Relationships among *Prochlorococcus* ecotypes across oceanic temperature ranges

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For all my immediate and extended family and for Werner C.Von Dohlen (grandfather, scientist and organic chemist), Helga Von Dohlen (grandmother), and John M. Chandler(grandfather, scientist, and nuclear engineer) who never saw me reach my full development in my research and academic pursuits.

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Abstract

Prochlorococcus, the world's smallest known photosynthetic organism, is an open ocean cyanobacterium, thought to be globally significant in nutrient cycling. Genetically and physiologically distinct "ecotypes" of *Prochlorococcus* populate the world's subtropical and tropical oceans. A few of these key ecotypes comprise the majority of these populations, with the dominant ecotypes frequently varying as a function of depth and latitude. The mechanisms underlying the specific distributions of the ecotypes remain poorly understood, but temperature was believed to play a key role in latitudinal partitioning. Quantitative PCR (Q-PCR) was used to assess ecotypic abundances across transects spanning the Pacific Ocean from 27°N to 37°S latitude. We concentrated on an extremely warm region of the Western Pacific Warm Pool (WPWP), the warmest body of open ocean water (+30°C) in the Pacific basin, and on temperatures at transition zones in high north and south latitudes. Our studies indicated that, consistent with prior studies of Atlantic Ocean populations, areas of elevated temperatures are dominated by the eMIT9312 ecotype, and in cooler zones the eMED4 ecotypes prevail as the dominant *Prochlorococcus* representative. Contributions of other ecotypes varied as a function of latitude and/or depth, consistent with their physiological properties. We also show that the two dominant ecotypes, eMIT9312 and eMED4 co-exist in the surface mixed layer of the oligotrophic waters of the Atlantic and Pacific Oceans in a ratio that changes as a function of temperature. Finally, we developed a novel competition technique under laboratory settings to formally assess fitness of these ecotypes. Our preliminary results confirm that the relative fitness of the eMED4 and eMIT9312 ecotypes vary as a function of temperature.

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Chapter 1

Introduction

Introduction

The warm euphotic zones of the open oceans represent one of the world's largest and most important ecosystems. Sunlight supplies these indispensable floating pastures with the energy potential that drives life itself in the open oceans, and through energy transfer far beyond their local environment. Photosynthetically active members within these oceanic communities together form the basis of open ocean food chains through the chemical conversion of light energy into organic carbon compounds for the broad heterotrophic and photosynthetic oceanic microbial community, which by some estimates comprises 70% of organic carbon of the photic zone [1]. Those compounds pass through viral loops, mortality, and through higher trophic relationships with grazers are disseminated throughout the oceanic food chain [2].

Prochlorococcus is Earth's most abundant photosynthetic organism [3], with a global abundance estimated at one trillion trillion cells or 10^{24} cells on Earth. (personal comm. Sallie Chisholm) The habitat of this unicellular cyanobacterium is the oligotrophic, nutrient deprived, open oceans that persist between 40ºN and 40ºS latitude from the subtropics through the tropic zones. Their size of less than 1 micron in diameter distinguishes them as the smallest photosynthetic organisms on the planet, but because of their numerical dominance in these regions, also as arguably one of the most important. Their small size gives them a high surface area to low volume ratio, which can confer a competitive advantage in nutrient scavenging in oligotrophic environments [4].

The *Prochlorococcus* genus has additional traits understood to be advantageous for the photic oligotrophic ocean. They have unique divinyl chlorophyll pigments, that are especially efficient at absorbing the blue light wavelengths that penetrate deepest in the euphotic zone [3]. They have also lost the phycobilisome light harvesting complexes that are

common in cyanobacteria, replacing them with transmembrane chlorophyll-containing proteins to reduce the costs associated with light absorption [3]. What's more, they have replaced phospholipids with sulfolipids as the major lipid class in their membranes, lowering the P quota for the cell [5]. In addition, they have pushed the limits of what is possible for photosynthetic life in terms of minimalistic genome content [6, 7]. With a genome size ranging from 1.6- 2.4 Mb they have the smallest genome of any known photosynthetic autotroph, and lack mobile elements such as insertion sequences, prophages, and plasmids that are common in other prokaryotes [7]. One class of genes lost in *Prochlorococcus* are those involved in oxidative stress response. All *Prochlorococcus* genomes sequenced thus far lack catalases, and it appears that this lineage lost this gene to reduce its quota for Fe and/or N, taking advantage of the ability of the community as a whole to remove hydrogen peroxide from the environment [8-10].

