm^3 and were slightly larger than those grown in other N treatments (0.86 to 0.78 μm^3) (ANOVA, F= 4.1, d.f.=537, p=0.001, Tukey's HSD, Table 8). Cell volumes for all N treatments were greater than the volume of 0.53 μm^3 observed for the control.

Cell lengths of the coastal-spring bloom strain (CC9311) grown in N treatments ranged from 1.36 μm to 1.50 μm compared to 1.27 μm for the control. Alanine, proline, and glutamine treatments produced the longest cells with lengths ranging from 1.45- 1.50 μm; these cells were significantly longer than control cells (ANOVA, F=3.9, d.f.=536, p=0.001, Tukey's HSD). Nitrate-grown and ammonium-grown cell lengths were approximately 1.40 μm, similar to glycine-grown and urea-grown cell lengths at 1.37 μm.

Cell widths were not distributed similarly to cell lengths among the coastal spring bloom strain cells (CC9311) grown in different N sources. Most N treatments yielded cells with similar widths at approximately 1.03 μm, and with the exception of cells grown in alanine, were significantly narrower than control CC9311 cells (ANOVA, F=7.2, d.f.=536, p=0.001 Tukey's HSD). In addition, nitrate and glycine-grown cells were wider than alanine-grown cells.

Similar to the ocean-oligotrophic strain (WH8102), CN and C:N ratios of the coastal-spring bloom strain (CC9311) did not significantly differ among N source-grown cells. However C:N ratios represented a broad range from an ammonium-grown minimum of 5.1 to an alanine-grown maximum 10.9 (Table 8).

Table 8. Coastal-spring bloom *Synechococcus* strain (CC9311) cell dimensions (n>100) carbon and nitrogen content (fmol cell^{-1}, n=2,3), and molar C:N ratio (n=2,3). Cell volumes, lengths, and widths differed among N sources (ANOVA ($p<0.001$)). Carbon, nitrogen and C:N ratios did not differ among N sources (ANOVA ($p>0.05$)). Treatments with shared letters (A or B) did not differ significantly (Tukey's HSD, $p<0.05$). SD=1 standard deviation.

	Cell Volume (μm^3)	Cell Length (μm)	Cell Width (μm)	Carbon (fmol^{-1}cell)	Nitrogen (fmol^{-1}cell)	C:N Ratio (molar)
Nitrate	1.04 +/- 1.19[a]	1.41+/- 0.43[ab]	1.06 +/- 0.3[a]	33.0 +/- 9.0	4.8 +/- 1.72	6.9 +/- 0.8
Ammonium	0.86 +/- 0.37 [a]	1.40 +/- 0.34 [ab]	1.02 +/- 0.25 [ab]	73.1 +/- 81.7	14.4 +/- 18.1	5.1 +/- 3.2
Urea	0.78 +/- 0.28 [a]	1.38 +/- 0.26 [ab]	1.02 +/- 0.12 [ab]	53.9 +/- 17.9	6.2 +/- 1.2	8.8 +/- 1.5
Alanine	0.84 +/- 0.81 [a]	1.50 +/- 0.46 [a]	0.93 +/- 0.28 [abc]	6.6 +/- 0.8	0.4 +/- 0.3	10.9 +/- 1.9
Proline	0.88 +/- 0.81 [a]	1.45 +/- 0.42 [a]	1.02 +/- 0.22 [ab]	8.0 +/- 3.0	0.9 +/- 0.3	8.9 +/- 0.6
Glutamine	1.03+/- 1.03 [a]	1.50 +/- 0.46 [a]	1.02 +/- 1.02 [ab]	21.3 +/- 8.9	1.5 +/-1.4	9.6 +/- 1.7
Glycine	1.02 +/- 1.13 [a]	1.36 +/- 0.44 [ab]	1.07+/- 0.32 [a]	24.8 +/- 2.7	3.3 +/- 2.3	7.5 +/- 5.0
Control, CC9311	0.53+/- 0.28 [b]	1.27 +/- 0.29 [b]	0.87 +/- 0.17 [c]	24.4 +/- 4.9	3.0 +/- 1.1	8.4 +/- 1.4

Analysis of morphological and physiological coastal-spring bloom (CC9311) cell data demonstrated that relationships existed between CN and cell volume, CN and growth rate, and C:N and cell shape. Unexpectedly, there was no linear relationship between cell volume and CN. Instead the nutrient densities of several N source-grown cells appeared to cluster together (Fig. 7). For instance, alanine-grown and proline-grown cells showed smaller cell volumes (0.84 and 0.88 μm) and correspondingly lower CN (6.6, 0.4 and 8.0,0.9 fmol $cell^{-1}$, Table 8). In contrast, glycine-grown, nitrate-grown, and glutamine-grown cells showed the largest cell volumes (1.02, 1.04, and 1.03 μm), with intermediate CN (24.8, 3.3; 33, 4.8; and 21.3, 1.5 fmol $cell^{-1}$). Interestingly, urea-grown and ammonium-grown cells had comparably smaller cell volumes (0.78 and 0.86 μm) than most other N source-grown cells, but had the highest CN (53.9, 6.2 and 73.1, 14.4 fmol $cell^{-1}$), suggesting cells grown in ammonium and urea were the most nutrient dense.

Correlation analysis was used to explore the relationship between C:N ratio and cell shape (l:w ratio) among N source-grown coastal-spring bloom (CC9311) cells. Length:width ratios were not correlated to C:N ratio when all N source-grown cell data were included (Fig. 8, correlation coefficient r=0.72, p=0.067). However, because cells grown in ammonium seemed to have elevated CN for their size (exceptionally high nutrient density) correlation analysis excluding ammonium-grown cells was performed. When ammonium-grown cells were excluded, the increase in C:N correlated to the increase in l:w ratio (correlation coefficient r=0.93, p=0.008). Therefore, with the exception of ammonium-grown cells, cells with higher C:N ratios tended to be more elongated.

Not only did coastal-spring bloom (CC9311) growth rates vary among N sources, but the relationship between cell CN and growth rate was markedly different between cells grown in nitrate, urea, and ammonium versus amino acid treatments. The relationship between CN and growth rate was examined using linear regression. When the data were analyzed as a whole set, there was no clear relationship between CN and growth rate (Fig. 9, C: r^2=0.1, N: r^2=0.13). However, when data were separated into dominant natural N sources (nitrate, urea, and ammonium) versus amino acids (proline, glutamine, glycine, and alanine), CN increased as a function of growth rate within each N

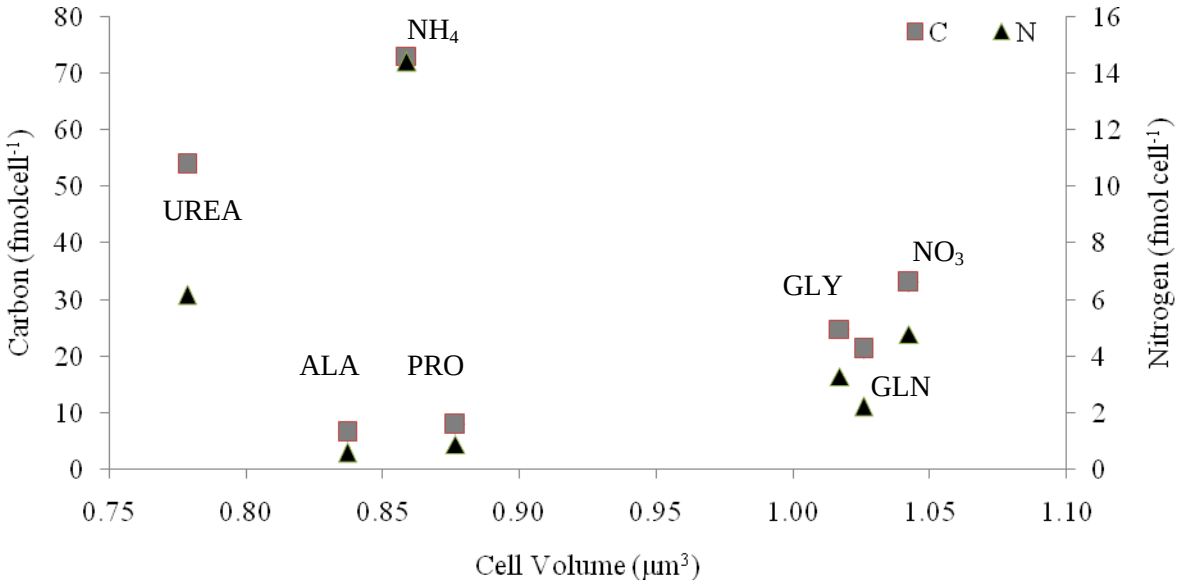

Figure 7. The mean carbon and nitrogen content (n=2,3; fmol/cell) versus cell volume for *Synechococcus* CC9311 (coastal spring-bloom) grown in single N sources.

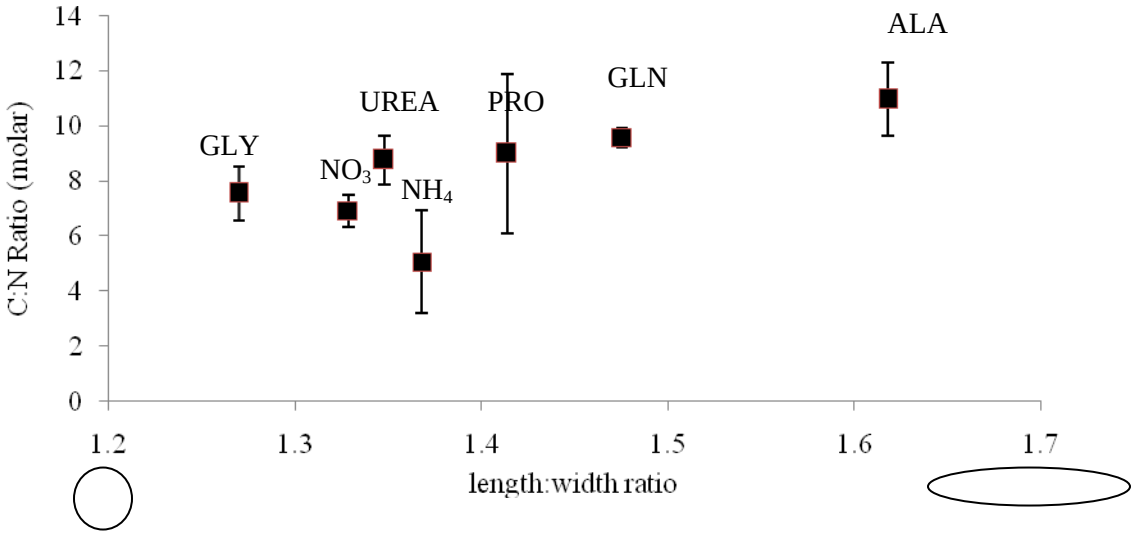

Figure 8. The relationship between mean cell length: width ratio (n>100) and mean cell C:N ratio(n=2,3, +/- 1 SE).

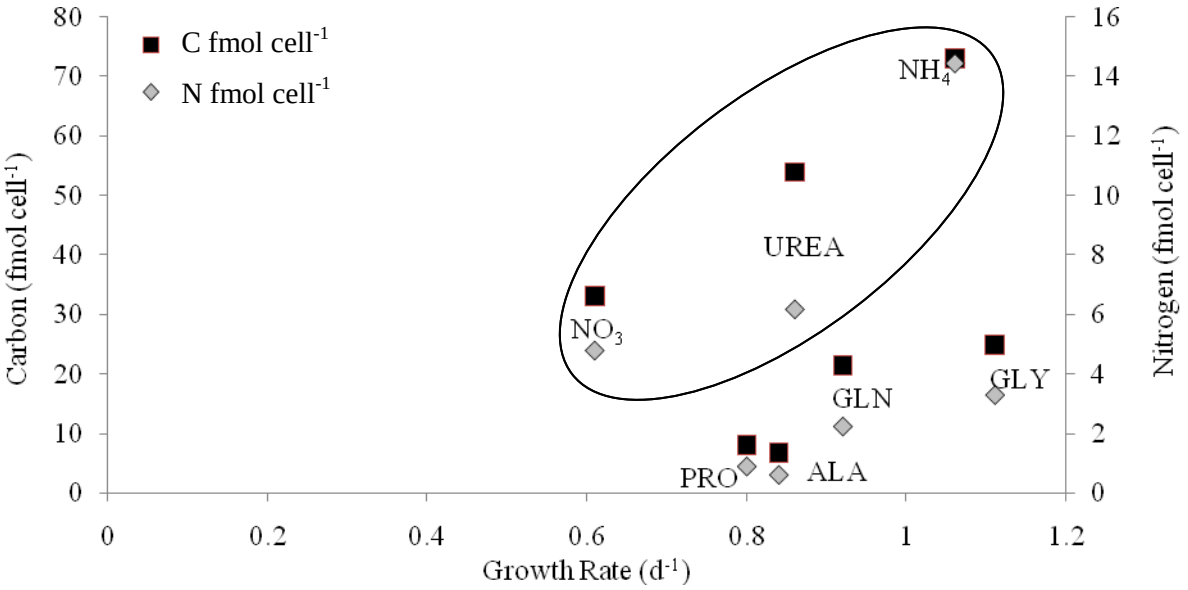

Figure 9. The relationship between mean cell carbon and nitrogen content (n=2,3 fmol) and growth rate (n=3, d^{-1}) for CC9311 (coastal-spring bloom strain) cells grown in single N sources. Carbon (NO_3,NH_4,UREA): y = 88.8x - 21.5, r^2 = 0.99. Nitrogen (NO_3,NH_4,UREA) y = 20.8x - 9.1, r^2 = 0.81. Carbon (amino acids): y = 59.4x - 39.3, r^2 = 0.78. Nitrogen (amino acids): y = 8.6x - 6.1, r^2 = 0.90.

source subset (Fig. 9). Means of CN and growth rate data were used because each N source-grown growth replicate did not have a direct CN match. The CN of the dominant N source-grown cells was substantially higher and increased with a steeper slope (C: r^2=0.99, N: r^2=0.81), than that of amino acid N source-grown cells (C: r^2=0.78, N: r^2=0.90). Results suggested the growth response of the coastal-spring bloom (CC9311) strain to more common N sources (ammonium, nitrate, and urea versus amino acids differed in regards to the amount of C and N incorporated as a function of growth rate.

