Title

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Journal

The ISME Journal: Multidisciplinary Journal of Microbial Ecology, 6(3)

ISSN

1751-7362

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Publication Date

2012-03-01

DOI

10.1038/ismej.2011.127

Peer reviewed

Seasonal dynamics of *Synechococcus* and *Thaumarchaeal* populations resolved with high resolution with remote *in situ* instrumentation

Running title: High resolution microbial oceanography

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ABSTRACT

Monterey Bay, California is an Eastern Boundary upwelling system that is nitrogen limited much of the year. In order to resolve population dynamics of microorganisms important for nutrient cycling in this region, we deployed the Environmental Sample Processor with quantitative PCR assays targeting both ribosomal RNA genes and functional genes for subclades of cyanobacteria (*Synechococcus*) and ammonia-oxidizing Archaea (*Thaumarchaeota*) populations. Results showed a strong correlation between *Thaumarchaea* abundances and nitrate during the spring upwelling but not the fall sampling period. In relatively stratified fall waters, the *Thaumarchaeota* community reached higher numbers than in the spring, and an unexpected positive correlation with chlorophyll concentration was observed. Further, we detected drops in *Synechococcus* abundance that occurred on short (i.e. daily) time scales. Upwelling intensity and blooms of eukaryotic phytoplankton strongly influenced *Synechococcus* distributions in the spring and fall, revealing what appear to be the environmental limitations of *Synechococcus* populations in this region. Each of these findings has implications for Monterey Bay biogeochemistry. High-resolution sampling provides a better-resolved framework within which to observe changes in the plankton community. We conclude that controls on these ecosystems change on smaller scales than are routinely assessed, and that more predictable trends will be uncovered if they are evaluated within seasonal (monthly), rather than on annual or interannual scales.

keywords: *Crenarchaeota/*Environmental Sample Processor/Monterey Bay/*Synechococcus/Thaumarchaeota/*time series

INTRODUCTION

Ocean time series are essential for accurate predictions of climate scenarios in this age of anthropogenic change (Doney 1999). Marine biological time series have proven invaluable for uncovering major oceanographic trends that would not have been observed otherwise (Chavez 1999, Chavez *et al.,* 2003, Karl *et al.,* 1995, Karl 1999, Karl 2007, McGowan *et al.,* 1998). Although monthly time series are essential for providing data on annual and decadal patterns, the factors controlling changes in microbial populations are known to occur on shorter time, and smaller space scales (i.e. seconds to months, depending on the process: Karl and Dore 2001; Johnson *et al.*, 2009). However, sustaining high frequency assessments of microbial populations is generally not possible given logistical and practical constraints, particularly for remote locations. With few exceptions, acquisition of detailed information concerning microbial distributions and activities is typically limited to ship-based surveys. The reliance on traditional ship-based sampling has driven the development and testing of new biological sensors (Paul *et al.,* 2007, Scholin 2010, Shade *et al.,* 2009).

The Environmental Sample Processor (ESP) is among this new generation of sensors. Modeled as an 'ecogenomic sensor' (Scholin 2010), the instrument provides for subsurface, hands-off collection of discrete water samples, particle concentration and application of various molecular biological analyses, including quantitative PCR (Preston *et al*., 2011) as well as DNA and protein probe arrays for detection of a variety of organisms, genes and metabolites (Goffredi *et al.,* 2006; Greenfield *et al.* 2008; Haywood *et al.,* 2007; Doucette *et al.,* 2009; Preston *et al.,*

66 2009; Scholin *et al.,* 2009; Scholin 2010). At present, the instrument can perform
67 these types of assays with daily resolution and in real-time, and transmit data to a
68 remote location. The ESP can also archive samples for more in-depth analyses upon
69 instrument retrieval (Ottesen *et al.,* 2011; Scholin *et al.,* 2009).

70 Monterey Bay, California serves as an optimal location for a high resolution
71 time series study, as it has been historically monitored by the Monterey Bay
72 Aquarium Research Institute (MBARI) time series (Pennington and Chavez 2000),
73 and is a region of enormous biogeochemical interest with strong seasonality in
74 environmental conditions (Breaker and Broenkow 1994). Productivity in Monterey
75 Bay is nitrogen-limited for much of the year (Johnson *et al.,* 2006). Upwelling is the
76 primary source of nitrate, and remineralization within the surface waters adds to
77 the nitrogen pool during productive and stratified periods (Dugdale and Goering
78 1967; Johnson *et al.,* 2006). Eukaryotic and some cyanobacterial phytoplankton can
79 assimilate nitrate as well as ammonia, and compete with each other for limiting
80 nitrogen in the bay.

81 The *Thaumarchaeota* (or Group I Archaea, formerly the *Crenarchaeota*
82 (Brochier-Armanet *et al.,* 2008; Spang *et al.,* 2010)) also play an important role in
83 the nitrogen cycle in coastal marine systems by oxidizing ammonia to nitrite
84 (Konneke *et al.,* 2005) , thus altering the form of nitrogen available for
85 photosynthesis and other processes in upwelled waters and deeper in the photic
86 zone (where they reside: Mincer *et al.,* 2007; Santoro *et al.,* 2010; Preston et al.,
87 2011) . The *Thaumarchaeota* have widespread global distributions, and have been
88 estimated to comprise over 20% of marine picoplankton (Karner *et al.,* 2001).

These organisms are key nitrifiers in Monterey Bay (Francis *et al.,* 2005; Mincer *et al.,* 2007; Santoro *et al.,* 2010) and maintain large, but temporally variable populations in coastal California waters (Massana *et al.,* 1997; Mincer *et al.,* 2007; Murray *et al.,* 1999).

The cyanobacteria are significant contributors to marine primary production (Goericke and Welschmeyer 1993; Liu *et al.,* 1997) and perform many essential functions to maintain the health of food webs (Wilhelm and Trick 1994; Zehr *et al.,* 2001). The cyanobacterial genus *Synechococcus* can assimilate a diversity of nitrogen compounds, including ammonium and nitrate, and reside in relatively nutrient-rich waters at coastal sites throughout the world (Scanlan and West 2002). The *Synechococcus* populations in coastal California waters are genetically diverse (Paerl *et al.,* 2008; Palenik *et al.,* 2009; Toledo and Palenik 1997) and strain abundances vary over the course of the year (Paerl *et al*., in review; Tai and Palenik 2009).