Despite these commonalities, *Prochlorococcus* is a diverse genus composed of genetic lineages with distinct ecological distributions in the ocean (Fig 1.1). Importantly these "ecotypes" have been shown in laboratory studies to have growth characteristics that are consistent with their distributions in nature. The *Prochlorococcus* genus is divided primarily into two larger groups encompassing high light (HL) adapted ecotypes and low light adapted ecotypes (LL) (Fig1.1)[11, 12]. These adaptations, observed in laboratory experiments with representative strains, is also observed in the field: the HL ecotypes dominate the upper euphotic zone, while the LL ecotypes peak in the poorly-illuminated lower euphotic zone [13], [14], [15]. eMIT9312, eMED4, and the newly identified HNLC (high nutrient low chlorophyll) specialist clades comprise the HL ecotypes, while eMIT9313, eSS120, and some uncharacterized lineages [16], [17] make up the (LL) ecotypes. One of the original LL ecotypes eMIT9211 was recently incorporated into the eSS120 ecotype due to its highly similar genomic structure [18]. eNATL2A, is a special LL ecotype, as it can tolerate brief exposures to high light [19], which gives it a unique ability amongst the LL ecotypes to exist in the surface mixed layer, as long as the mixed layer is sufficiently deep [18]. The recently identified HNLC clades HNLC1 and HNLC2 are found predominantly in areas of the open oceans where micro nutrient conditions are replete but often associated with

regions of low overall iron; these ranges are predominately found near regions of equatorial upwelling [19, 20].

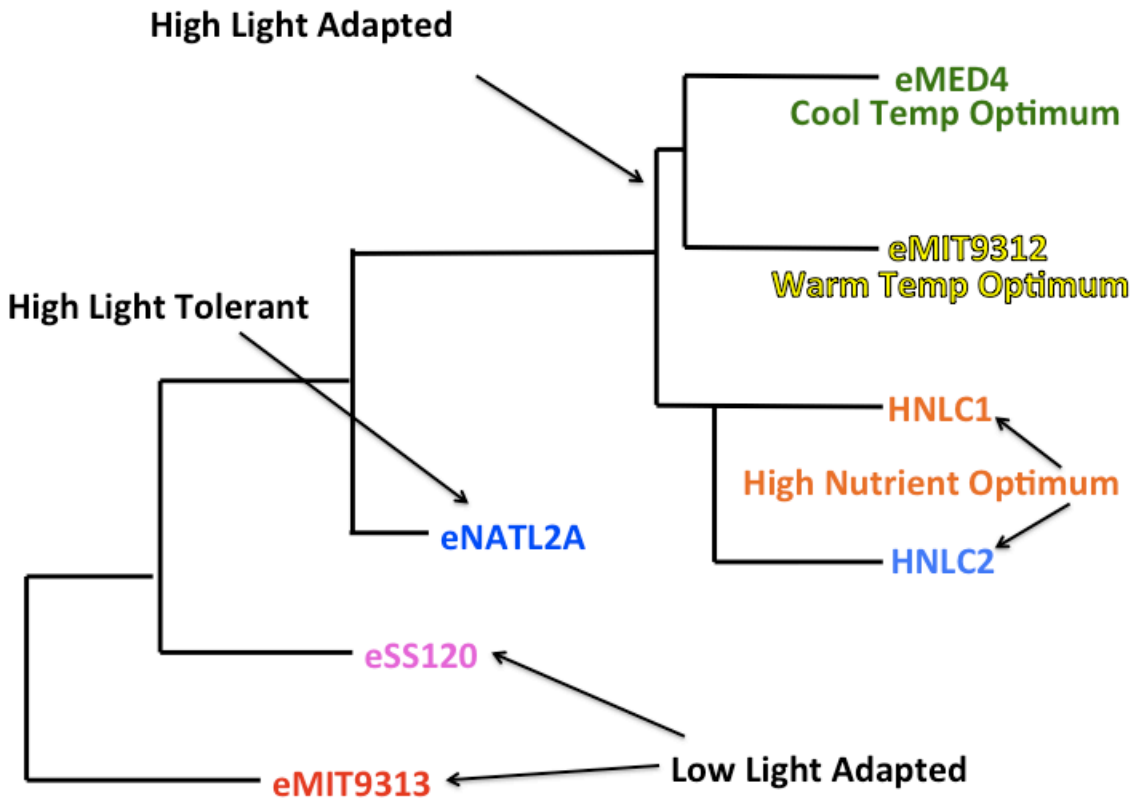


Fig 1.1. Schematic of ecotype phylogeny and niche partitioning in *Prochlorococcus*. Branch lengths are not to scale. Adapted from (Malmstrom et al. 2012)