Short Term, Timed Grazing Experiments

Overall, N source for ocean-oligotrophic strain (WH8102) growth had little effect on *O. marina* feeding rates regardless of incubation time period. Feeding rates of *O. marina* remained low on all N source-grown ocean-oligotrophic strain cells (WH8102). Only the fraction of the *O. marina* population feeding on ocean-oligotrophic strain cells (WH8102) implied that N source of prey may affect the termination (i.e. the timing of feeding cessation), but not the rate of grazing. Conversely, the growth-supporting N source of the coastal-spring bloom strain (CC9311) affected *O. marina* grazing rates for both time intervals examined (0-10 and 10-30 min). The fraction of the *O. marina* population feeding did not change over time. It appeared the growth-supporting N source of the coastal-spring bloom strain (CC9311) affected grazing rates, but not termination of grazing.

Ocean-oligotrophic *Synechococcus* Strain (WH8102) Grazed by *O. marina*

Average grazing rates of *O. marina* (OX) on ocean-oligotrophic strain (WH8102) grown in different N sources were uniformly low (0.01 to 0.02 Syn OX^{-1} min^{-1}) over the first 10 min of incubation (ANOVA, F=42, d.f.=18, $p<0.001$, Fig. 10, Table 9). Grazing rates on ocean-oligotrophic

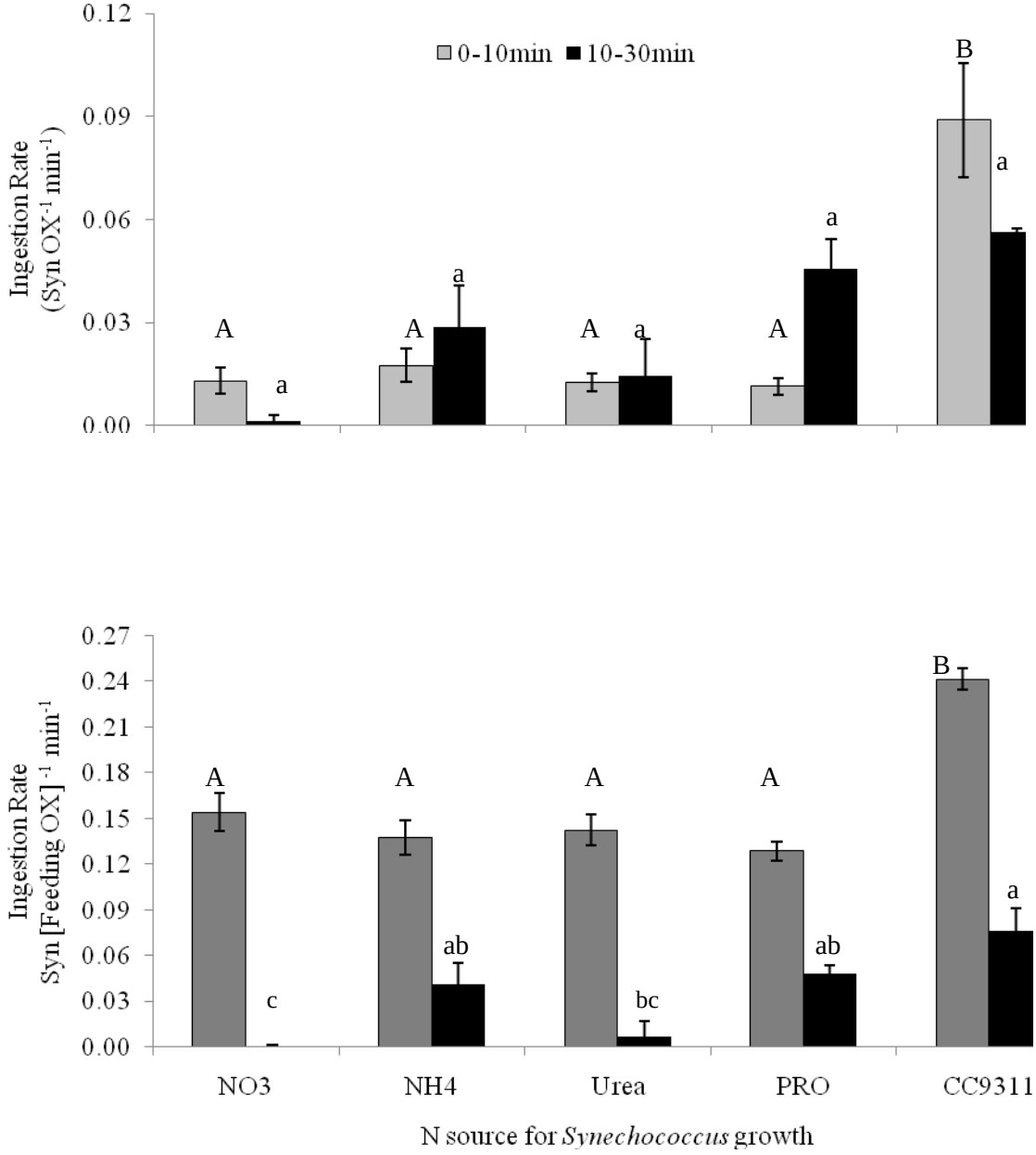

Figure 10. Ingestion rates (Syn OX^{-1} min^{-1}(top) Syn [Feeding OX]$^{-1}$ min^{-1} (bottom)) of *O. marina* (n=4, +/- 1 SE) on ocean-oligotrophic *Synechococcus* strain (WH8102) grown on single N sources and on control diet CC9311. Rates for 0-10 (A,B) and 10-30 (abc) min time intervals are shown. Treatments with shared letters (A,B or a,b) are not significant (Tukey's HSD, $p<0.05$).

Table 9. *Oxyrrhis marina* mean grazing rate on the ocean-oligotrophic *Synechococcus* strain (WH8102) (n=4). Grazing rates differed among N sources (one-way Anova ($p<0.001$)). Treatments with shared letters (A or B) did not differ significantly (Tukey's HSD, $p<0.05$). SD=1 standard deviation

	Ingestion Rate Syn OX $^{-1}$ min^{-1}		Ingestion Rate Syn [FeedingOX $^{-1}$] min^{-1}	
Time	0-10 min	10-30 min	0-10 min	10-30 min
N Source	Mean +/- SD	Mean +/- SD	Mean +/- SD	Mean +/- SD
Nitrate	0.01 +/- 0.02^{A}	0.00 +/- 0.00^{a}	0.15 +/- 0.03^{A}	0.01 +/- 0.02^{c}
Ammonium	0.02 +/- 0.01^{A}	0.03 +/- 0.02^{a}	0.14 +/- 0.02^{A}	0.04 +/- 0.03^{ab}
Urea	0.01 +/- 0.01^{A}	0.02 +/- 0.02^{a}	0.14 +/- 0.02^{A}	0.01 +/- 0.02^{bc}
Proline	0.01 +/-0.00^{A}	0.05 +/- 0.02^{a}	0.13+/- 0.01^{A}	0.05 +/- 0.01^{ab}
CC9311 Control	0.09 +/- 0.08^{B}	0.06 +/- 0.00^{a}	0.24+/- 0.01^{B}	0.08 +/- 0.02^{a}

strain (WH8102) grown in all N sources were significantly less than the rate on control CC9311 cells (0.09 Syn OX^{1} min^{-1}, Tukey's HSD).

Similarly, grazing rates of *O. marina* on ocean-oligotrophic strain (WH8102) were comparable among N sources for 10-30 min time period. Mean grazing rates increased, decreased, or remained the same between 0-10 and 10-30 min time intervals on ocean-oligotrophic strain (WH8102) grown in N sources (Fig. 10, Table 9). *Oxyrrhis marina* grazing on the control CC9311 and nitrate-grown ocean-oligotrophic strain (WH8102) decreased from 0.09 to 0.06 and from 0.02 to <0.01 Syn OX^{-1} min^{-1} respectively. In contrast, proline-grown ocean-oligotrophic strain (WH8102) cells experienced an increased grazing rate from 0.01 to 0.05 Syn OX^{-1} min^{-1}. Grazing rates on ammonium-grown and urea-grown ocean-oligotrophic strain (WH8102) cells remained constant from 0-10 and 10-30 min.

When considering only the fraction of the *O. marina* population feeding, grazing rates of *O. marina* on the ocean-oligotrophic strain (WH8102) grown in N treatments (0.13 to 0.15 (Syn [Feeding OX^{-1}] min^{-1}) were significantly lower than the grazing rates on control CC9311 cells (0.24 Syn [Feeding OX^{-1}] min^{-1}) (ANOVA, F=18, d.f.=18, p=0.001, Tukey's HSD, Fig. 10, Table 9). However, there were no differences among *O. marina* grazing on ocean-oligotrophic strain (WH8102) cells grown in different N sources during the first 10 min of incubation.

In contrast to observations with the total *O. marina* population, mean grazing rates of the fraction of the *O. marina* population feeding decreased substantially (to 0.01 to 0.05 Syn [Feeding OX^{-1}] min^{-1}) between 0-10 and 10-30 min for all N source-grown ocean-oligotrophic (WH8102) cells (ANOVA, F=9.77, d.f.=18, p=0.004, Tukey's HSD, Fig. 10, Table 9). Furthermore, while all N source-grown ocean-oligotrophic strain (WH8102) cells were grazed at lower rates than control CC9311 for the initial 10 min, only *O. marina* grazing rates on nitrate-grown and urea-grown ocean-oligotrophic (WH8102) cells remained significantly lower than grazing on control CC9311 for 10-30 min. Within N source-grown ocean-oligotrophic strain cells (WH8102), grazing rates on nitrate-grown cells were significantly less than grazing rates on ammonium-grown and proline-grown cells.

The fraction of the *O. marina* population feeding on the ocean-oligotrophic (WH8102) strain changed over time, but the direction of change depended on N source. Regardless of N source for growth, the fraction of *O. marina* population feeding on the ocean-oligotrophic strain (WH8102) was similar at 10 and 20 min. However, the fraction feeding differed at 30 min and was dependent on N source (repeated measures ANOVA, Time: $F=20.0$, d.f.=1.895, $p<0.001$, Tim*N source: $F=3.84$, d.f.=7.58, $p=0.005$, Fisher's LSD, Fig. 11, Table 10). At 10 min, approximately 0.10 of the total *O. marina* population was feeding on the ocean-oligotrophic strain (WH8102) across all N treatments. By 20 min, the fraction of the *O. marina* population feeding had significantly increased to approximately 0.20. In contrast, 0.35 of the total *O. marina* population fed on control CC9311 over the entire time course. The fraction of the *O. marina* population feeding on all ocean-oligotrophic (WH8102) N-grown cells at 20 min was not different from 30 min. This is due to the significant interaction term of N source and time, where N source controlled variation in the fraction of the *O. marina* population feeding at 30 min; this fraction ranged from the minimum of 0.14 on nitrate-grown ocean-oligotrophic (WH8102) cells to the maximum of 0.46 on proline-grown ocean-oligotrophic (WH8102) cells.

When comparing the average fraction of the *O. marina* population feeding among N source-grown ocean-oligotrophic (WH8102) cells and control strain CC9311 across all time points, feeding on control strain CC9311 was significantly greater than the fraction of the *O. marina* population feeding on any ocean-oligotrophic strain (WH8102) diet. In addition, the fraction of the *O. marina* population feeding on proline-grown cells was significantly greater than on nitrate-grown cells (Tukey's HSD). Temporal variation in the fraction of the *O. marina* population feeding was dependent on *Synechococcus* N source for growth. *Oxyrrhis marina* individuals stopped feeding on nitrate-grown ocean oligotrophic (WH8102) cells between 20 and 30 min, whereas more *O. marina* started feeding on proline-grown and no change was seen in the fraction of *O. marina* feeding on urea-grown cells between 20 and 30 min.