Despite their important roles in marine nutrient cycling, *Thaumarchaeota* and *Synechococcus* population dynamics in Monterey Bay are not well resolved. In an effort to better understand controls on these organisms' distributions, we deployed an ESP in the spring and fall months of 2009 in shallow waters at the MBARI's coastal time series station M0. This study applies qPCR assays to detect both general groups of organisms as well as specific subclades to determine trends in microbial distribution relative to environmental change. Physical, chemical and biological parameters were measured over 57 days, along with the corresponding changes in the prokaryotic communities using the ESP. *Thaumarchaeota* were

112 detected with a general 16S rRNA assay (Suzuki *et al*., 2000) and subclades were
113 enumerated with a functional gene assay targeting the ammonia monooxygenase α-
114 subunit gene for the marine shallow water ecotype (*amoA,* group A; Mosier and
115 Francis 2011). *Synechococcus* were detected with a comprehensive *rbcL* assay
116 targeting the ribulose-1,5-bisphosphate carboxylase gene that accounts for ca. 89%
117 total *Synechococcus* in Monterey Bay when compared with flow cytometry derived
118 cell counts (Paerl *et al.,* in review) (Preston *et al.,* 2011), while *Synechococcus*
119 subclades were detected with a functional gene assay coding for the assimilatory
120 nitrate reductase gene (*narB*) and targeting subclades C C1 and D C2 (associated
121 with Synechococcus clades I and IV, respectively from cultured isolates: Paerl *et al.,*
122 2011).
123 The utility of the ESP for quantifying microbial population dynamics in
124 Monterey Bay has been demonstrated by Preston et al., 2011, where microbes with
125 predictable dynamics were quantified during the upwelling season, for technical
126 validation of the ESP technology. Here it was shown that *Thaumarchaeota*
127 abundances increase with upwelling, as expected based on their deeper
128 distributions. Aside from this proof-of-concept field study, their abundance near
129 shore has not been addressed, and it is generally assumed based on previous
130 findings offshore (Santoro et al., 2010; Mincer et al., 2007) that the *Thaumarchaea*
131 are scarce, if at all present in shallow waters, and that any distribution to shallow
132 waters is determined by upwelling and mixing alone. Paerl et al., have shown that
133 *Synechococcus* populations are abundant for most of the year near the coast, with
134 population decreases corresponding with times of intense upwelling (Paerl et al., in

review), and a fairly consistent presence in surface water otherwise. With daily sampling, Preston et al. 2011 further resolved the period of *Synechococcus* appearance in coastal waters, at the relaxation of upwelling. Little is known, however, regarding the drivers of specific *Synechococcus* subclade distributions over the rest of the year, nor whether their populations are consistently present when upwelling intensity is low. This study sought to obtain higher resolution of the abundance of both *Thaumarchaeal* and *Synechococcus* populations and subclades, within and between seasons. The resulting data illustrate a highly dynamic microbial community reflective of regional scale physical processes, such as upwelling, as well as localized processes in stable conditions, where we posit that inter-organismal interactions play a determinant role in dictating microbial distributions.

METHODS

Instruments were deployed in Monterey Bay, CA at Station M0 (36.83N, 121.90W) on a mooring as described previously (Doucette *et al.,* 2009). ESP deployments occurred in the spring (May 14 to June 11) and fall (September 24 to October 28) of 2009, and included an attached CTD (Seabird SBE 16+CTD, Bellevue, WA) with fluorometer (Turner Cyclops-7) and transmissometer (WetLABS Cstar). An *in situ* ultraviolet spectrophotometer (ISUS; (Johnson and Coletti 2002) was also coupled to the ESP for *in situ* nitrate analyses. Data was recorded every 12 minutes from accompanying sensors. Data was transmitted to shore in real time over a radio

158 modem. The instrument was deployed slightly deeper in the spring (10-11.4 m)
159 than the fall (5.8-8.8 m).
160 *Instrument preparation*
161 To prepare the ESP, tubing was cleaned and reagents were loaded as
162 described (Preston *et al.,* 2011). Quantitative PCR assays were loaded onto the
163 microfluidic block for the following genes: *Thaumarchaeota* 16S rRNA (which
164 amplifies all ammonia oxidizing Archaea known; Preston et al., 2011), and *amoA*
165 water column group A (which target all known *amoA* group A sequences from a
166 Monterey Bay database, representing 825 sequences from a total of 9634): Mosier
167 and Francis 2011), S*ynechococcus* RuBisCO (*rbcL,* targets all cultivated
168 *Synechococcus* clades I-IV and all known *rbcL* sequences in Monterey Bay (Paerl *et*
169 *al.,* in review)), *narB* group C_C1 and group D_C2 ((Paerl *et al.,* 2011). The *narB* and
170 *rbcL* assays were the same: each 30µl reaction contained 1x Accuprime Supermix II
171 (Invitrogen, Carlsbad, CA), 0.333 µM primers, and 2µM probe. The *amoA* group A
172 assay contained 1x Accuprime, 2.5 mM additional $MgCl_2$, 0.5 µM forward primer,
173 and 0.333 µM reverse primer. 16S rRNA amplification and cycling conditions were
174 as described previously (Preston *et al.,* 2011). For enumeration, the deployed
175 reagents were used on the microfluidic block of the ESP to create duplicate standard
176 curves from 10-fold linearized plasmid standard dilutions ranging from 10^2-10^5 or
177 10^6 gene copies per reaction. Amplification plots were analyzed and standard
178 curves generated in Excel. Quantitative PCR assay cross-reactivities were low or
179 nonexistent (published in Paerl *et al.,* in review; Preston *et al.,* 2011) and Mosier and
180 Francis 2011) and detection limits on the microfluidic block were ca. 10-20

copies/ml for *Synechococcus* targets, 10-50 copies/ml for *Thaumarchaeal* targets. qPCR standard curve information for the spring deployment are reported elsewhere (Preston *et al.,* 2011). For the fall deployment, copy numbers for the standards in each assay were linear with cycle threshold (C_t). Linear regressions for all assays had r^2 values of >.98, and amplification efficiencies were 90-110% with the exception of the *narB* group C C1 assay, which had an efficiency of 88.6%. Analyses of this target group are discussed with consideration of the implications of this lower reaction efficiency. The DNA extraction efficiency in the ESP microfluidic block was 98-104% compared to parallel extractions performed in the lab with a Qiagen DNeasy Kit.

ESP operations included 24 discrete sampling events in which particles retained on 0.22 μm pore size filters were subjected to qPCR analyses, and an equal number of discrete sampling events where particulates were preserved for later analysis (after Ottesen *et al.,* 2011). The day before each deployment (on May 14 and September 23), 50 ml of sterile water (Sigma-Aldrich) was sampled by the intake of the instrument in order to determine the degree of internal contamination, if any. Negative controls were also run during each deployment (on May 17 and 30 in the spring and October 9 and 16 in the fall), each containing a core system negative control (where a "negative lysate" is created and run through the entire system) and a no-template control (NTC: where the PCR is run with the elution water used for DNA extractions in normal operating conditions).