An Atlantic Ocean transect encompassing 40N and 40S latitude identified the HL ecotypes eMIT9312 and eMED4 as the two numerically dominant lineages [15]. Importantly, these two ecotypes appear to partition the niche with respect to latitude, with eMIT9312 dominating the low latitudes, and eMED4 dominating the high latitudes.[15]. This distribution pattern was consistent with the growth characteristics of the ecotypes, with eMIT9312 adapted for warmer waters and eMED4 adapted to cooler waters. Interestingly, while these ecotypes have different ranges of temperatures permissive for growth (10°C to 28°C for eMED4 and 19°C to 31°C for eMIT9312) both have the same optimum temperature for growth, around the 24°C to 25°C [18]. Thus, there is an apparent tradeoff for growth at one temperature extreme that impacts growth at the other extreme, but apparently does not impact growth at the optimum temperature. Genomic and physiological analyses have yet to reveal the mechanistic bases of these temperature adaptations, but are believed to involve photosynthesis [21, 22], [23], [24] and membrane fluidity [25, 26], based on studies in other systems.

Objectives of this study

Captive in the knowledge that temperature is a driving force influencing niche partitioning and community structures in *Prochlorococcus* community in the Atlantic Ocean, we sought to improve understanding of how this abiotic variable may influence diversity globally, by analyzing the *Prochlorococcus* community in the Pacific Ocean. The primary goal of the study was to understand the relationship between temperature and ecotype abundance, with particular attention given to the numerically-dominant eMIT9312 and eMED4 ecotypes. We employed qPCR techniques to quantify natural populations of *Prochlorococcus* and from this data made inferences in relation to mapped physical characteristics of the surrounding water column.

When the dominance of the surface mixed layer by two ecotypes eMIT9312 and eMED4 became apparent for both the Atlantic and Pacific Ocean, with temperature playing a significant role in establishing the ratio of these two ecotypes, we developed a novel

laboratory culturing assay to test competition between eMIT9312 and eMED4 ecotypes across a range of temperatures.

In this study **we addressed the following hypotheses** in our investigation of temperature effects on *Prochlorococcus*:

1. Niche organization in the water column of the Pacific basin is constrained by temperature

Null: Niche organization in the Pacific basin is not constrained by temperature.

2. Temperature impacts population structure in the dominant eMIT9312 and eMED4 population structures in a predictable fashion.

Null: Temperature does not impact population structure and is randomly associated with eMIT9312 and eMED4 population structures.

3. Laboratory competitions at ecologically relevant abundances are competitive relationships impacted by temperature.

Null: Laboratory competitions at ecologically relevant abundances are not competitive relationships impacted by temperature.

Chapter 2

Prochlorococcus diversity and niche partitioning along a North to South Pacific Ocean transect through the Western Pacific Warm Pool

Many thanks to my good friend and colleague Jackson Gainer who graciously constructed standards, conducted QPCR, and processed raw data for the HNLC and new SS120 ecotypes.

This section is a version of an article being prepared for submission to the journal of Applied Environmental Microbiology in conjunction with the lab of Dr. Zackary Johnson

Abstract

Here we detail the first investigation of *Prochlorococcus* ecotype abundance along a major latitudinal transect of the Pacific Ocean. This is also the first quantification of two recently-identified ecotypes (HNLC1, HNLC2) in the context of the other ecotypes. The vast majority of the transect data from the Pacific ocean pointed to a clear dominant trend that was either dominated by the high light adapted eMIT9312 or eMED4. HNLC1 and HNLC2 were found in significant abundances at equatorial regions as expected from conditions of their discovery in high nutrient low chlorophyll regions associated with equatorial upwelling. In large part ecotypes eSS120 and eMIT9313 of the low light adapted strain were found to contribute a minimal fraction to the euphotic zones with periodic spikes in abundance at lower depths. eNATL2A abundances were found at sustainably low abundances in low latitudes, and increased in abundance in higher latitudes, dominating the total abundance of the *Prochlorococcus* population at some depths.