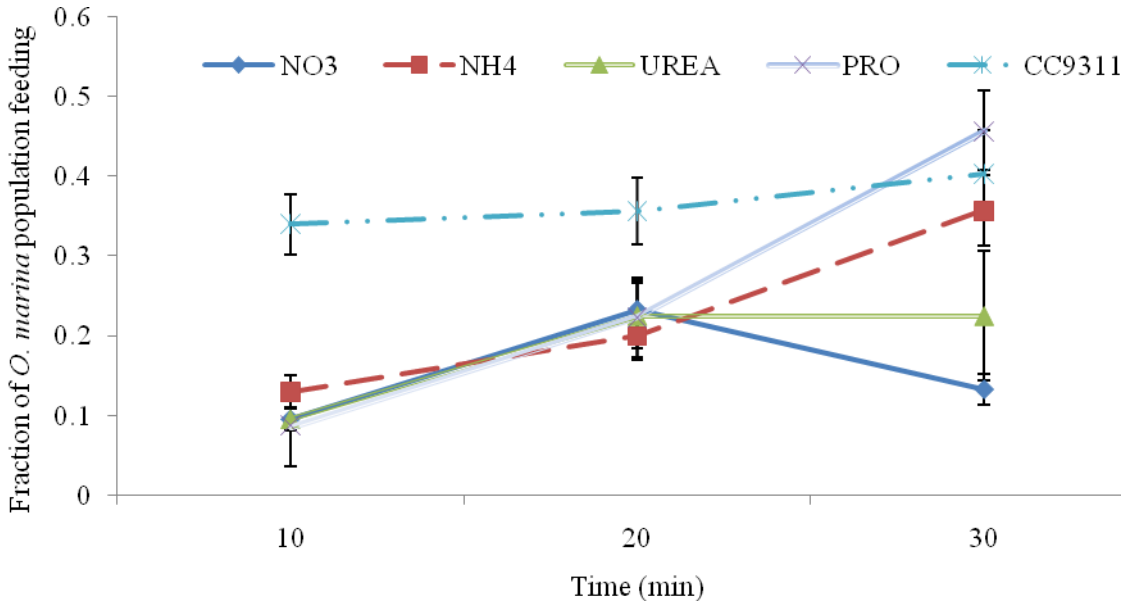

Figure 11. The mean fraction of the *O. marina* population feeding on the ocean-oligotrophic *Synechococcus* strain (WH8102) grown in N treatments for times 10, 20, and 30 min (n=4, +/- 1 SE).

Table 10. Fraction of *O. marina* population feeding on the ocean-oligotrophic *Synechococcus* strain WH8102 for time points 10, 20, and 30 min (n=4). The fraction of *O. marina* feeding were different based on the factors time, N source, and the interaction term (time*N source) (ANOVAR ($p<0.001$)). Treatments (N Source factor only) with shared letters (A or B) did not differ significantly (Fischer's LSD ($p<0.05$). SD=1 standard deviation

N source	Mean (+/- SD) fraction of *O. marina* population feeding at 10 min	Mean (+/- SD) fraction of *O. marina* population feeding at 20 min	(Mean +/- SD) fraction of *O. marina* population feeding at 30 min
Nitrate	0.10 +/- 0.03 [c]	0.23 +/- 0.07 [c]	0.13 +/- 0.04 [c]
Ammonium	0.13 +/- 0.04 [bc]	0.20 +/- 0.06 [bc]	0.36 +/- 0.09 [bc]
Urea	0.10 +/- 0.03 [bc]	0.23 +/- 0.08 [bc]	0.23 +/- 0.16 [bc]
Proline	0.09 +/- 0.03 [b]	0.22 +/- 0.09 [b]	0.46 +/- 0.0 [b]
CC9311, Control	0.34 +/- 0.08 [a]	0.36 +/- 0.09 [a]	0.40 +/-0.11 [a]

Synechococcus* Strain CC9311 Grazed by *O. marina

Oxyrrhis marina grazed several N source-grown coastal-spring bloom (CC9311) diets at higher rates than others, but similar to *O. marina's* grazing behavior while feeding on the ocean-oligotrophic strain (WH8102), grazing rates on all diets changed between 0-10 and 10-30 min. During the first 10 min *O. marina* grazed urea-grown coastal-spring bloom (CC9311) cells at a significantly higher rate than all other N source-grown coastal-spring bloom (CC9311) cells (0.26 versus 0.04 Syn OX^{-1} min^{-1}) and the control CC9311 (0.05 Syn OX^{-1} min^{-1} , ANOVA, F=3.3, d.f.=30, p=0.015, Tukey's HSD, Fig. 11, Table 11). Similar to the ocean-oligotrophic strain (WH8102) data, average grazing rates of *O. marina* decreased between 0-10 and 10-30 min. In contrast to the initial 10 min, grazing rates between 10-30 min on urea-grown cells (-0.02 Syn OX^{-1} min^{-1}) were lower than grazing rates on ammonium-grown and glutamine-grown cells (0.04 Syn OX^{-1} min^{-1}, ANOVA, F=2.18, d.f.=25, p=0.08, Fig. 12, Table 11).

When considering only the feeding portion of the *O. marina* population (10 min), *O. marina* grazing rates on urea-grown cells were again significantly higher than on cells grown in all other N treatments and the control (ANOVA, F=5.5, d.f.=26, p<0.001, Tukey's HSD, Fig. 12, Table 11). Grazing rates were highest on urea-grown cells (0.38 [Syn Feeding OX^{-1}] min^{-1}) and lowest on proline-grown cells (0.16 Syn [Feeding OX^{-1}] min^{-1}); grazing rates on the control (0.17 Syn [Feeding OX^{-1}] min^{-1}) were only slightly higher than this minimum. For 10-30 min, grazing on urea-grown cells (0.01 Syn [Feeding OX^{-1}] min^{-1}) was significantly lower than grazing on other N source-grown coastal-spring bloom (CC9311) cells (0.05 to 0.10 Syn^{-1} [Feeding OX^{-1}] min^{-1}). As in the majority of grazing data sets, average *O. marina* grazing rates during 10-30 min decreased relative to the initial 10 min for all coastal-spring bloom (CC9311) N source-grown diets (ANOVA, F=3.1, d.f.=25, p=0.026, Fig. 12, Table 11).

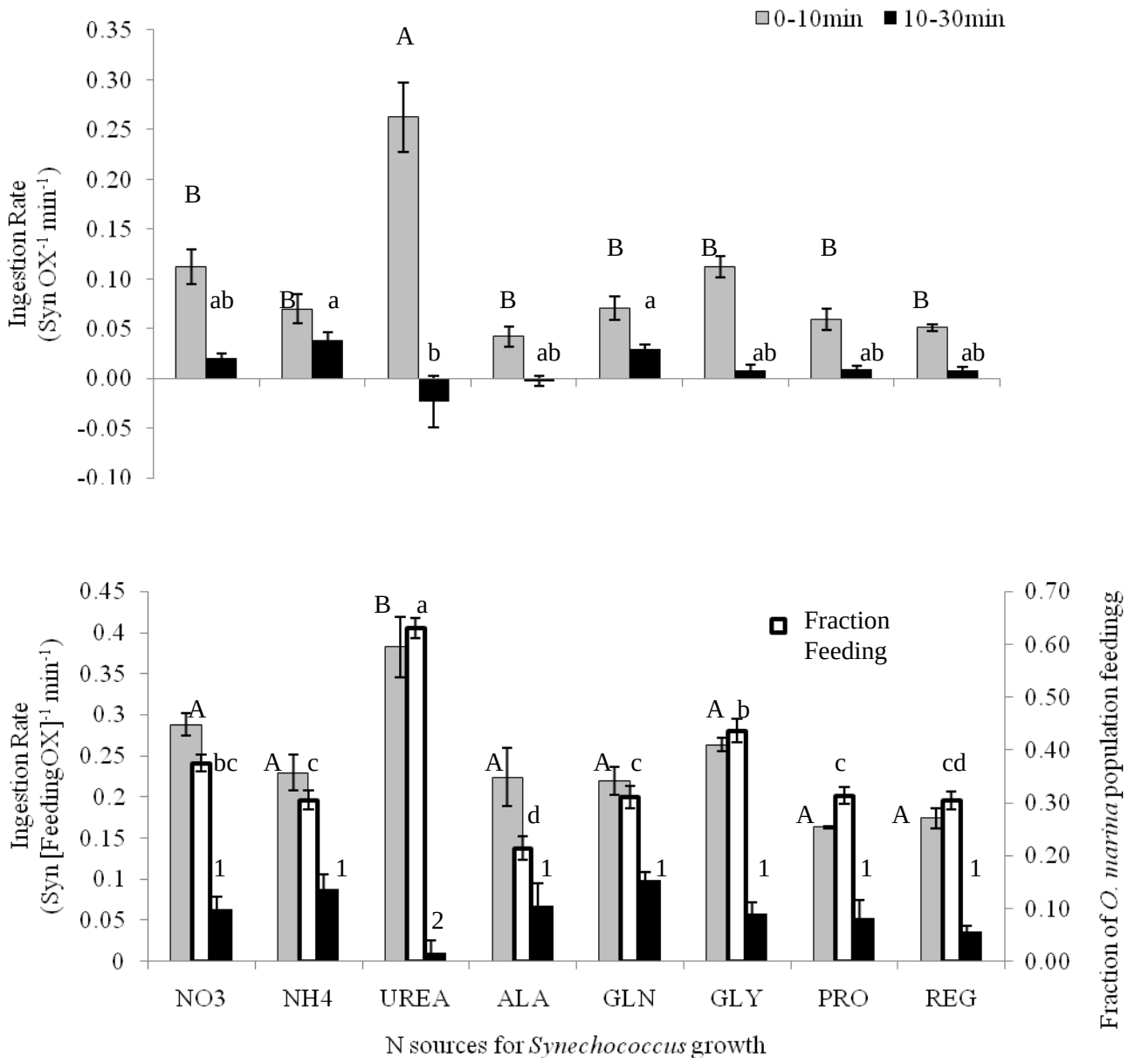

Figure 12. The ingestion rates of the *O. marina* population (Syn $OX^{-1}min^{-1}$; AB,ab) and of the feeding *O. marina* population (Syn [Feeding OX^{-1}] min^{-1}; 1,2) are displayed during two time intervals [0-10 (AB) and 10-30 min (ab) n=4, +/- 1 SE]. The mean fraction of the *O. marina* population feeding is also shown (1,2). *O. marina* diets were coastal-spring bloom *Synechococcus* strain (CC9311) grown on single N sources, and control diet CC9311 (REG). Treatments with shared letters or numbers (A,B; a,b; or 1,2) are not significantly different.

Table 11. *Oxyrrhis marina* mean grazing rates on the coastal-spring bloom *Synechococcus* strain (CC9311) grown in single source N treatments for both the total grazer population (Syn OX $^{-1}$ min^{-1}) and the feeding portion of the grazer population (Syn [Feeding OX $^{-1}$] min^{-1}). Rates are shown for two time intervals (n=4). Grazing rates differed among N sources (ANOVA ($p<0.001$)). Also shown is the mean fraction of the *O. marina* population feeding on *Synechococus* strain CC9311 (n=3). The fraction of *O. marina* feeding differed among N sources (ANOVA ($p<0.001$). Treatments with shared letters (A or B) did not differ significantly (Tukey's HSD, $p<0.05$). SD=1 standard deviation.

	Mean Ingestion Rates +/- SD (Syn OX $^{-1}$ min^{-1})		Mean Ingestion Rates +/- SD (Syn [Feeding OX $^{-1}$] min^{-1})		Mean fraction of feeding *O. marina* population +/- SD
N source	0-10 min	10-30 min	0-10min	10-30min	0-30 min
Nitrate	0.11 +/- 0.03^{B}	0.02 +/- 0.01^{ab}	0.29 +/-0.02^{A}	0.06 +/- 0.03^{1}	0.38 +/- 0.03^{bc}
Ammonium	0.07 +/- 0.03^{B}	0.04 +/- 0.02^{a}	0.23 +/-0.04^{A}	0.09 +/- 0.03^{1}	0.31 +/- 0.03^{c}
Urea	0.26 +/- 0.07^{A}	0.03 +/- 0.05^{b}	0.38 +/- 0.07^{B}	0.01+/- 0.03^{2}	0.63 +/- 0.04^{a}
Alanine	0.04 +/- 0.01^{B}	0.00 +/- 0.01^{ab}	0.22 +/-0.05^{A}	0.07 +/- 0.06^{1}	0.21 +/-0.04^{d}
Glutamine	0.07 +/- 0.02^{B}	0.03 +/- 0.00^{a}	0.22 +/-0.03^{A}	0.1 +/- 0.02^{1}	0.31 +/- 0.04^{c}
Glycine	0.11 +/-0.02^{B}	0.01+/- 0.01^{ab}	0.26+/-0.02^{A}	0.06 +/- 0.02^{1}	0.44 +/- 0.04^{b}
Proline	0.06+/- 0.02^{B}	0.01 +/- 0.01^{ab}	0.16 +/-0.00^{A}	0.05 +/- 0.04^{1}	0.31 +/- 0.03^{c}
CC9311, Control	0.05+/-0.01^{B}	0.01+/- 0.00^{ab}	0.17 +/-0.03^{A}	0.04 +/- 0.01^{1}	0.30 +/- 0.03^{cd}

The fraction of the *O. marina* population feeding on the coastal-spring bloom strain (CC9311) remained constant over time within N treatments, but varied widely among treatments (Fig. 12, Table 11). The average fraction of *O. marina* feeding on coastal-spring bloom (CC9311) cells ranged from 0.21 on alanine-grown cells to 0.63 on urea-grown cells. The fraction of *O. marina* feeding on urea-grown cells was significantly greater than the fraction feeding on cells grown in all other N treatments and control. Similarly, a greater fraction of the *O. marina* population fed on cells grown in the glycine treatment compared to cells grown in treatments ammonium, glutamine, proline, alanine and CC9311 control cells (ANOVA, $F=30.5$, d.f.=84, $p<0.001$, Tukey's HSD). The fraction of the *O. marina* population feeding on cells grown in the treatments nitrate, ammonium, glutamine, and proline were significantly greater than cells grown in the alanine treatment.