Assay performance during deployment

Though the ESP does not allow standard curve generation for all samples taken for all assays while deployed, internal positive controls give some degree of confidence in target quantification on a per sample basis. For each sample, the degree of PCR inhibition was assessed using a positive control reaction as described in Preston *et al.,* 2011. Samples were only included in our analyses if the C_t of the internal positive control (IPC) was between 33.5 and 36.5 (spring) or 27.2 and 29.7 (fall). These ranges were within the C_t variability encountered for each PCR module in reactions with water; the template of the IPC is included in the primer/probe reagent. None of the NTC reactions amplified indicating uncontaminated reagents and sufficient cleaning of the PCR microfluidics between samples.

In core system negative control reactions, amplification occurred for a few of the targets. However, in each case, the C_t values translated to copy numbers that were at least an order of magnitude lower than the sample, which does not affect our interpretations of the trends. The fall deployment allowed interrogation of subpopulations of both *Synechococcus* and *Thaumarchaeota*. Subclade dynamics correlated with total clade dynamics, providing support that these groups were adequately quantified. Pearson and Spearman correlation analyses were performed using SigmaPlot 11.2 (Systat Software, Inc.).

Additional data

Mooring data from station M1 (Pennington and Chavez 2000) was used to create the water column profiles for Monterey Bay, and chlorophyll satellite images were obtained from Moderate Resolution Imaging Spectroradiometer (MODIS) on NOAA's Coastwatch/SWFSC Oceanwatch Live Access Server

(http://coastwatch.pfeg.noaa.gov).

RESULTS/DISCUSSION

Seasonal conditions at station M0

Seasonal upwelling dominated spring conditions at Station M0, leading to two major bloom periods that were clearly distinguished by increased chlorophyll-*a* fluorescence (Figure 1). During this deployment period, conditions developed from initial intense upwelling to relaxation and eventual stratification of the water column (Figures 1, 2). The first week of the deployment, when upwelling was sustained, high nitrate concentrations (near 25μM), high salinity (ca. 33.9 PSU) and low temperatures (<11°C) were observed as expected (Breaker and Broenkow 1994); (Figures 1, 2). As a reflection of deep-water transport to surface waters (Bolin and Abbott 1963), chlorophyll-*a* concentrations were low and constant (below 10 μg/L) until the relaxation of upwelling around May 24. The following seven ESP-collected biological samples after this date were from periods of high chlorophyll-*a*/low transmissivity (until ca. June 2: black-outlined boxes Figure 3), indicating abundant eukaryotic phytoplankton at station M0 (Behrenfeld and Boss 2006; Green and Sosik 2004; Green *et al.,* 2003). These blooms were not adequately captured by satellite imaging (http://coastwatch.pfeg.noaa.gov; Figure 1), but microscopy of Monterey Bay seawater during this period supports this interpretation with the identification of a bloom of *Chaetoceros* spp. on May 27 [HABMAP: http://cimt2007plankton.wordpress.com/]. A tongue of nitrate-rich, low salinity, offshore water entered the bay displacing the bloom (Preston *et al.,*

2011), and this was followed by increased stratification that persisted through the end of the deployment when nitrate dropped to 5-10 μM, salinity to 33.6 PSU, and temperatures raised to 13-14°C (Figure 1). There was only trace precipitation over the course of the spring deployment.

After an initial unstable phase that lasted 3 days, fall conditions were well-stratified relative to spring, with warmer (12-15°C), fresher (33.2-33.5 PSU) water, steady physical and chemical conditions and no deep-water supply to the surface detected (Figures 1, 2). Nitrate was less variable in the fall than the spring, with concentrations ranging from 8-12 μM with the exception of several transient (hourly) spikes to ca. 17μM. Chlorophyll-*a* concentrations increased over time, beginning at an average of 7 μg/L (with a range from approximately 3-20 μg/L), and increasing to an average of 17 μg/L (9-38 μg/L range; Figure 1, 2). The ratio of chlorophyll-*a* to % transmission during ESP sampling periods was high, indicating abundant eukaryotic phytoplankton for most of the fall (Figure 3; (Behrenfeld and Boss 2006, Green and Sosik 2004); (Green *et al.,* 2003). Microscopy of Monterey Bay seawater for this period also documented high abundances of eukaryotic phytoplankton, with dinoflagellates (*Ceratium* spp.) dominating for the majority of the deployment period (sampled at Santa Cruz Wharf on Sep 23, 29; Oct 5, 12, 19: http://cimt2007plankton.wordpress.com/), and *Pseudonitszchia*-like diatoms observed in high numbers for one of the five samples (on October 5 http://cimt2007plankton.wordpress.com/). There was only trace precipitation over this period, with the exception of 4.54 inches of rain on October 13. This rain event

was coincident with the onshore influx of a chlorophyll-poor water mass (Figure 3, outlined diamonds).

Microbial population dynamics

Upwelling was the major driver of microbial distributions in the spring, as expected, but population abundances fluctuated despite the relative stability of the water column in stratified fall conditions. Our data lead us to hypothesize that blooms of eukaryotic phytoplankton have an effect on the *Thaumarchaeota* and *Synechococcus* clade dynamics over this period.

Thaumarchaeal *population dynamics*

For much of the spring period, the *Thaumarchaeota* were abundant in surface waters, based on 16S rRNA gene copy numbers (with a range of undetectable to ca. 6.0×10^4 copies per ml; (Preston *et al.,* 2011). A weak negative correlation with chlorophyll-*a* during this upwelling period likely reflects the transport of chlorophyll-poor, *Thaumarchaea*-rich deep-water to the surface (Pearson's r= -0.448, P= 0.037; Spearman's correlation insignificant). *Thaumarchaeota* abundances were positively correlated with the magnitude of upwelling, using temperature (Pearson's r=-0.636, P=0.001; Spearman's ρ=-0.853, P<0.0001), salinity (Pearson's r=0.558, P=0.007; Spearman's ρ=0.704, P=0.0002) and nitrate as a proxy (Figure 4A; Pearson's r=0.769, P<0.0001; Spearman's ρ=0.831, P<0.0001). Aside from one exception, all quantified genes show correlations with these three upwelling proxies in the spring, demonstrating the impact of upwelling on these microbial populations. *Synechococcus*, however, did