Introduction

The marine cyanobacterium *Prochlorococcus* numerically dominates the euphotic zones (regions of net primary production) of the oligotrophic oceans [3]. It is found in high abundance, often peaking above 10^5 cells ml^{-1} in the upper euphotic, within the 40N-40S latitudinal band of the oceans, and is often detectable down to the base of the euphotic (~200 m). Phylogenetic analyses of laboratory isolates and field clones revealed that *Prochlorococcus* is composed of distinct lineages, whose unique ecological distributions (see below) indicate that they are ecotypes [11, 27]. Original analyses identified 6 ecotypes: two high-light (HL) adapted (eMED4 and eMIT9312) and four low-light (LL) adapted (eNATL2A, eMIT9313, eSS120, and eMIT9211) [11]. Subsequent characterizations have

identified two new HL ecotypes (HNLC 1 and 2) and multiple new LL ecotypes [19, 28]. It was also recently proposed that the eSS120 and eMIT9211 ecotypes be merged into a single ecotype (eSS120) due to their highly similar genomic composition [7], [19].

Methods to quantify the natural abundance of these ecotypes were developed using quantitative PCR (QPCR) and dot blot hybridization [29], [14], [13]. The former allows for absolute quantification of the 6 original ecotypes, while the latter cannot resolve all of the ecotypes; despite their different genetic targets and level of resolution, the two methods provide similar results [14]. Recent discovery of the HNLC HL ecotypes (see below) and single cell sequencing of their genomes has facilitated the development of QPCR primers for these ecotypes as well [28](currently there are no cultured representatives of these new HL ecotypes, or any of the newly-discovered LL ecotypes).

Studies in the Atlantic Ocean, including a transect from the United Kingdom to the Falkland Islands, demonstrated 4 distinct distribution patterns of the ecotypes that are also consistent with their growth physiologies. The HL ecotypes eMED4 and eMIT9312 are found closer to the surface than the LL ecotypes, consistent with their ability to grow at higher light intensities. Two LL ecotypes, eMIT9313 and eSS120, are only found below the surface mixed layer, consistent with their inability to grow at high light intensity. eNATL2A, an evolutionary intermediate between the LL and HL lineages, is found in mixed layers that are deep only, and this is consistent with their ability to tolerate brief exposure to high light (but cannot grow at high light) [18], [19]. Finally, the meridional transect from the UK to the Falklands revealed that the eMIT9312 ecotype dominates the surface (and total) population in the mid-latitudes, white the eMED4 ecotype dominates in the higher latitudes of both the N. and S. Atlantic Ocean [15]. This latitudinal partitioning was consistent with the ecotype-specific growth rates at different temperatures: the eMED4 strains grew faster than eMIT9312 at low temperatures, while the opposite was true at higher temperatures [15].

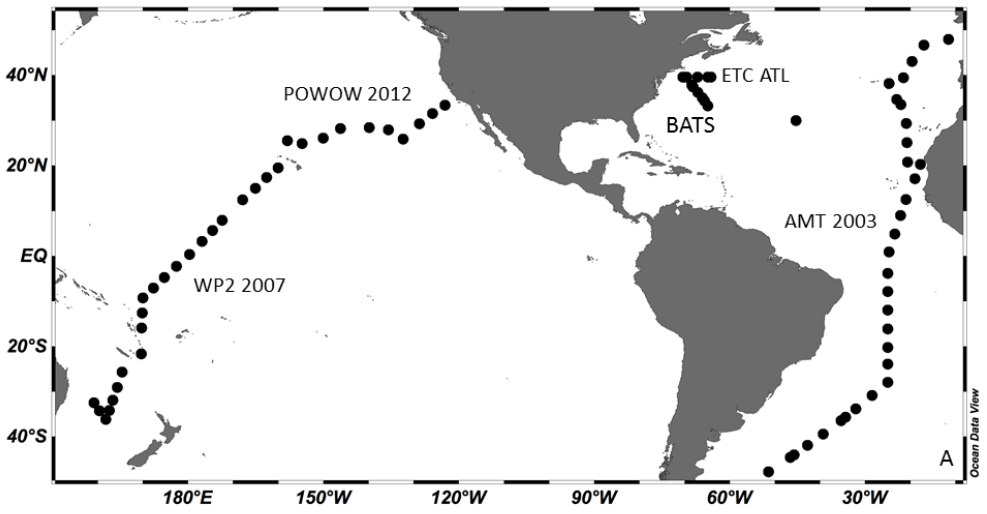
Relative to the Atlantic Ocean, the Pacific Ocean is less well characterized. At Station Aloha near Hawaii, the eMIT9312 ecotype dominates year-round, consistent with the invariable warm temperature [19]. In the high latitudes of the S. Pacific Ocean, dot blot hybridization studies showed that the eMED4 and an unidentified LL ecotype(s) dominate. Interestingly, the HLNC ecotypes were discovered in high abundance just south of the