DISCUSSION

As a diverse and abundant genus of the picophytoplankton, *Synechococcus* strains are expected to show diverse growth responses to N source and to exhibit this in their cell morphology and physiology. Variation in susceptibility to grazing should also exist among strains and possibly even within a strain under various growth conditions. Interestingly, while many biogeography studies have described clades as "nutrient-replete" or "oligotrophic," variation in strain response to N source was not consistent within nutrient-replete or oligotrophic clades in my study. Clades associated with either nutrient-replete or oligotrophic conditions were composed of strains that grew on all N sources at high growth rates, as well as strains that did not grow, strains that grew at lower growth rates, or strains that were inhibited by several amino acids. While similarities existed among nutrient-replete and oligotrophic clades, in-depth morphological and physiological analysis of an ocean-oligotrophic strain (WH8102) and a coastal-spring bloom strain (CC9311) revealed differences in N response on the cellular level. Among single N sources, the coastal-spring bloom isolate (CC9311) showed a wide range of cell sizes, cell shapes, cell CNs, and cell C:N molar ratios. In contrast, ocean-oligotrophic (WH8102) cells showed only subtle variation among single N sources.

Additionally, in contrast to *O. marina* grazing rates on the ocean-oligotrophic strain (WH8102), *O. marina* grazing rates on the coastal-spring bloom strain (CC9311) were comparatively higher and markedly different among N sources. Results suggest *O. marina's* elevated and variable grazing rates on the coastal-spring bloom strain (CC9311) were related to the strain's cell shape, CN (fmol cell^{-1}), and C:N ratio. Despite *O. marina's* consistently low grazing rates on the ocean-oligotrophic strain (WH8102), there were subtle differences in *O. marina* grazing rate on N source-grown ocean-oligotrophic cells (WH8102). These subtle differences in grazing rates may be due to the minimal variation of the prey cell's shape, size, CN, and C:N ratio among N source-grown ocean-oligotrophic cells (WH8102). In any case, *O. marina* grazing rates varied among N source-grown

cells for both strains, but the magnitude and apparent source of variation were not consistent between strains. Nitrogen source clearly plays a role in the morphology, physiology, and grazing susceptibility of *Synechococcus*; however, its magnitude of impact is strain dependent.

Synechococcus Response to Single N Sources

Several growth features were common to all *Synechococcus* strains in this study. Before growth in single N source media, *Synechococcus* growth rates differed significantly among strains. My results contrast with previous data showing that rates did not differ among strains; however, previous growth was in SN medium in 12:12 L:D cycles (Apple et al., submitted). The differences in growth rates of *Synechococcus* strains cultured in continuous light compared to 12:12 L:D cycles were not consistent. Compared to their 12:12 SN medium counterparts, growth rates of continuous light-grown coastal-spring bloom (CC9311) and coastal-oligotrophic (CC9605) strains increased, while coastal-dominant (CC9902) and ocean-oligotrophic (WH8102) strain growth rates decreased. Mutant strains (JMS40 and SIO7B) growth rates remained similar to previous values (data not shown). Variation in growth rates between studies is most likely due to the differing photoperiods.

Regardless of their growth rate under N-replete conditions (pre-add growth rates), results suggest *Synechococcus* strains grew faster after N limitation (e.g. when transferred into the single N source experiments)**.** A previous study measured N uptake and growth rates for both a coastal and an open ocean *Synechococcus* strain. Similar to my findings, both uptake and growth rates were higher in cultures that had been transferred from N deplete to N replete conditions when compared to rates prior to transfer. In addition, within 15 min of transfer from N-deplete to N-replete medium, *Synechococcus* cultures had incorporated 50% of the total NH_4 they would accumulate in the next 60 min (Glibert, 1990). Flynn (2009) argued that severe N limitation can disrupt N transport systems, but mild N limitation increases N transport, potentially by an order of magnitude. While my data support the latter interpretation, my pilot studies illustrate the former concept. For example, in pilot studies,

Synechococcus strains transferred several days after reaching N-limited stationary phase did not grow and the experiment had to be repeated with transfers made earlier in stationary phase.

The diverse growth responses of strains isolated from the California Current (CC9311, CC9902, and CC9605) supports N's important role in the niche differentiation that creates microdiversity in planktonic communities (Ahlgren et al., 2006). For example, the environmental clades I and IV make up the majority of the *Synechococcus* population in the California Current. While clade IV is often dominant, clade I dominates prior to the spring bloom. Often associated with oligotrophic conditions, clades II and III remain at low levels throughout the year (Tai et al., 2009). From these observations, the coastal-dominant strain (CC9902), the clade IV representative in my study, would be expected to grow on a wider range of N sources than the coastal-spring bloom (CC9311) and coastal-oligotrophic (CC9605) strains. Unexpectedly, the coastal-dominant strain (CC9902) grew on fewer N sources and grew more slowly than the coastal-spring bloom (CC9311) and coastal-oligotrophic (CC9605) strains. In further contrast to the other coastal strains, for unexplained reasons the amino acids alanine and glycine inhibited coastal-dominant strain (CC9902) growth; both amino acids are suggested substrates for transporters in that strain. The coastal-dominant strain (CC9902) did grow on proline and glutamine, which interestingly have not been hypothesized as substrates for transporters (Paulsen et al., 2010).

Despite its relatively limited ability to grow on a broad range of N sources, the low growth rates observed in the coastal-dominant strain (CC9902) may be under advantageous selection and contribute to clade IV's dominance in temperate global oceans, in addition to its dominance in the California Current (Scanlan, et al. 2009; Zwirglmaier et al., 2008). Flynn (2009) has recently proposed that low maximum growth rates are advantageous because slow growing cells avoid the repercussions of short and long-term exposure to nutrient stress that can affect cell viability and the ability to recover during nutrient pulses. According to the model (Flynn, 2009), high nutrient acquisition rates are not important to a slow growing-adapted organism; rather, the rate of nutrient supply versus cellular demand will determine its competitive success. Therefore having excess

transporters or ability to grow on diverse N sources may not be as important for the coastal-dominant strain (CC9902) as maintaining its slow growth rate, especially if it has defenses against mortality.

In contrast, the coastal-spring bloom strain (CC9311) may employ a high growth rate strategy. Compared to all other studied strains, the coastal-spring bloom strain (CC9311) has the greatest number of genes encoding membrane transport proteins (Paulsen et al., 2010), which could possibly explain its ability to grow on all N sources tested in this study. The coastal-spring bloom strain's (CC9311) high growth rates in response to N resupply may give it a selective advantage over the coastal-dominant strain (CC9902) and contribute to clade I's dominance in the California Current during periods of increased nutrient input, prior to the spring bloom (Tai et al., 2009). Genomic studies of the coastal-spring bloom strain (CC9311) have further revealed it has many opportunist adaptations including metal-dependent and ammonium/nitrate transporters and the ability to use diverse organic matter sources, light levels, and iron concentrations (Palenik et al., 2006; Palenik et al., 2003).

Similar to the coastal-dominant and coastal-spring bloom strains (CC9902 and CC9311), the coastal-oligotrophic and ocean-oligotrophic strain exhibited diverse N responses, suggesting that diverse growth strategies are widely distributed. In contrast to clades I and IV, clades II and III appear in stratified waters; the coastal-oligotrophic strain (CC9605) has been observed to be more prevalent in oligotrophic offshore sites and the ocean-oligotrophic strain (WH8102) is found in the oligotrophic Sargasso Sea (Toledo, 2003). Interestingly, the coastal-oligotrophic strain's (CC9605) N response was more similar to that of coastal-spring bloom strain (CC9311) than to that of the ocean-oligotrophic strain (WH8102). The coastal-oligotrophic strain (CC9506) also had a higher growth rate than the ocean-oligotrophic (WH8102) and coastal-dominant strains (CC9902), again similar to the coastal-spring bloom strain (CC9311). Maximum growth occurred on glycine for the coastal-spring bloom (CC9311), coastal-oligotrophic (CC9605), and ocean-oligotrophic (WH8102) strains, suggesting that this amino acid may be particularly relevant for *Synechococcus* growth in diverse environments. Coastal-oligotrophic (CC9605) and ocean-oligotrophic (WH8102) strains both grew on

all N sources offered, but the ocean-oligotrophic strain (WH8102) and mutants (JMS40 and SIO7B) were the only strains to grow in the No-N control. It is possible that N was present below detection limits in the seawater used to make media and that the ocean-oligotrophic strain (WH8102) was able to use N at these low levels. Blooms of clade III *Synechococcus* have been observed in low N areas. For example, Glover et al. (2007) showed that nanomolar N levels stimulated *Synechococcus* spp. blooms and luxury consumption, in which N uptake exceeds the cells metabolic requirement, in the Sargasso Sea.

Slight genetic differences that affect the cell surface and motility of ocean-oligotrophic strain (WH8102) and mutants (JMS40 and SIO7B) appear to affect their ability to use N sources. Specifically, mutant JMS40 lacks SwmB, the punctate 1.126 MD protein, and mutant SIO7B lacks SwmA, the S-layer protein. In contrast to the ocean-oligotrophic strain (WH8102), mutant strain growth was inhibited by alanine, glycine, and/or glutamine. Different cell surface proteins among the mutants and the ocean-oligotrophic strain (WH8102) could be directly or indirectly involved in the synthesis or stabilization of membrane transport proteins essential for N uptake or metabolism. However, visualization of the cell surface showed that major and minor cell surface proteins were not affected by the disappearance of SwmA or SwmB (McCarren 2005, 2007; Brahamsha, 1996). The varying levels of motility, conferred by the presence of SwmA and SwmB among ocean-oligotrophic (WH8102) and mutant strains, could explain the mutants' inability to grow in amino acids. In contrast to the non-motile mutants, the ocean-oligotrophic strain (WH8102) has the ability to chemotax to N gradients (Waterbury, 1989). Mutant SIO7B does retain some rotation abilities, but loses all thrusting ability (McCarren et al., 2005, 2007). Increased movement capabilities could increase diffusion across the cell surface, thus increasing the available N to the cell. A recent study hypothesized that motility reduced the cell boundary layer thickness in instances where N uptake rates exceeded maximum diffusion rates (Sunda et al., 2010). Motility may have only affected amino acid-supported growth because, for whatever reason, the cell needs to acquire a higher concentration of amino acids

then it does other N sources for growth. The different growth responses of mutants and WH8102 suggest that SwmA and SwmB may play direct and/or indirect roles in N acquisition.

In addition to growth rates, cell morphological and physiological features varied among N source treatments to a greater extent for the coastal-spring bloom strain (CC9311) than the ocean-oligotrophic strain (WH8102). Morphological characteristics included cell volume and cell shape [length:width ratio (l:w)], while physiological characteristics included CN and C:N ratios. Overall, N source-grown ocean-oligotrophic strain (WH8102) and coastal-spring bloom strain (CC9311) were comparable in volume, ranging from 0.73 to 1.59 and 0.78 to 1.04 μm^3, respectively. However, l:w ratios indicated N source-grown ocean-oligotrophic strain cells (WH8102) were generally rounder (l:w 1.24-1.33) than the more elongated N source-grown coastal-spring bloom strain (CC9311) cells (l:w 1.27-1.62). There are many diverse abiotic and biotic factors that can affect in cell size and shape of *Synechococcus.* It is unlikely that slight variations in WH8102 cell shape and size were due to differing nutrient limitation as cell CN and C:N were not related to cell volume or shape. In addition, the ocean-oligotrophic strain (WH8102) grew in the No-N control, indicating it had adequate N supply. The ability of the ocean-oligotrophic strain (WH8102) to grow efficiently at sub-micromolar N levels may explain its lack of variability relative to the coastal-spring bloom strain (CC9311), which may have a higher or more specific N demand than its ocean-oligotrophic counterpart (WH8102).

The coastal-spring bloom strain (CC9311) contrasted with the ocean-oligotrophic strain (WH8102), in that the coastal-spring bloom strain (CC9311) morphology reflected its physiological state. With the exception of ammonium-grown cells, the CN content of the coastal-spring bloom strain (CC9311) increased as cell volume increased. Urea and ammonium-grown cells both had higher CN in relation to their cell volumes (i.e higher nutrient densities than cells from other N sources). This may indicate that CC9311 is better suited to acquire and assimilate these two N sources. Ammonium has been suggested to be the preferred N source of *Synechococcus*; however, less is known about urea-supported production.

Previous work has also shown that N sources alter the biochemistry of autotrophic cells. For instance, Liotenberg et al. (1996) demonstrated that N source affected intracellular proteins involved in pigmentation and glycogen reserves using a filamentous cyanobacteria strain, *Calothrix sp.* Madariaga et al. (1992) found elemental composition of *Pavlova lutheri* was similar among ammonium, nitrate, and glycine-grown cells, the composition of carbohydrates, amino acids, and lipids was dependent on a growth-limiting factor. The coastal-spring bloom strain (CC9311) in this study could be experiencing similar changes in intracellular compounds, explaining its variable nutrient density among N sources.

Despite the scattered distribution of CN relative to cell volume for N source-grown coastal-spring bloom strain cells (CC9311), CN was not clearly related to cell shape (l:w). However, elongated coastal-spring bloom strain (CC9311) cells in my experiment were suggested to be N-limited in comparison to their spherical counterparts; thus cell shape did reflect nutritional status to some extent. Specifically, C:N ratios of N source-grown coastal-spring bloom strain cells (CC9311) increased with increasing l:w ratios with the exception of ammonium-grown cells, which had an anomalously low C:N for their given l:w ratio (Fig. 7). Nitrogen limitation in elongated cells is not an uncommon phenomenon as previous studies have shown that N concentration is inversely correlated with bacteria cell length (Malitis, 2004). Hahn et al. (1999) suggested that, because cell size is affected by increase in biomass and frequency of replication, elongated cells result from increased growth rate or decreased cell division. The most elongated CC9311 cells in my study were associated with the highest C:N ratios. This indicates that N-limited CC9311 cells decreased cell division while still accumulating biomass, which resulted in an increase in length.