294 not correlate with nitrate (see below), and the observation that *Thaumarchaeal*
295 abundances correlate most strongly with nitrate (vs. temperature and salinity) may
296 reflect their role in marine nitrification.
297 *Thaumarchaeota* were more abundant in the fall (16S rRNA gene range of ca.
298 $2.7x10^3$ to $1.3x10^6$ per ml), and again correlated with cold (Pearson's r=-0.754,
299 P=0.0002; Spearman's ρ=-0.805, P<0.0001), saline waters (Pearson's r=0.625,
300 P=0.004; Spearman's ρ=0.853, P<0.0001), but the connection between
301 *Thaumarchaeal* abundance and nitrate concentration was not supported in these
302 stratified conditions. Also in contrast to the spring, *Thaumarchaea* 16SrRNA genes
303 showed a significant positive correlation with chlorophyll-*a* in the fall (Pearson's
304 r=0.554, P=0.014; Spearman's ρ=0.721, P=0.0003) (Figure 4A). During this time,
305 large *Thaumarchaeal* populations were sustained in the surface waters and 16S
306 rRNA gene copy numbers grew to become two orders of magnitude more abundant
307 over time (Figure 4A). These data suggest that *Thaumarchaeal* populations can
308 thrive in surface waters during blooms of eukaryotic phytoplankton. The decrease
309 in abundance that correlates with a drop in chlorophyll towards the end of the
310 deployment may be a reflection of the chlorophyll-poor water mass that moved
311 onshore at this time (which was warmer and less saline, two conditions previously
312 suggested to be unfavorable based on marine *Thaumarchaea* distributions ((Mincer
313 *et al.,* 2007, Murray *et al.,* 1999, Santoro *et al.,* 2010)).
314 In order to determine whether the *Thaumarchaea* targeted by the 16S rRNA
315 gene primers used in this study have the genetic potential to oxidize ammonia, we
316 correlated their abundance with the abundance of ammonia monooxygenase

317 subunit A functional genes (*amoA*). There is now considerable evidence for the
318 existence of two distinct clades of marine ammonia oxidizing Archaea (AOA),
319 namely the 'shallow' (group A) and 'deep' (group B) water column AOA (Francis *et al.,*
320 2005; Hallam *et al.,* 2006; Beman *et al.,* 2008; Mosier and Francis 2011). It is worth
321 noting that both marine water column *amoA* subclades would be detected by the
322 described *Thaumarchaeal* 16S rRNA qPCR assay. In this study, we specifically
323 targeted the shallow (group A) water column AOA ecotype, and during stratification
324 in the fall season. Previous work in the Monterey Bay region shows relative
325 consistency between total *amoA* gene and marine Group I 16S rRNA gene
326 abundances (Santoro *et al.,* 2010), implying that most, if not all, of the
327 *Thaumarchaea* contain at least one copy of the *amoA* gene.
328 Though *Thaumarchaeota* subclade populations have not been quantified at
329 depths relevant to this study (5-10 m), or near shore within Monterey Bay, trends
330 from past studies in the region (Mincer *et al.,* 2007, Santoro *et al.,* 2010) would
331 imply that the shallow water subclade A might dominate in surface waters at station
332 M0. We find that this subclade is abundant for the duration of the fall deployment
333 (ca. 2.1×10^3 to 7.5×10^4 copies per ml), but the ratio of *amoA*, group A to 16S rRNA
334 gene copy numbers ranges from 0.05 to 1.39, with an average of 0.26 (these
335 numbers are not directly comparable to ratios found in past studies, since those
336 enumerated total *amoA* group A and B abundances rather than that of group A only
337 (Mincer *et al.,* 2007; Santoro *et al.,* 2010)). For just six time points, the shallow
338 subclade A makes up >50% of the *Thaumarchaea* 16S rRNA copy number. This
339 corresponds with the lowest chlorophyll-*a* concentrations (Figure 4B) after

movement of the offshore water mass into the bay, leading to the hypothesis that the shallow subclade is dominant in shallow waters offshore, but that we are missing the *amoA* diversity (e.g., 'deep' subclade or otherwise), closer to the coast. Future deployments will measure *amoA* diversity at this site and target all subclades in stratified fall conditions, in order to determine the relative contribution of each to the total *Thaumarchaeal* community.

In a previous California coastal time series study, *Thaumarchaeal* abundances correlated with the characteristics of deeper waters, including low temperature, high salinity and low chlorophyll (Murray *et al.,* 1999). Murray *et al.,*. present correlative data from 32 months integrated over the course of the time series, so any potential short-term effects may have been overlooked. Furthermore, though longer in duration than this study, the previous study has monthly temporal resolution, while this study has daily resolution. Despite these differences, we expected the trends to be similar: large *Thaumarchaeal* abundances in the spring that correlate with upwelling, and low numbers in the fall as a result of increased water column stratification. While the expected trends were indeed observed in the spring, the higher relative abundance of the *Thaumarchaea* populations in the fall points to additional unforeseen factors dictating their distributions. Repeated observation of this phenomenon as a result of higher resolution sampling, affords sufficient confidence to investigate the cause behind this finding in the near future.

The high abundance of *Thaumarchaea* in the surface waters at this coastal station during stratification has far reaching implications. If this correlation between the *Thaumarchaea* population and chlorophyll-*a* holds true during

nutrient-rich stratified conditions in Monterey Bay, then we would expect near shore regions to experience several periods of *Thaumarchaeal* dominance annually, perhaps up to 3-4 months per year (Breaker and Broenkow 1994). Assuming that this population is oxidizing ammonia, it is competing with phytoplankton for this preferred nitrogen source where nitrogen is limiting. Furthermore, the relative decrease in ammonia and increase in nitrite (and therefore nitrate) might sustain growth of larger phytoplankton in the bay (L'Helguen *et al.,* 2008) and thereby contribute to increased carbon export during these periods (Yool *et al.,* 2007).

Synechococcus *population dynamics*

Over the two months (spring and fall) of ESP sampling at station M0, *rbcL*-based *Synechococcus* abundance estimates ranged from undetectable to 10^4 genes per ml. The only correlations in the spring included a positive relationship between *Synechococcus* abundance and temperature (Pearson's r=0.708, P=0.0002; Spearman's ρ=0.708, P=0.0002), as well as a negative relationship with salinity (Pearson's r=-0.567, P=0.006; Spearman's ρ=-0.758, P<0.0001), likely reflecting the inability of *Synechococcus* to remain in surface waters during active upwelling. Once upwelling ceased and conditions became more uniform, *Synechococcus* increased to $2.4x10^3$ copies per ml over the course of three days (Figures 1, 2).

We observed no correlation between *Synechococcus* abundances and any of the environmental parameters measured over the fall period. Though the water column was stratified throughout the fall and there was very little change in the physico-chemical environment, major drops in *Synechococcus* abundance (from 10^4 to <10 per milliliter over one day) were observed twice during this time. These

386 crashes coincided with spikes in chlorophyll (Figure 5, time points with thick black
387 dotted outlines), and may be the result of competition or grazing. In a southern
388 California coastal time series, total *Synechococcus* abundances correlated weakly
389 with temperature, and a weaker negative correlation was observed with phosphate
390 concentration (Tai and Palenik 2009). It is likely that different parameters control
391 *Synechococcus* distribution over the course of the year in Monterey Bay. In the
392 future we hope to deploy more physical and chemical sensors, including phosphate
393 and ammonia sensors, in order to further resolve the environmental context during
394 these time series.