equator; their abundances dropped off sharply away from this zone. This restricted location to the "high nutrient low chlorophyll" region of the Pacific, where iron is believed to limit phytoplankton production, as well as the genomic analyses of these new ecotypes, revealing novel mechanisms of iron scavenging in these cells, provided the impetus for naming these "HNLC" ecotypes [19].

To date no basinal transects through the Pacific Ocean have been conducted which examined niche partitioning in *Prochlorococcus* across an array of geographical regions. Over the extent of the oligotrophic regions of the Pacific Ocean, where *Prochlorococcus* thrives from 40N to 40S latitude, temperatures seen in the euphotic zones can range between 10 and 31 ºC. Of particular interest is a specific area of the Pacific basin range known as the western pacific warm pool. This region is part of the massive Indo-Pacific warm pool region spanning close to 50% of the equatorial global oceans, and is the warmest region of the open ocean in the world [30]. This region is also thought to be globally important in regulation of climactic events such as El Niño [31-33]. Given the increased levels of evapotranspiration from this region and known biogeochemical implications of phytoplankton in DMS cloud forming cycles, the climactic as well as ecological significance of populations residing in this region could be of future key interest [31, 34, 35].

We sought to examine the *Prochlorococcus* population structure in the Pacific Ocean across a broad temperature range from 31N latitude approaching 40S latitude, using QPCR to quantify the eMIT9312, eMED4, eNATL2A, eMIT9313, eSS102, and HNLC 1 and HNLC 2 ecotypes. Timing of the 2007 (WP2) cruise coincided with decadal warming cycle of the Western Pacific Warm pool and provided a point of great interest given the known permissive temperature maxima of predominant *Prochlorococcus* ecotypes in laboratory culture[14]. It was reasonable to hypothesize that given temperatures predicted in excess of 30 ºC for this region; we might observe unique *Prochlorococcus* populations if present on the upper temperature limits of known survivability for the species from lab cultivation experiments. The northern transect of this 2007 cruise, ending just south of Hawaii, was extended by a 2012 (POWOW) cruise from Hawaii to San Diego, to investigate the population transitions as the *Prochlorococcus* community approached its low-temperature habitat limit in the northern Pacific Ocean.

Results



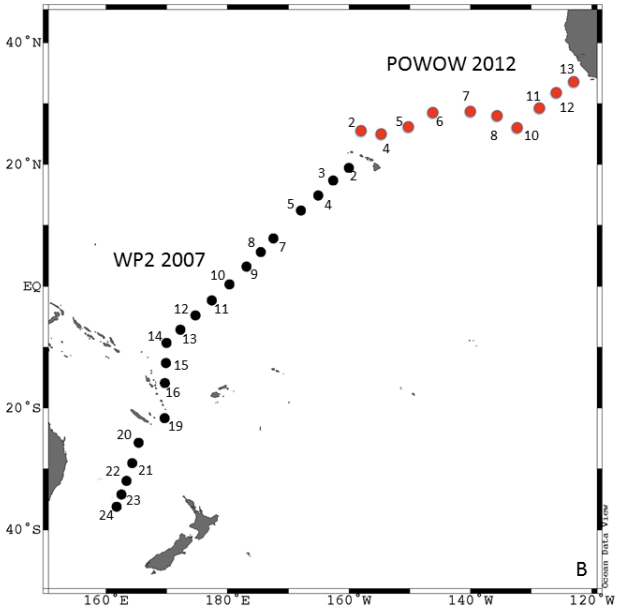


Fig 2.1 A. Transects considered in this study. B Pacific transects with station numbers.