In addition, the linear relationship between the coastal-spring bloom strain's (CC9311) growth rate and cell CN (Fig. 8) further showed that the coastal-spring bloom strain (CC9311) may prefer certain N sources to others (i.e. ammonium and urea), while the ocean-oligotrophic (WH8102) cells appear to have similar physiology and morphology when grown in all N sources. Growth rate of coastal-spring bloom strain (CC9311) did not appear to be related to morphological characteristics (v,

l:w), but there was an overall tendency for cells growing at higher rates to have higher CN. However, cells grown in more common N sources such as nitrate, urea, and ammonium had higher CN for their growth rates than cells grown on amino acids. This phenomenon was not observed in the ocean-oligotrophic strain (WH8102). Madariaga et al. (1992) showed similar effects in which *Pavlova lutheri*'s photosynthetic efficiency and its assimilation rates of C and N rates were related to growth in N sources (ammonium, nitrate, and glycine). As representatives of contrasting environments, the ocean-oligotrophic strain (WH8102) and the coastal-spring bloom strain (CC9311) display diverse growth responses to N source, apparent in growth rate, cell dimension, cell CN content and C:N ratio.

Results suggest that N is not the predominant driver, but is a formidable factor that governs *Synechococcus* distribution on spatial and temporal scales. Clearly, demonstrated by their contrasting physiological and morphological features, a coastal strain and an oceanic strain responded to N sources differently. Furthermore, within the California Current environment, the predominant coastal clade's (IV) representative is the slow growing, picky N user and the minor clades' (I,II) representatives are the fast growing opportunistic N users. While physiology and genomic study of isolates from a particular region are useful, one cannot expand conclusions from one or a few strains to an entire clade. The process of isolation and culturing of strains is selective in nature and underestimates microdiversity (Lakeman et al., 2009). Furthermore, while in culture many strains can alter ecologically relevant characteristics, including growth rate (Flynn, 2009), nonetheless, based on a large amount of information, from metagenome transects to physiological study of laboratory isolates, it is often suggested that natural selection occurs on *Synechococcus* strains on the "scale of physiochemically defined oceanic provinces" such as open ocean gyres (oligotrophic) or upwelling areas (coastal) (Hewson et al. 2009, p. 1989). Nitrogen responses of strains used in the present study did not conclusively support the hypothesis that strains isolated from different environments retain characteristic N adaptations that appear to be relevant to the geographical province where they predominantly occur. In particular, elevated growth rates and inhibition by amino acids were not exclusive to either the oligotrophic or the coastal strains. While the in-depth study of an ocean-

oligotrophic strain (WH8102) and a coastal-spring bloom strain (CC9311) showed that N source affects their morphological and physiological features differently, strains from contrasting environments shared similar growth strategies in response to N source, suggesting that N physiology is not a defining feature of geographically defined ecotypes.

Oxyrrhis marina Grazing Response to N Source-Grown Ocean and Coastal *Synechococcus* Strains

While the diverse responses of *Synechococcus* to N may not be relevant on geographic spatial scales, the changing physiological and morphological states of the cell, defined by its environment, are relevant to a microzooplankton grazer. Because strains differed in their morphological and physiological characteristics when grown on different N sources, it was expected that grazer feeding rates would vary among and within strains. My results suggest that the raptorial feeder, *O. marina,* was sensitive to changes in prey morphology (shape, size) and quality (elemental composition), both of which are partially controlled by N source. However, several other common features of *O. marina's* behavior were unexpected, including reductions in feeding rate over time when grazing on the two contrasting strains.

The range of grazing rates sustained by both strains were comparable to rates from prior grazing experiments that used the coastal-spring bloom *Synechococcus* strain (CC9311) and the ocean-oligotrophic *Synechococcus* strain (WH8102) with the grazer *O. marina.* The broad range of grazing rates on the coastal-spring bloom strain (CC9311) in this study suggests that N source affects grazing rate for the coastal-spring bloom strain (CC9311); however, due to the narrow range of grazing rates on the ocean-oligotrophic strain (WH8102), N source effects on grazing for that strain appear less important. When *O. marina* grazing rates on the coastal-spring bloom strain (CC9311) were converted to hr^{-1}, prior experimental estimates (of 4.5 and 9.6 SN medium-grown CC9311 OX^{-1} hr^{-1}) (Apple et al., submitted) fell within the current study's range (2.7 to 15.8 CC9311 Syn OX^{-1} hr^{-}

^{1}). The majority of N source-grown coastal-spring bloom (CC9311) cells were grazed at 3-5 Syn OX $^{-1}$ hr^{-1}; however cells grown in glycine, nitrate, and urea were grazed at comparatively elevated rates (7.2-15.8 Syn OX^{-1} hr^{-1}). *Oxyrrhis marina* grazing rates on the ocean-oligotrophic strain (WH8102) in the current study (0.7-1.1 WH8102 Syn OX $^{-1}$ hr^{-1}) were slightly below those of the previous study (1.7 and 6.2 WH8102 Syn OX $^{-1}$ hr^{-1}) and 2x to 5x lower than grazing rates on all coastal-spring bloom (CC9311) cells. It appears that, regardless of the growth-supporting N source, the ocean-oligotrophic strain (WH8102) is not as readily eaten as the coastal-spring bloom strain (CC9311) by the grazer *O. marina.* Even though *O. marina* grazing experiments with ocean-oligotrophic strain (WH8102) and coastal-spring bloom strain (CC9311) were conducted on separate days in my study, variability in *O. marina* populations most likely did not account for its different grazing rates between the two *Synechococcus* strains. My results agree with previous *O. marina* grazing experiments on the coastal-spring bloom strain (CC9311) and the ocean-oligotrophic strain (WH8102) that were conducted on the same day (Jude et al., submitted), supporting that my results are largely affected by strain characteristics rather than grazer heterogeneity. Previous experiments have suggested that the ocean-oligotrophic strain (WH8102) is resistant to microzooplankton with diverse feeding mechanisms including the heterotrophic dinoflatellate *O. marina,* the cryptophyte *Goniomonas pacifica*, and the ciliate *Eutintinnis* sp. This resistance has been attributed to its characteristic S-layer, rather than motility or cell size (Apple et al., submitted). S-layers in other bacteria have been hypothesized to conceal outer membrane components and receptors and were shown to protect proteobacteria from bacterial predation, but not ciliate predation (Koval, 1993) indicating the importance of considering cell-surface interactions in the context of specific predator-prey pairs.

Oxyrrhis marina demonstrated similar behavior while grazing the ocean-oligotrophic strain (WH8102) and the coastal-spring bloom strain (CC9311) in that grazing rate (Syn [FeedingOX^{-1}] min^{-1}) decreased between 0-10 and 10-30 min for all N treatments. This may potentially be explained by properties inherent to *O. marina* such as feeding mechanism or chemotaxis rather than the cell surface properties of the ocean-oligotrophic (WH8102) and/or coastal-spring bloom (CC9311) strains.

Ultrastructure studies of *O. marina* have shown the dinoflagellate contains trichocysts: dense, threadlike carbohydrate structures common to this taxonomic group (Clarke et al., 1976). Trichocysts have been hypothesized to aid in predation, possibly enhancing adhesion to prey; however, their function in prey capture remains unknown. In contrast, it is well known that trichocysts aid in escape from predation in other taxa (Lukes et al., 2009; Wolfe, 2000; Sakaguchi, 1998). In addition to trichocysts, *O. marina* engulfs the cell via phagotrophy, which requires a large volume of membranous material that limits the amount of prey a protist can engulf or "catch" over time (Opik et al., 1989).

In addition to difficulty in prey capture, a recent study suggests that *O. marina*'s prey location devices, such as its chemotaxis apparatus and motility behavior, are strongly affected by batch culture conditions, specifically exposure to regenerated ammonium and prey vacuole saturation (Martel, 2010). Although *O. marina* in my experiment showed reduced feeding rates after the first 10 min, this is unlikely to have arisen from food vacuole saturation. In previous studies, *O. marina* food vacuoles have been observed to exceed the number of ingested *Synechococcus* cells seen in my study (Strom and Apple, personal communication). Prior to the experiment, *O. marina* were cultured in the dark and starved, preventing any regenerated NH_4 uptake by *O. marina's* maintenance autotrophic prey. Therefore, *O. marina* were most likely exposed to high NH_4 levels for several days; duration and intensity of NH_4 exposure has been hypothesized to impair chemotaxis. While *O. marina*'s batch culture conditions may have considerably influenced their behavior, chemotaxis is only one element of prey selectivity. In addition chemotaxis may not be relevant to prey concentrations of 1×10^6 *Synechococcus* ml^{-1} as *O. marina* feeding rates were most likely not encounter-rate limited. Clearly, despite batch culture conditions, *O. marina* retained the ability to locate and capture prey differently, as the coastal-spring bloom strain (CC9311) was consistently grazed at higher rates than the ocean-oligotrophic strain (WH8102), an observation that has been repeatedly documented.

While it appears the growth-supporting N source of *Synechococcus* may affect grazing behavior of *O. marina* on both strains, the interaction was much more pronounced for the coastal-

spring bloom strain (CC9311), suggesting that strain diversity plays a larger part in grazing resistance than does growth condition. Similarly, prior experiments have demonstrated that the prey type affects grazing rates more than nutritional status, but nutritional status within prey types also affects grazing rates. For example, the chrysomonad *Paraphysomonas vestita* grazed on *Isochrysis galbana* at higher rates than *Pavlova lutheri* regardless of growth condition, but within prey types *P. vestita* consistently grazed faster on N-replete than N-deplete cells (John et al., 2001). The interaction between *O. marina* grazing behavior and growth-supporting N source for the coastal-spring bloom strain (CC9311) may appear stronger because the coastal-spring bloom strain's (CC9311) physiological characteristics varied to a greater degree in response to N source than those of the ocean-oligotrophic strain (WH8102). Due to the magnitude of grazing response and degree of variation in cell characteristics among the N source-grown coastal-spring bloom strain (CC9311), it is not surprising that many morphological and physiological cell characteristics were related to both the grazing rate and fraction *O. marina* feeding on this strain, while a subset of those appeared to affect only the fraction of *O. marina* feeding on the ocean-oligotrophic strain (WH8102).

Studies have shown that diverse protist grazers, in particular *O. marina*, are to some degree size-selective; therefore microscale variations in cell volume and/or shape of N source-grown cells are important factors to consider in explaining feeding rate variations (Gonzalez et al., 1990; Simek et al., 1992; Hansen et al., 1996; Hahn et al., 1999). If grazing rates and fraction of the feeding *O. marina* population were based on size alone, *O. marina* should feed on the larger cells, as demonstrated in previous studies with the coccolithophore *Emiliana huxleyi* as prey (Hansen et al., 1996). However, in my study, coastal-spring bloom strain (CC9311) cell shape, reflected in l:w, appeared to be related to *O. marina* grazing behavior more strongly than cell volume (v). Excluding urea-grown cells, it appeared that the fraction of the *O. marina* population feeding on the coastal spring-bloom strain CC9311 increased as a function of decreasing l:w ratios cells (Fig. 13). Urea-grown cells were excluded from the analysis

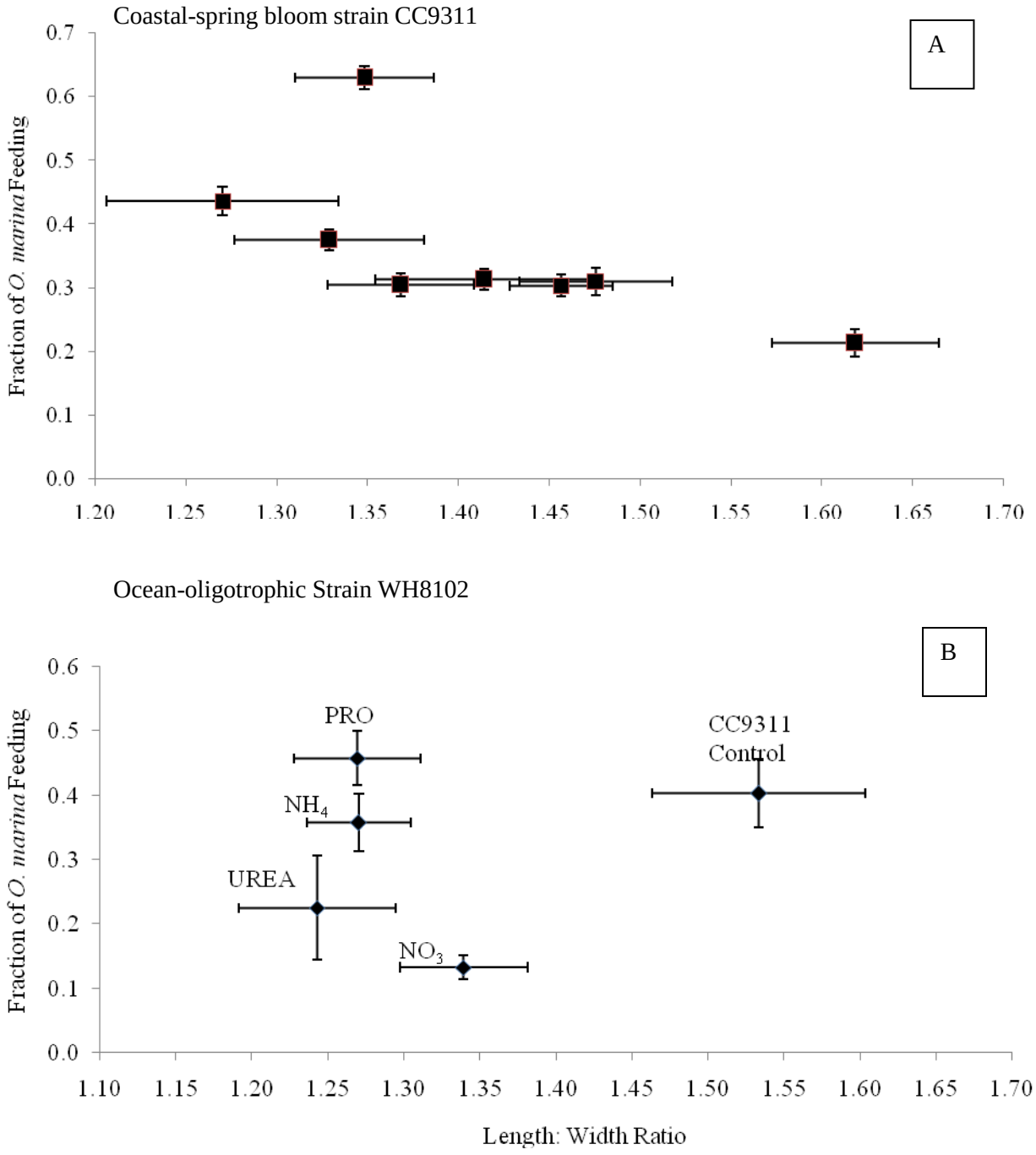

Figure 13. The relationship between the fraction of the feeding *O. marina* population and cell length:width ratio of the A) coastal-spring bloom *Synechococcus* strain CC9311 and B) ocean-oligotrophic strain grown on various N sources. Excluding urea from the linear regression: y=-0.567x+1.127, r^2=0.87, including urea: y=-0.77x+1.446, r^2=0.43.