395 Only a narrow range of chlorophyll-*a* and nitrate concentrations were
396 sampled during the spring and fall ESP series, representing 18.5% of the range
397 sampled by MBARI's Biological Oceanography Group at station M0 over the course
398 of four years (2006-2010; Figure 5). We found that *Synechococcus* abundances were
399 highest over a predictable range, specifically less than ca. 16μM nitrate and 15μg/L
400 chlorophyll-*a*. The only exceptions to this included the period of upwelling
401 relaxation in the spring when conditions were still erratic and *Synechococcus* was
402 not yet abundant (demarcated in Figure 1A, and corresponding to the few small
403 dashed circles within the box in Figure 5), and a single time point in the fall where
404 chlorophyll concentrations were highest (26.6 μg/L). It appears that the
405 environmental parameters recorded during our deployments were more extreme
406 than typical conditions at this coastal station, and we may have sampled the upper
407 limits of *Synechococcus* distribution with respect to nitrate and chlorophyll-*a*
408 concentration at Station M0. We intend to continue deploying over various times of

409 year in order to sample a higher diversity of conditions, to gather data on
410 *Synechococcus* distributions under more common circumstances and to test whether
411 the observed trends hold true.

412 Previous time series in Monterey Bay demonstrate the degree of variation
413 observed for *Synechococcus* populations over the year, with total abundances
414 ranging from undetectable to 10^5 copies per ml seawater, and peak abundances
415 occurring over the summer and throughout the fall and winter (Paerl *et al.,* in
416 review). Daily sampling with the Environmental Sample Processor further resolves
417 *Synechococcus* population dynamics, and it is apparent that the population does not
418 maintain continuously high abundances over the summer and fall periods in
419 Monterey Bay.

420 *Synechococcus* clades I and IV appear to be common in temperate coastal
421 waters (Paerl *et al.,* in press, Zwirglmaier *et al.,* 2008). In the fall of our time series,
422 *narB* was used to differentiate between the dominant *Synechococcus* groups C_C1
423 (part of clade I) and D_C2 (of clade IV). Previously in Monterey Bay, these groups
424 maintained relatively high abundances, with subgroup D_C2 being slightly more
425 abundant during 2006 and 2008 (Paerl *et al.,* in review). These *narB* subgroups
426 were also found to be most abundant in the coastal-transition and upwelling zones
427 of the California Current System (CCS), and low in abundance in oligotrophic waters
428 further offshore (Paerl *et al.,* 2011).

429 Unlike average conditions in the previous study, here we find that group
430 C_C1 (clade I) is slightly numerically dominant over clade D_C2. Representative
431 *Synechococcus* strains from clade I and IV differ in their gene repertoires (Dufresne

et al., 2008) so there is the genetic potential for the presence of these different subgroups to have implications for ecosystem function or nitrogen cycling in this region. Both groups are lower in abundance in offshore waters (Figure 6A, and low salinity waters in Figure 6B). This contradicts what might be expected based on previously described higher distributions of Synechococcus in offshore waters (Paerl *et al.,* in press), though a likely explanation is that these sampled waters were not from CCS transition waters. The relative population sizes of each subclade demonstrate no clear differences with respect to temperature or salinity preferences for either subclade (Figure 6B). Though these were the only subclades quantified, the *rbcL* copy number remains high when *narB* groups C_C1 and D_C2 numbers decrease, indicating the presence of additional subclade(s) in offshore waters.

Conclusions

Owing to high-resolution sampling, we have demonstrated that high *Thaumarchaea* abundances can be seen in the stratified fall surface waters, and that these abundances can exceed *Thaumarchaea* numbers transported to surface waters during active upwelling. Such high abundances of ammonia oxidizers likely have significant implications for local nitrogen cycling. If the mass of data we retrieved from the *Thaumarchaea*ota in the fall series were instead a single data point in one month, over the course of a year time series, we may have disregarded it as an outlier, since the conditions corresponding with these high abundances are not conditions that normally coincide with abundant *Thaumarchaeota*. These data demonstrate that *Thaumarchaeota* reside with abundant phytoplankton in the

surface waters over long periods of time, however it is currently unknown whether this co-occurrence is typical in stratified waters in Monterey Bay. It is now possible to test this theory by sampling high and low chlorophyll regimes within the same water mass during stratified conditions, using adaptive sampling with robotic instrumentation.

The daily resolution of the Environmental Sample Processor also allowed us to demonstrate higher variability in *Synechococcus* populations in the fall than anticipated based on previous time series with approximately monthly resolution. Due again to the high number of samples within this season, we were successful in linking low *Synechococcus* numbers to high chlorophyll concentration, indicating a possible inverse relationship between bloom conditions and *Synechococcus* abundances. Here, we have demonstrated the deeper insight gained through high-resolution biological sensing, without the need for continuous on-station ship-time.

The sampling frequencies of most time series programs necessitate a more general interpretation of microbial dynamics and though seasonal trends may be investigated, factors that are important for biogeochemistry are easily obscured by the low likelihood of their continued observation due to intermittent sampling. We have shown that the factors driving *Synechococcus* and *Thaumarchaea* population dynamics changed between seasons, and such effects that occur over shorter scales can be easily overlooked when only investigating the dominant drivers on annual or interannual cycles.

ACKNOWLEDGEMENTS

This work was partially funded by the MEGAMER facility grant by the Gordon and Betty Moore Foundation and the NSF Center for Microbial Oceanography, Research and Education (C-MORE) (to J.P.Z.). Development and application of the ESP has been funded in part by grants (to C.A.S.) from the David and Lucille Packard Foundation (through funds allocated to MBARI), NSF, NASA Astrobiology, and the Gordon and Betty Moore Foundation. Work was also supported in part by an EPA STAR Graduate Fellowship and Stanford University DARE Fellowship (to A.C.M.), as well a National Science Foundation grant (OCE-0825363) to C.A.F. We thank the science and engineering technicians and machinists at MBARI (in particular Cheri Everlove and Roman Marin III) for their invaluable help and dedication toward instrument development, and the crew of the R/V Zephyr for their support and expertise during field operations. Thanks also to the MBARI Biological Oceanography Group, especially Reiko Michisaki for help with MBARI mooring data. Raphael Kudela and lab, John Ryan, Heather Kerkering, Tom Wadsworth, Tracy Cote and the NOAA CoastWatch Program and NASA's Goddard Space Flight Center, OceanColor Web provided and aided in analyses. Thanks to Jim Birch for help with manuscript preparations.

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FIGURE LEGENDS:

Figure 1. **Environmental data for Monterey Bay** in A) Spring and B) Fall. First panel includes MODIS 8-day composite satellite images of chlorophyll-*a* for selected dates during each deployment (most were not available for the spring dates). Star in right panel is approximate location of Station M0. Color legends range from (purple to red): *5/18/09*: -0.6 to 2.0, *6/3/09*: -0.7 to 2.1, *6/12/09*: -0.5 to 2.3, *10/01/09*: -0.4 to 2.4, *10/08/09*: -0.4 to 2.4, and *10/14/09*: -0.8 to 1.8. Units are log mg/m^3. Panels below contain continuous temperature, nitrate, chlorophyll-*a* and salinity data gathered from ISUS, CTD sensors coupled with the ESP during each deployment. The two bloom periods in the spring are designated with a box from ca. May 24 to June 2 and after June 8. Arrows point to periods of offshore water influx into the bay.

Figure 2. **Abundances of bacterial and archaeal gene copy numbers per ml seawater over time** for A) spring and B) fall, 2009, plotted on a log scale. Panels below show Monterey Bay water column temperature and salinity characteristics over this period (note the differences in scales between the spring and fall). Dotted line within each of the bottom panels corresponds with the average deployment depth for each season.

Figure 3. **Eukaryotic phytoplankton abundances.** Percent transmission vs. chlorophyll-*a* for both spring (squares) and fall (diamonds) periods. Top left of graph indicates high phytoplankton relative to bottom right. The size of the data

731 point within each season corresponds to the time of sampling: the larger the data
732 point the later it is in the deployment. As described in the text, dark outlined points
733 are those depicting upwelling relaxation in the spring (squares, mid-deployment)
734 and of the influx of the offshore water mass in the fall (diamonds, end of
735 deployment).
736
737 Figure 4. ***Thaumarchaea* population dynamics.** A) Bubble plot of *Thaumarchaea*
738 abundances (i.e. 16S rRNA gene copy numbers) in the spring (thick outlined) and
739 fall (no outline) vs. nitrate and chlorophyll-*a* concentrations. Size of bubble
740 corresponds to the population size; brightness corresponds with time (the darker
741 the bubble, the earlier in the deployment). Bubbles with dotted outlines indicate
742 time points corresponding with the period of upwelling relaxation in the spring. B)
743 *Thaumarchaea* total population abundances (i.e.16S rRNA gene copy numbers in
744 dark), and *amoA* water column A gene copy numbers (in light) over the fall period,
745 plotted vs. nitrate and chlorophyll.
746
747 Figure 5. ***Synechococcus* populations and environmental parameters.** Bubble
748 plot of *Synechococcus* total abundances (i.e. *rbcL* copy numbers) in the spring (dark
749 outlined) and fall (no outline) vs. nitrate and chlorophyll-*a* concentrations. Size of
750 bubble corresponds to the population size; brightness corresponds with time (the
751 darker the bubble, the earlier in the deployment). Bubbles with dotted outlines
752 correspond to time points corresponding with the period of upwelling relaxation in
753 the spring. Plus signs correspond with chlorophyll-*a* and nitrate concentrations

754 sampled by the MBARI Monterey Bay time series at Station M0 over the course of 4
755 years with ca. 3-week resolution. Dotted box indicates the range of chlorophyll-*a*
756 and nitrate concentrations where we sampled abundant *Synechococcus*.
757
758 Figure 6. ***Synechococcus* subclade dynamics.** A) *Synechococcus* clade abundances
759 over time. *narB*, group D_C2 (black) and group C_C1 (grey) gene copy numbers
760 correspond to the *Synechococcus* subclades enumerated in the fall (Sept to Oct,
761 2009). Arrow points to time of offshore water influx. B) *Synechococcus* total (filled
762 circles) and subclade (black solid and dotted open circles) abundances with respect
763 to temperature and salinity in the fall.
764
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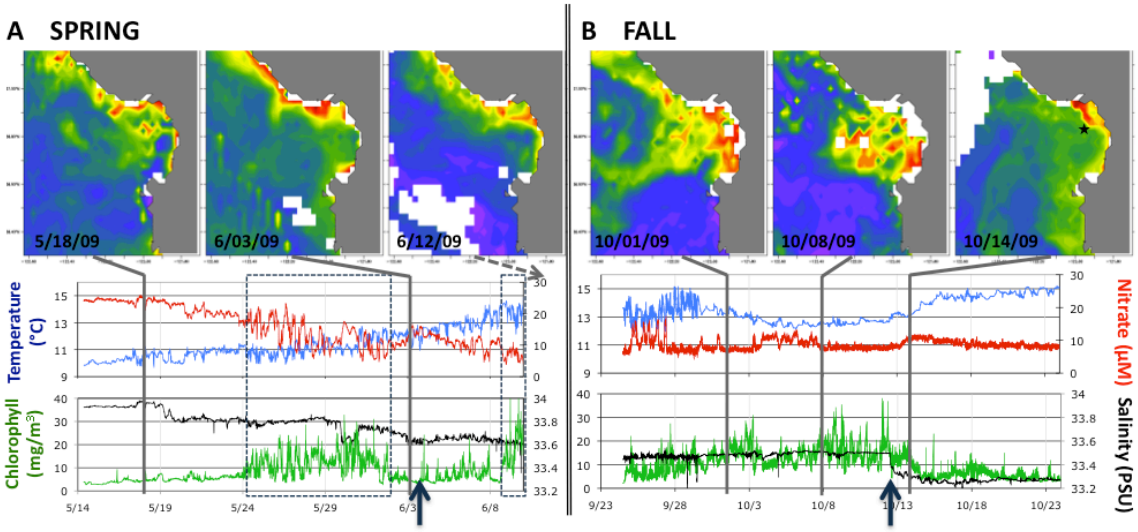
A SPRING
B FALL
5/18/09
6/03/09
6/12/09
10/01/09
10/08/09
10/14/09
Temperature (°C)
Chlorophyll (mg/m³)
Nitrate (µM)
Salinity (PSU)
5/14
5/19
5/24
5/29
6/3
6/8
9/23
9/28
10/3
10/8
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10/18
10/23