because comparison of linear regression statistics with and without the urea treatment showed urea-grown cells may be affected by a unknown variables such that, in contrast to other N source-grown cells, unknown cell characteristics were positively affecting the grazing behavior of *O. marina*. For instance, *O. marina* grazing rates on urea-grown cells followed the general trend in the linear relationship, in which a higher fraction of *O. marina* fed on rounder cells than on their more elongated counterparts; however, the fraction of *O. marina* feeding on urea-grown cells was higher than the linear regression would predict given the l:w of urea-grown cells. Cell size of urea-grown cells could possibly have enhanced feeding rates; urea-grown cells were the smallest in volume. However, *O. marina* has been shown to prefer larger cells, and when considering all data, cell volume was not related to grazing behavior. Clearly, the unexpected elevated fraction of the *O. marina* population feeding on urea-grown cells was affected by another factor, but its identity remains unknown. Despite data suggesting *O. marina* eats spherical cells faster than the elongated cells of the coastal-spring bloom strain (CC9311), neither grazing rates nor fraction of *O. marina* feeding were related to the morphology of the spherical ocean-oligotrophic strain cells (WH8102), supporting the conclusion that strain-specific differences other than shape determine first-order grazing dynamics.

There is a variety of predator-prey size/shape interactions within the marine microbial realm. For instance, dependent upon the specific predator-prey pair, grazers have been shown to select, as well as avoid, smaller, larger, longer, or wider cells (Young, 2006). Therefore, it is interesting to contemplate why cell shape, rather than cell size, would affect the grazing behavior of *O. marina*. What could potentially be inhibiting *O. marina* from feeding on elongated cells? Perhaps elongated cells are more difficult to capture or ingest than spherical cells; however, elongated, rod-shaped cells were observed in the food vacuoles of *O. marina*. Similar to the observation in this study, previous studies have shown that, even though interception-feeding nanoflagellates (*Ochromonas* sp. and *Spumella*) ingest filamentous bacteria, cell length was negatively correlated to ingestion rate (Wu et al., 2004; Matz et al., 2002). Researchers using high-resolution video microscopy further showed that ingestion efficiency, rather than contact and capture rate, caused the negative correlation. In *O.*

marina's case, elongated cells could possibly entangle *O. marina*'s capturing mechanism or require more cellular material for a larger food vacuole, thus decreasing the grazing rate. In any case, *Synechococcus* cells 1-2 μm are bellow the lower limits of *O. marina's* preferred size spectrum (5-10 μm) (Hansen et al, 1996). Therefore, it is reasonable to observe sensitive responses of *O. marina* to the coastal-spring bloom strain's (CC9311) slight changes in shape.

Because the physiological components of a cell constrain its morphology and the coastal-spring bloom strain's (CC9311) nutritional quality is reflected in the size/shape characteristics as previously discussed, both of these factors are important to a microzooplankter. However, there was no apparent relationship between the coastal-spring bloom strain's C:N and grazing behaviors, despite the coupling between C:N and l:w and between l:w and grazing rates or fraction *O. marina* feeding. But when considering coastal-spring bloom strain (CC9311) cell CN rather than C:N, it appears there is a size-independent nutritional effect on *O. marina* grazing rates and fraction *O. marina* feeding (Fig. 14). With the exception of ammonium-grown cells, grazing rates and fraction of *O. marina* feeding increased as a function of increasing CN. Ammonium-grown cells were designated as an outlier after comparing several linear regressions. Unlike the exception of urea-grown cells (discussed above), ammonium-grown cells did not follow the pattern of the linear trend. Rather, ammonium-grown coastal-spring bloom cells were grazed by an anomalously low fraction of the *O. marina* population feeding. To a lesser extent, a similar size-independent cell CN effect was also observed in the fraction of *O. marina* feeding at t=30 min on the ocean-oligotrophic strain; however, this relationship was non-linear (WH8102) (Fig. 15). The highest fraction of *O. marina* fed on N source-grown WH8102 with the highest cell CN (proline-grown cells).

Previous studies have also observed predator-prey interactions related, in part, to prey size and quality. For example, in a previous study, ingestion rates on live cells seemed to be driven by cell length; however, complementary experiments showed that nanoflagellates fed on similar-sized beads with various biochemical compositions at different ingestion rates (Matz et al., 2002). Shannon et al. (2006) also found that, even though cell size affected ingestion rates of a flagellate, food quality

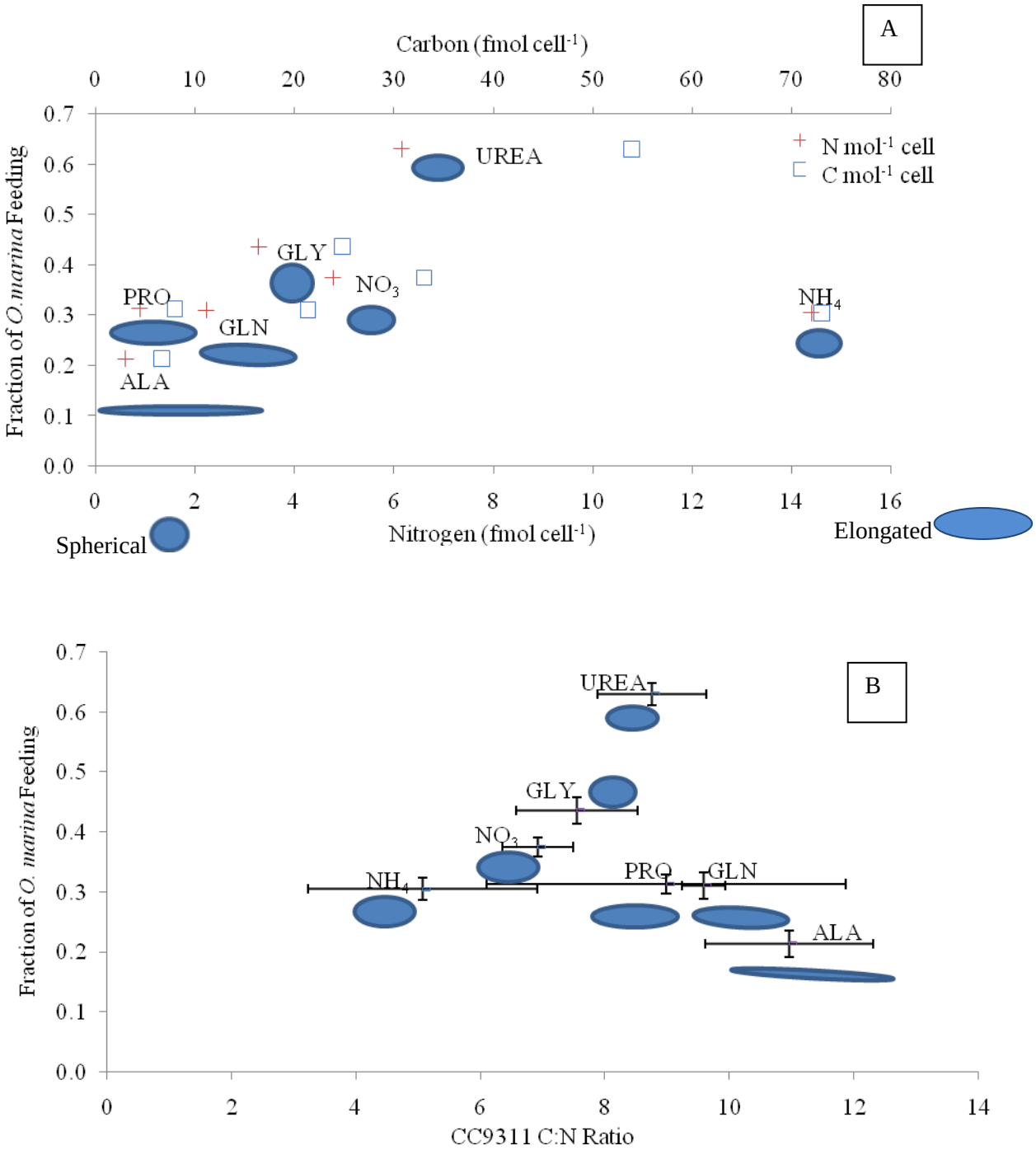

Figure 14. A) The linear relationship between the fraction of *O. marina* population feeding and the nitrogen and carbon content (n=2,3; fmol cell) of CC9311 (coastal-spring bloom). Excluding ammonium from the linear regression, C: y=0.007x + 0.194, r^2=0.85; N: y=0.57x+0.206, r^2=0.783. Including ammonium, C: y=0.002x+0.293 r^2=0.19, N: y=0.004x+ 0.346, r^2=0.029. B) The relationship between fraction of *O. marina* population feeding and the C:N ratio of CC9311 (coastal-spring bloom strain). When elongated N source-grown cells (ALA, PRO, GLN) were excluded from linear regression, y=0.84x-0.160, r^2=0.862. When all data were included: y=-0.011x+0.465, r^2=0.028

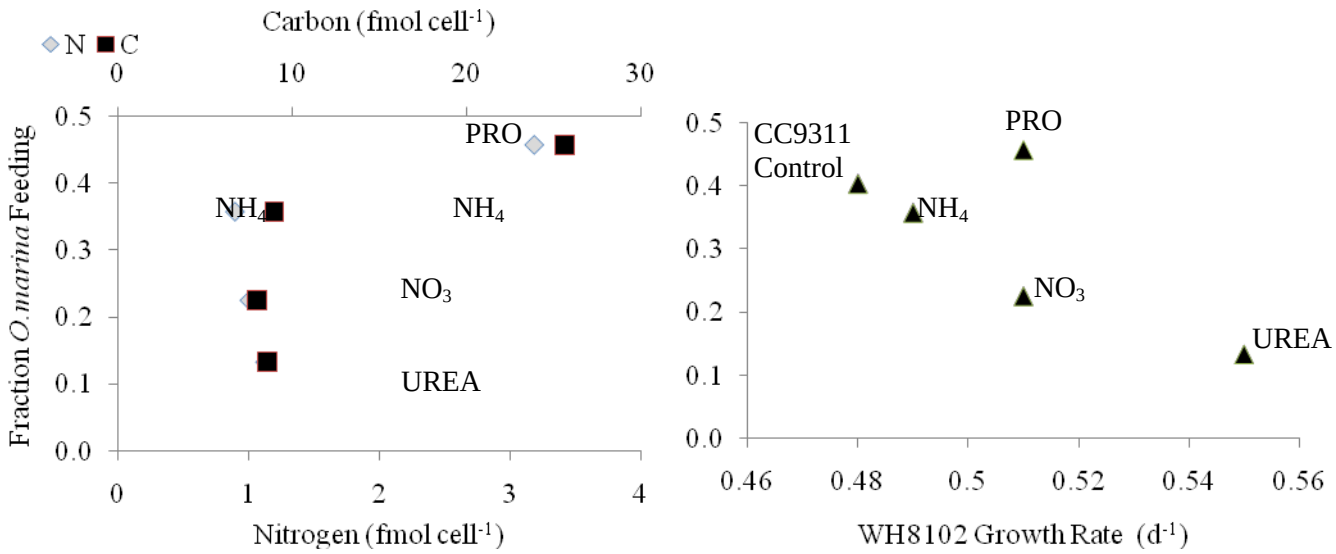

Figure 15. A) The mean fraction of the *O. marina* population feeding at t=30 min (n=4) on single N source-grown WH8102 cells versus their mean cell nitrogen and carbon content (n=2,3 fmol/cell) and mean growth rate (n=3, d^{-1}). B) The relationship between the fraction of *O.marina* population feeding at t=30 min (n=4) on N source-grown WH8102 and the WH8102 growth rates (n=3, d^{-1}). When excluding proline from the linear regression, y = -3.879x + 2.248 R^2 = 0.940. Including all the data, y = -3.749x + 2.219, R^2 = 0.57.