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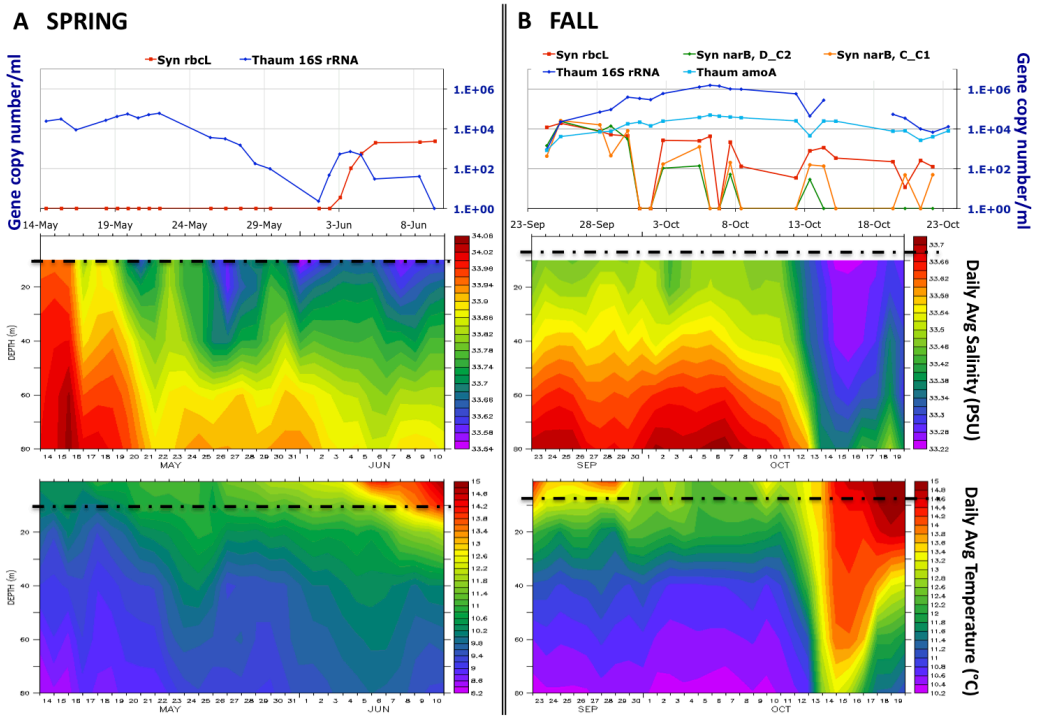
A SPRING
B FALL
Syn rbcL
Thaum 16S rRNA
Syn narB, D_C2
Syn narB, C_C1
Thaum amoA
Gene copy number/ml
1.E+06
1.E+04
1.E+02
1.E+00
14-May
19-May
24-May
29-May
3-Jun
8-Jun
23-Sep
28-Sep
3-Oct
8-Oct
13-Oct
18-Oct
23-Oct
DEPTH (m)
MAY
JUN
SEP
OCT
Daily Avg Salinity (PSU)
Daily Avg Temperature (°C)

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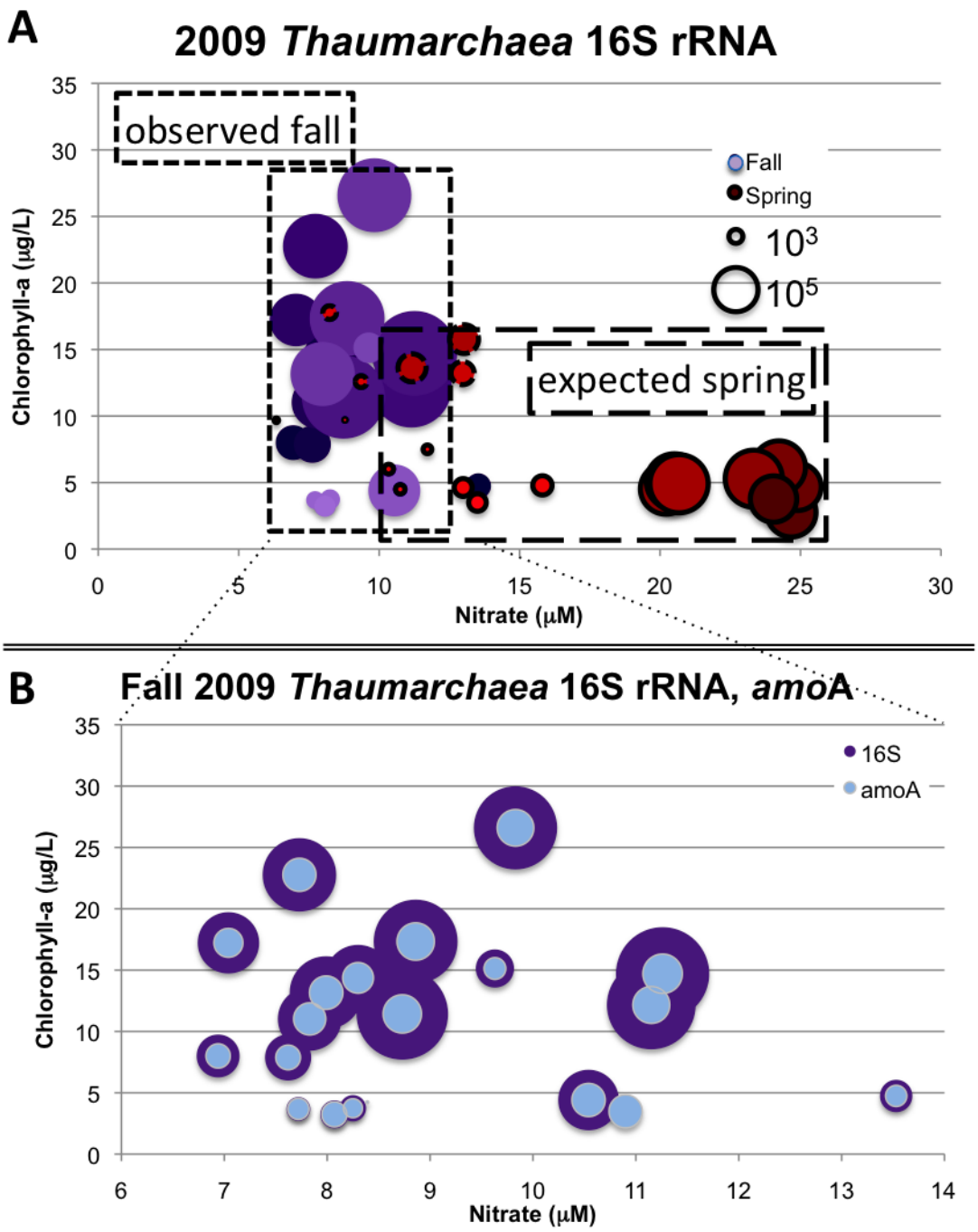
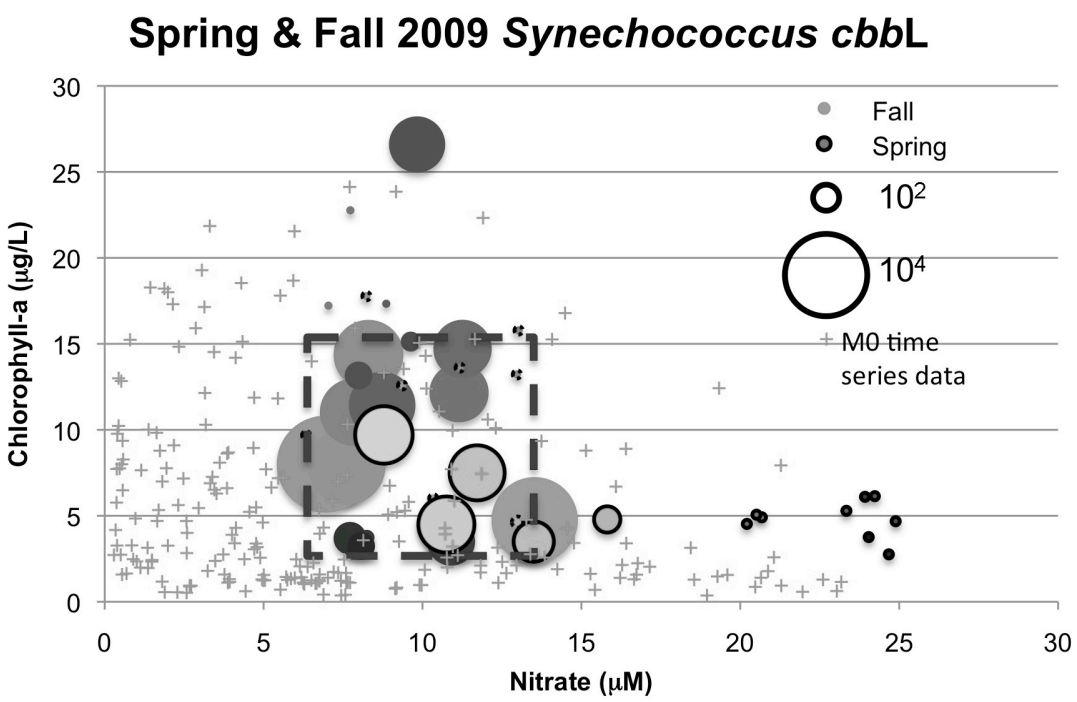

A
2009 Thaumarchaea 16S rRNA
observed fall
expected spring
Fall
Spring
10^3
10^5
Chlorophyll-a (µg/L)
Nitrate (µM)
B
Fall 2009 Thaumarchaea 16S rRNA, amoA
16S
amoA
Chlorophyll-a (µg/L)
Nitrate (µM)

770
771


Spring & Fall 2009 Synechococcus cbbL
Chlorophyll-a (µg/L)
Nitrate (µM)
Fall
Spring
10^2
10^4
M0 time series data
0
5
10
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772
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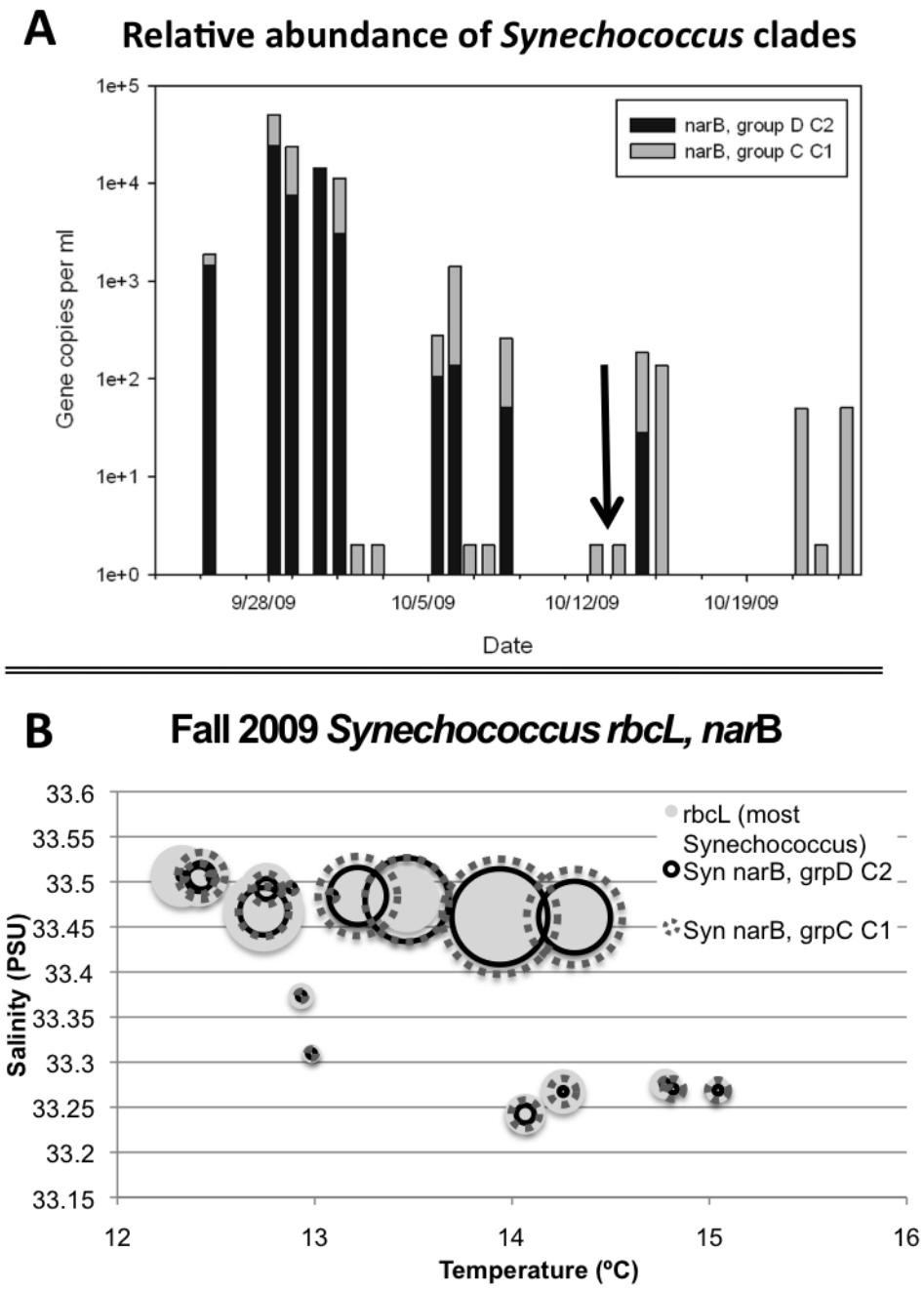

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