(high vs. low C:N) explained more variability than did cell size. Martel et al. (2006) observed that *O. marina* showed "specific distaste to N-deplete *Isochrysis*" (p. 210) and hypothesized that prey quality may be learned over time. While there is no conclusive data on the mechanism that enables protists to select quality prey, Martel demonstrated that pre-exposing *O. marina* to specific prey resulted in different grazing rates. Others, cited in Martel, have suggested that *O. marina* remembers prey as undesirable through "biochemical feedback from prey items that are being assimilated" (p. 217). Therefore, the timescale of a predator-prey interaction would supposedly be based on the life of macromolecules associated with selectivity, but this hypothesis remains unexplored (Martel, 2006). My results agree with the general consensus that *O. marina* ingestion rates, along with those of diverse protozoan grazers, are affected by prey quality; however, the mechanisms underlying this relationship remain unknown.

Growth rates of primary producers have direct impacts on morphological and physiological cell characteristics and have, in some cases, also been shown to be positively correlated with grazing behavior in diverse taxa. As discussed above, the coastal-spring bloom strain's (CC9311) CN increased as a function of increasing growth rate for different N sources (common vs. amino acids) and the fraction of the *O. marina* population feeding increased as a function of increasing CN of the coastal-spring bloom strain (CC9311). Despite these observations, growth rates of the coastal-spring bloom strain (CC9311), regardless of their relationship to CN, were not related to *O. marina* grazing behavior. Perhaps because growth rates were not consistently related to coastal-spring bloom strain (CC9311) cell characteristics, they were not related to grazing. In contrast to the coastal-spring bloom strain (CC9311), the ocean-oligotrophic strain (WH8102) growth rates were not related to cell CN, but were inversely related to the fraction of the *O. marina* population feeding at t=30 min (Fig 15). Proline was identified as an outlier in this relationship based on comparison of regression statistics. Similar to the exception-case of urea-grown coastal-spring bloom cells (as discussed above), the

proline-grown ocean-oligotrophic cells followed the general trend, but supported a much larger fraction of the *O. marina* population feeding than the overall growth rate versus feeding relationship would predict. In the case of proline-grown cells, it seemed that their comparatively high CN content, relative to other N source-grown ocean-oligotrophic cells, was related to their observed elevated susceptibility to grazing.

Despite the fact that *Synechococcus* growth rates were related to only a small subset of the grazing data, it is interesting to contemplate how growth rate may affect the grazing susceptibility of the ocean-oligotrophic strain (WH8102). Previous studies have concluded that diverse protists graze on metabolically active heterotrophic bacteria, more so than on dead or dormant cells (Koton-Czarnecka et al., 2003). Single N source growth experiments suggested that motility may affect the growth of the ocean-oligotrophic strain (WH8102); motility may also impact grazing. Cells in all N treatments were motile; however, the degree of motility may have been affected by N source. If the ocean-oligotrophic strain (WH8102) had a reduced capacity to take up a given N source, cells may have increased their activity in order to meet the higher N demand. Hypothesized increased movement could increase contact rates between *O. marina* and the ocean-oligotrophic strain (WH8102). While variable cell motility may have increased contact rates and the fraction of the *O. marina* population feeding over time, in general grazing rates on the ocean-oligotrophic strain (WH8102) remained uniformly low.

In contrast to prey motility (only relevant to the ocean-oligotrophic strain WH8102), the motility of *O. marina* is pertinent to grazing on both *Synechococcus* strains. *Oxyrrhis marina* may have increased its activity in an effort to chemotax towards particular unidentified biogenic sources leaking out in any of the N source-grown cells of either the coastal-spring bloom strain (CC9311) or the ocean-oligotrophic strain (WH8102). Nitrogen source-grown cells of ocean-oligotrophic (WH8102) and coastal-spring bloom strains (CC9311) may be inherently leaky based on cell surface characteristics or may become exceptionally leaky as they become environmentally stressed (due to

extremes of pH, UV, nutrient limitation, etc.). For example, Sunda et al. (2010) demonstrated in a recent study that diverse single-celled algae grown in low N lost approximately 50% of N assimilated. This is plausible because, as discussed above, ammonium and other unknown compounds were demonstrated to elicit positive chemotaxis in *O. marina* and, under certain conditions, to overwhelm *O. marina*'s ability to sense other chemical stimuli (Martel, 2010; Martel, 2006). Data from this study do not provide any clear insight into the leakage phenomenon; however, leakiness could possibly be a factor contributing to the increased grazing rates on the urea-grown cells of the coastal-spring bloom strain (CC9311) or perhaps the proline-grown cells of the ocean-oligotrophic strain (WH8102).

In addition to the success of search and find mechanisms, the efficiency of capture and handling of prey items will also determine *O. marina's* ingestion rate for a given prey type. While the cell surface of the ocean-oligotrophic strain (WH8102) is covered by a large matrix of S-layer proteins, punctuated by an even larger randomly distributed protein, little is known of the surface of the coastal-spring bloom strain (CC9311) cells. Despite the available information regarding potential membrane transport proteins and LPS complexes specific to both strains, the information these elements convey to a grazer is unknown. Furthermore, there is a lack of information that describes how cell surfaces of the picoplankton change as a function of CN, ratio, size, shape, or growth rate, all of which fluctuate with growth condition (i.e. N source). It is arguable that multiple combinations of these cell surface features are relevant to selective protozoa, including *O. marina*. Further study is needed to characterize the dynamic cell-surface boundary and its fluctuations on predator-prey interaction time scales.

Overall, my data suggest that cell shape and CN play a large role in *O. marina* grazing behavior with few exceptions. Prey quality, indicated by C:N ratio, was clearly reflected in l:w ratios. It would be expected that, because l:w ratios were tightly coupled with grazing rates, C:N ratios would be related to grazing rates, but this was not the case. Instead, a prey cell CN effect on grazing behavior was observed for both the resistant ocean-oligotrophic strain (WH8102) and the susceptible coastal-spring bloom (CC9311) *Synechococcus* strains. Clearly the elemental composition and/or

shape of cells affects *O. marina* grazing behavior within strains, but between-strain variation remains a primary driver of predator-prey interaction. In addition to cell shape and/or size effects, a plethora of studies have shown grazers feed on higher quality prey faster or preferentially, but it is unclear how these cellular properties are manifested in the cell and are potentially recognizable to a microzooplankton.

Summary

Coastal and oligotrophic strains showed diverse growth responses to single N sources. In addition, variation in morphological and physiological cell characteristics were neither consistent among strains, nor were they consistently correlated to one another. Unsurprisingly, grazing rates of the heteroflagellate *O. marina* varied among N sources within and between a coastal and oceanic *Synechococcus* strain.

1. *Synechococcus* strains belonging to coastal and oligotrophic clades exhibited diverse growth responses to single N sources. While all strains showed growth on ammonium, nitrate, and urea, several strains did not grow on, or were inhibited by several amino acids. Decreased growth in response to amino acids was not consistent among strains. For instance, the amino acid glycine supported maximal growth for the coastal-spring bloom (CC9311), the coastal-oligotrophic (CC9605) and ocean-oligotrophic (WH8102) strains; but glycine supported reduced growth for the coastal-dominant strain (CC9902) and WH8102 mutant (SIO7B) and inhibited growth for the WH8102 mutant JMS40. Variability in growth rate, the ability to grow on broad range of N sources, and no growth in or inhibition of amino acids were not consistent features of either oligotrophic or coastal strains, which suggests that N-based growth strategy is not confined to a specific environmental condition.
2. In contrast to wildtype WH8102, mutants JMS40 and SIO7B either did not grow on or were actively inhibited by several N sources, specifically amino acids. This suggests that

characteristic cell surface proteins associated with the S-layer may also affect N uptake. Varying levels of motility among the mutants and the ocean-oligotrophic strain (WH8102) could explain the discrepancy between growth rates; however, this remains unknown.

3. The morphology and physiology of the ocean-oligotrophic strain (WH8102) and the coastal-spring bloom strain (CC9311) varied depending upon N source for growth. The coastal-spring bloom strain (CC9311) showed the greatest amount of variation in cell shape (l:w), CN, and C:N ratio. In addition, several of these morphological and physiological traits were related to the fraction of the *O. marina* population feeding and to the grazing rates of *O. marina*. Specifically, with few exceptions, a larger fraction of the *O. marina* population feeding fed at higher rates on spherical cells with higher CN than elongated cells with lower CN. In contrast, for the ocean-oligotrophic strain (WH8102), only the growth rate was related to the fraction of grazers feeding.
4. *O. marina* consistently grazed on the coastal-spring bloom strain (CC9311) at higher rates than on the ocean-oligotrophic strain (WH8102); furthermore, grazing rates on the coastal-spring bloom strain (CC9311) were affected by cell size and CN. A number of factors could possibly contribute to these observations; however, the specific mechanism remains unknown.

For a ubiquitously distributed autotrophic picophytoplankter, such as *Synechococcus*, N is just one of the many potentially growth-limiting elements that fluctuate in concentration and available form on diverse timescales in marine environments. Results of this study suggest that there are diverse *Synechococcus* responses to available N sources and the cell characteristics associated with this response affect *O. marina* grazing rates. Understanding how a primary producer's cell-specific response to environmental variability affects both growth and grazing will elucidate the mechanisms by which bottom up and top down factors influence net population growth and hence competitive success. Grazers have the potential to be powerful regulators of primary production; however their regulation may be influenced by the growth condition of their prey.

References

Agawin NSR, Duarte CM, Agusti S, Vaque D (2004) Effect of N:P ratios on response of Mediterranean picophytoplankton to experimental nutrient inputs. *Aquatic Microbial Ecology* 34: 57-67.

Ahlgren N, Rocap G (2006) Culture isolation and culture independent clone libraries reveal new marine *Synechococcus* ecotypes with distinctive light and N physiologies. *Applied and Environmental Microbiology* 72:7193-7204.

Ahlgren G, Hyenstrand P (2003) Nitrogen limitation effects of different nitrogen sources on nutritional quality of two freshwater organisms, *Scenedesmus quadricauda* (chlorophyceae) and *Synechococcus sp.* (Cyanophyceae). *Journal of Phycology* 39:906-917.

Anderson RA (2005) *Algal Culturing Techniques*. Burlington, Massachusetts: Elsevier Academic Press

(2008) BD Facscalibur Operator Course Workbook. San Jose, CA: Becton Dickinson Co.

Berninger UG, Wickham SA (2005) Response of the microbial food web to manipulation of nutrients and grazers in the oligotrophic Gulf of Aqaba and northern Red Sea. *Marine Biology* 147:1017-1032.

Brahamsha B (1996) An abundant cell-surface polypeptide is required for swimming by the nonflagellated marine cyanobacterium *Synechococcus*. *Proceedings of the National Academy of Sciences* USA 93:6504-6509.

Brahamsha B (1996) A genetic manipulation system for oceanic cyanobacteria of the genus *Synechococcus*. *Applied and Environmental Microbiology* 62: 1747-1751.

Clarkel KJ, and Pennick NC (1976) The occurrence of body scales in *Oxyrrhis marina Dujardin*. *European Journal of Phycology* 11:345-348.

Collier JL, Brahamsha B, Palenik B (1999) The marine cyanobacterium *Synechococcus* sp. WH7805 requires urease (urea amidohyrolase, EC 3.5.1.5) to utilize urea as a nitrogen source: molecular-genetic and biochemical analysis of the enzyme. *Microbiology* 145:447-459.

Dupont C, Barbeau K, Palenik B (2008) Ni uptake and limitation in marine *Synechococcus* strains. *Applied and Environmental Microbiology* 74: 23-31.

Epstein SS, Shiaris MP (1992) Size selective grazing of coastal bacterioplankton by natural assemblages of pigmented flagellates, colorless flagellates and ciliates. *Microbial Ecology* 21: 211-225.

Flynn KJ (2009) Going for the slow burn: why should possession of a low maximum growth rate be advantageous for microalgae? *Plant Ecology and Diversity* 2:179-189.

Friaz-Lopez J, Thompson A, Waldbauer J, and Chisholm SW (2008) Use of stable isotope-labeled cells to identify active grazers of picocyanobacteria in ocean surface waters. *Environmental Microbiology* 11: 512-525.

Garrison TS (2005) *Oceanography: An Invitation to Marine Science.* Thompson Brooks/Cole.

Glibert PM, Kana TM, Olson RJ, Kirchman DL, Alberte RS (1986) Clonal comparisons of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp. *Journal of Experimental Marine Biology and Ecology* 101:199-208

Glibert PM, Heil CA, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S (2004) Evidence for dissolved organic nitrogen and phosphorous uptake during a cyanobacteria bloom in Florida Bay. *Marine Ecology Progress Series* 280: 73-83.

Glibert PM, Ray RT (1990) Different patterns of growth and nitrogen uptake in two clones of marine *Synechococcus* spp. *Marine Biology* 107: 273-280.

Glibert PM, Burkholder JM, Kana TM, Alexander J, Skelton H, Shilling C (2009) Grazing by Karenia brevis on *Synechococcus* enhances its growth rate and may help to sustain blooms. *Aquatic Microbial Ecology* 55:17-30.

Glibert PM, Garside C, Fuhrman JA, Roman MR (in press) Uptake of nitrogenous nutrients by plankton in the plume of the Chesapeake Bay estuary, USA: Time dependent relationships between inorganic and organic nitrogen utilization. *Limnology and Oceanography*

Glover HE, Garside C, Trees C (2007) Physiological responses of Sargasso Sea picoplankton to nanomolar nitrate perturbations. *Journal of Plankton Research* 29:263-274.

Gonzalez JM, Sherr EB, Sherr BF (1990) Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Applied and Environmental Microbiology* 56:583-589.

Grover J, Chrzanowski T (2009) Dynamics and nutritional ecology of a nanoflagellate preying upon bacteria. *Environmental Microbiology* 58:231-243.

Guillou L, Jacquet S, Chrétiennot-Dinet MJ, Vaulot D (2001) Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. *Aquatic Microbial Ecology* 26:201-207.

Hahn, M.W. and M.G. Hofle. 1999. Flagellate predation on a bacterial model community: interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. *Applied and Environmental Microbiology* 65: 4863–4872

Hahn MW, Moore ERB, Hofle MG (1999) Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. *Applied and Environmental Microbiology* 65:25-35.

Hansen FC, White HJ, Passarge J (1996) Grazing in the heterotrophic dinoflagellate *Oxyrrhis marina*: size selectivity and preference for calcified *Emiliana huxleyi* cells. *Aquatic Microbial Ecology* 10:307-313.

Hollenstein K, Dawson RJP, Locher KP (2007) Structure and mechanism of ABC transporter proteins. *Current Opinion in Structural Biology*, 17:412-418.

Hewson I, Paerl RW, Tripp HJ, Zehr JP, Karl DM (2009) Metagenomic potential of microbial assemblages in the surface waters of the central pacific ocean tracks variability in oceanic habitat. *Limnology and Oceanography* 54: 1981-1994.

Jeong JH, Seong KA, Kang NS, Yoo YD, Nam SW, Park JY, Shin W, Glibert PM, Johns D (2010) Feeding by raphidophytes on the cyanobacterium *Synechococcus*. *Aquatic Microbial Ecology 58:181-195.*

Jezberova J, Komarkova J (2007) Morphometry and growth of three *Synechococcus*-like picoplankton cyanobacteria at different culture conditions. *Hydrobiologia* 578:17-27.

John EH, Davidson K (2001) Prey selectivity and the influence of prey carbon:nitrogen ratio on microflagellate grazing. *Journal of Experimental Marine Biology and Ecology* 260: 93-111.

Konton-Czarnecka 2003

Koval SF (1993) Predation on bacteria possessing S-layer. In Advances in Bacterial Paracrystalline Surface Layers pp.85-92

Lakeman MB, Dassow PV, Cattolico RA (2009) The strain concept in phytoplankton ecology. *Harmful Algae 8:*746–758.

Lepp P, Schmidt T (2004) Changes in *Synechococcus* Population Size and Cellular Ribosomal RNA Content in Response to Predation and Nutrient Limitation. *Microbial Ecology* 48: 1-9.

Liotenberg S, Campbell D, Ripkaa R, Houmard J, Tandeau de Marsac N (1996) Effect of the nitrogen source on phycobiliprotein synthesis and cell reserves in a chromatically adapting filamentous Cyanobacterium. *Microbiology* 142: 611-622.

Lukes J, Leander BS, and Keeling PJ (2009) Cascades of convergent evolution: The corresponding evolutionary histories of euglenozoans and dinoflagellates, *Proceedings of the National Academy of Sciences* 106:9963-9970

Martel C (2006) Prey location, recognition, and ingestion by the phagotrophic marine dinoflagellate *Oxyrrhis marina*. *Journal of Experimental Biology and Ecology* 335:210-220.

Martel C (2009) Conceptual bases for prey biorecognition and feeding selectivity in the microplanktonic marine phagotroph *Oxyrrhis marina. Microbial Ecology* 57:589-597.

Martel C (2009) Nitrogen-deficient microalgae are rich in cell-surface mannose: Potential implications for prey bioregonition by phagotrophic protozoa. *Brazilian Journal of Microbiology* 40:86-89.

Martel C (2010) Regenerated extracellular NH4+ affects the motile chemosensory responses of batch cultured *Oxyrrhis marina*. *Brazilian Journal of Microbiology* 41: 321-328.

Matz, C, Boenigk J, Arndt H, Jurgens K (2002) Role of bacterial phenotypic traits in selective feeding of the heterotrophic nanoflagellate *Spumella sp.* Aquatic Microbial Ecology 27:137-148.

McCarren J, Hueser J, Roth R, Yamada N, Martone M, Brahamsha B (2007). Inactivation of swmA Results in the Loss of an Outer Cell Layer in a Swimming *Synechococcus* Strain. *Journal of Bacteriology* 187:224-230.

McCarren J, Brahamsha B (2009) Swimming motility mutants of marine *Synechococcus* affected in production and locallization of the S-layer protein SwmA. *Journal of Bacteriology* 191: 1111-1114.

McCarren J, Brahamsha B (2007) SwmB, a 1.12 Megadalton protein is required for nonflagellar swimming motility in *Synechococcus*. *Journal of Bacteriology* 191:1111-1114.

McCarren J, Brahamsha B (2005) Transposon mutagenesis in a marine *Synechococcus* strain: isolation of swimming motility mutants. *Journal of Bacteriology* 187:4457-4462.

Mitra A, Flynn K (2005) Predator Prey Interactions: is ecological stoichiometry sufficient when good food goes bad? *Journal of Plankton Research* 27:393-399.

Montagnes DJS, Barbosa AB, Boenigk J, Davidson K, Jurgens C, Macek M, Parry JD, Roberts EC, Simik K (2008) Selective feeding behavior of key free-living protists: avenues for continued study. *Aquatic Microbial Ecology* 53:83-98.

Moore L, Post A, Roca G, Chisholm S (2002) Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Limnology and Oceanography* 47:989-996.

Moore CM, Mills MM, Langlois R, Milne A, Achterberg EP, Roche JL, Geider RJ (2008). Relative influence of nitrogen and phosphorous availability and productivity in the oligotrophic sub-tropcal North Atlantic Ocean. *Limnology and Oceanography* 53:291-305

Muro-Pastor MI, Reyes JC, Florecia F (2005) Ammonium assimilation in cyanobacteria. *Photosynthesis Research* 83:135-150.

Opik H, Flynn KJ (1989) The digestive process of the dinoflagellate *Oxyrrhis marina* Dujardin, feeding on the chlorophyte, *Dunaliella primolecta* Butcher: a combined study of ultrastructure and free amino acids. *New Phytologist* 113:143-151.

Palenik B, Qinghu R, Dupont CL, Myers GS, Heidelberg JF, Badger JH, Madupu R, Nelson WC, Brinkac LM, Dodson RJ, Durkin AS, Daugherty SC, Sullivan, SA, Khouri H, Mohamoud Y, Halpin R, Paulsen I (2006) Genome sequence of *Synechococcus* CC9311: Insights into adaptation to a coastal environment. *Proceedings of the National Academy of Sciences* 103:13555-13559.

Palenik B, Brahamsha B, Larimer F, Land M, Hauser L, Chain P (2003). The genome of a motile marine *Synechococcus*. *Nature 424*:1037-1041.

Palenik B, Ren Q, Tai V, Paulsen IT Coastal *Synechococcus* metagenome reveals major roles for horizontal gene transfer and plasmids in population diversity. *Environmental Microbiology* 11: 349-359.

Palenik B (2001) Chromatic adaptation in marine *Synechococcus* strains. *Applied and Environmental Microbiology* 67:991-994.

Paulsen IT, Elbourne L, Kang KH, Tchieu JGJ, Swansom M, Chen K, Chen J, Goldman S, Sliwinski MK (2010). Transport DB. http://www.membranetransport.org/

Pernthaler J (2005) Predation on prokaryotes in the water column and its ecological implications. *Nature Reviews: Microbiology* 3:537-546.

Rocap G, Distel DL, Waterbury JB, Chishom SW (2002) Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. *Applied and Environmental Microbiology* 68:1180-1191.

Sakaguchi M, Hausmann K, Suzaki T (1998) Food capture and adhesion by the heliozoon *Actinophrys sol*. *Protoplasma* 203:1615-6102.

Scanlan DJ (2002) Molecular ecology of the marine cyanobacterial genera. *FEMS Microbiology Review 40*:1-12.

Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, Post AF, Hagemann M, Paulsen I, Partensky F (2009) Ecological Genomics of Marine Picocyanobacteria. *Microbiology and Molecular Biology Reviews* 73:249-299.

Schattenhofer M, Fuchs B, Amann R, Zubkov MV, Tarran GA, Pernthaler J (2009) Latinudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. *Environmental Microbiology* 11:2078-2093.

Shannon S, Chrzanowski T, Grover J (2006) Prey Food Quality Affects Flagellate Ingestion Rates. *Microbial Ecology* 56:66-73.

Simek K, Chrzanowski TH (1992) Direct and Indirect Evidence of Size-Selective Grazing on Pelagic Bacteria by Freshwater Nanoflagellates. *Applied and Environmental Microbiology* 58:3715-3720.

Simek K, Verba J, Pernthaler J, Posch T, Harman P, Nedoma J, Psenner R (1997) Morphological and Compositional shifts in an Experimental Bacterial Community Influenced by Protists with Contrasting Feeding Modes. *Applied and Environmental Microbiology* 63:587-595

Snyder DS, Brahamsha B, Azadi P, Palenik B (2009) Structure of compositionally simple lipopolysaccharide from Marine *Synechococcus. Journal of Bacteriology,* published ahead of print.

Suttle CA (2007) Marine viruses-major players in the global ecosystem. *Nature Reviews Microbiology* 5:801-812.

SundaWG, Hardison DR (2010) Evolutionary tradeoffs among nutrients acquisition, cell size, and grazing defense in marine phytoplankton promote ecosystem stability. *Marine Ecology Progress Series* 401:63-76.

Stuart RK, Dupont CL, Johnson AD, Paulsen IT, Palenik B (2009) Coastal Strains of Marine *Synechococcus* Species Exhibit Increased Tolerance to Copper Shock and a Distinctive Transcriptional Response Relative to Those of Open Ocean Strains. *Applied and Environmental Microbiology* 75:5047-5057

Tai V, Palenik B (2009) Temporal variation of *Synechococcus* clades at a coastal Pacific Ocean monitoring site Time series of *Synechococcus* clade abundances. *Multidisplinary Journal of Microbial Ecology* 3:903-915.

Tarao M, Jezbera J, Hahn MW (2009) Involvement of cell surface structures in size-independent grazing resistance of freshwater Actinobacteria. *Applied and Environmental Microbiology* 75:4720-4726.

Toledo G, Palenik B (2003) A *Synechococcus* serotype is found preferentially in surface marine waters. *Limnology and Oceanography* 48:1744-1755.

Toledo G, Palenik B, Brahamsha B (1999) Swimming marine *Synechococcus* strains with widely different photosynthetic pigment ratios form a monophyletic group. *Applied and Environmental Microbiology* 65:5247-5251

Willey JM, Waterbury JB (1989) Chemotaxis toward nitrogenous compounds by swimming strains of marine *Synechococcus* spp. *Applied Environmental Microbiology* 55:1888-1894.

Wolfe GV (2000) The chemical defense ecology of marine unicellular plankton: constraints, mechanisms, and impacts. *Biology Bulletin* 198:225-244.

Wooten EC, Zubkov MV, Jones DH, Jones RH, Martel CM, Thorton CA, Roberts EC (2006) Biochemical prey recognition by planktonic protozoa. *Environmental Microbiology* 9:216-222.

Wilhelm SW (1995) Ecology of the iron-limited cyanobacteria: a review of physiological responses and implications for aquatic systems. *Aquatic Microbial Ecology* 9:295-303.

Wu J, Sunda W, Boyle EA, Karl DM (2000) Phosphate depletion in the Western North Atlantic Ocean. *Science* 289:459-762.

Wu, QL, Boenigk J, Hahn MW (2004) Successful Predation of Filamentous Bacteria by a Nanoflagellate Challenges Current Models of Flagellate Bactivory. *Applied and Environmental Microbiology* 70: 332-339.

Xu X, Khudyakov I, Wolk PC (1997) Lipopolysaccharide dependence of cyanophage sensitivity and aerobic nitrogen fixation in *Anabeana* sp. Strain PCC7120. *Journal of Bacteriology* 179:2884-2891.

Young KD (2006) The Selective Value of Bacterial Shape. *Microbiology and Molecular Biology Reviews*, 70(3):660-702

Zinovieva M, Fresneau C, Arrio B (1997) Nitrogen source dependent expression of a 126 kDA protein in the plasma membrane of the cyanobacterium *Synechococcus* PCC 7942. *FEBS Letters* 416(2): 179-182.

Zwirglmaier K, Heywood JL, Chamberlain K, Woodward MS, Zubkov MV, Scanlan DJ (2007) Basin-scale distribution patters of picocyanobacterial lineages in the Atlantic Ocean. *Environmental Biology* 9:1278-1290.

Zwirlgmaier KL (2008) Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals distinct partitioning of lineages among ocean basins. *Environmental Microbiology 10:*147-161.

Zubkov MV, Zollner E, Jurgens K (2001) Digestion of bacterial macromolecules by a mixotrophic flagellate, *Ochromonas sp.*, compared with that by two heterotrophic flagellates, *Pumella pudica and Bodo saltans. Journal of Protistology* 37:155-166.

Zwirglmaier K, Spence E, Zubkov MV, Scanlan D, Mann N (2009) Differential grazing of two heterotrophic dinoflagellates on marine *Synechococcus* strains. *Environmental Microbiology* 11:1767-1776.