●Black Circles WP2 Cruise (2007) ●Red Circles POWOW Cruise 2012

The WP2 transect covered an extensive set of depth profile casts from surface regions to 200m in depth from 25ºN to 36ºS (Fig 2.1a, Fig2.1b). Along this transect we observed several clear trends, highlighted by predictable shifts in *Prochlorococcus* population abundance throughout depth profiles. Ecotypes eMED4 and eMIT9312 contributed the overwhelming numerical majority of total *Prochlorococcus* populations at shallower depths in the euphotic zone and were taken over in numerical dominance at lower depths by the low light adapted ecotypes, eSS120, eMIT9313, and eNATL2a at varying degrees. The HNLC ecotypes peaked just south of the equator. These results fell in line with earlier studies examining depth profiles in many oligotrophic ocean points in the Atlantic [14, 18], and for the HNLC ecotypes, the Pacific [19].

Integrated abundance-

Ecotype-specific patterns of depth-integrated abundance, assessed down to 200 m that encompassed the euphotic zone, were observed along the Pacific transect (Fig 2.2). In general, the eMIT9312 in warmer waters numerically dominated until 36ºS latitude and 27ºN latitude, where the eMED4 ecotype became the dominant ecotype. In almost all cases where one dominant ecotype prevailed this difference was nearly a magnitude of order over that of secondary ecotypes. At only a single station (Station 12, Fig 2.1) was the total *Prochlorococcus* population numerically dominated by a different HL adapted ecotype, HNLC1, likely due to the unusual chemical environment of this location (see below). At higher north and south latitudes beyond these crossover points we observe the eventual drop of eMED4 populations in cold waters, but never see a recovery of the eMIT9312 populations once the eMED4 populations take over. eMED4 populations of the upper euphotic zone in take over events also are the only other ecotype to reach consistent concentrations well within 10^5 cells/mL seawater confirming their dominance of total *Prochlorococcus* population of take over events. This appears to indicate that temperature plays a larger role within the euphotic zone in facilitating eMED4 to eMIT9312 crossover and potentially in structuring at this crossover point. At both north and south crossover points across both transects we observed mean temperatures in the 18-20 ºC ranges.

Interestingly, new ecotypes of *Prochlorococcus,* HNLC1 and HNLC2 clades, recently identified displayed a persistent low level presence in the euphotic zone across the duration of the transect (Fig 2.2). As documented in previous discovery studies this ecotype appears to be a specialist that concentrates around the equatorial regions, especially in areas where macronutrient concentrations (e.g. inorganic phosphorus) are elevated due to equatorial upwelling events [20]. In the region of equatorial upwelling south of the equator at 4.7S latitude a one-time take over point was exhibited in which HNLC clades became the dominant ecotype present over the previously consistently dominant eMIT9312. In subsequent south latitude depth profiles eMIT9312 regained this dominance, particularly in in the Warm Pool Region where surface temperatures reached 31 ºC. In contrast, with a reported narrow range for these ecotypes restricted to a region just south of the equator, we found an unexpected persistent presence in many depth profiles up to and leading away from the equator with varying degrees of abundance ranging from 10^3 to 10^4 cells mL^{-1} for the WP2 transect.

Overall low light ecotypes eSS120 eMIT9313 played a minor role in integrated abundances of the euphotic zone to 200 m across the Pacific transect (Fig 2.2). Regions where integrated abundances were higher were often too deep euphotic zone population spikes in a narrow range of depth. eNATL2a was a special case that maintained a minor role in total integrated abundance, but among low light adapted ecotypes maintained a threshold in general about an order of magnitude above that of eSS120 and eMIT9313. This was not unexpected, as eNATL2a has been documented as having an elevated tolerance of high light exposure it may receive in upper euphotic zone intrusions [14, 18].

Interestingly, the equatorial regions dominated by the HNLC ecotypes also coincided with a precipitous drop in the eMIT9313 low light adapted ecotype, which was almost completely absent in both CTD casts around the equatorial regions. This is the first report of such an anomalous drop in eMIT9313 abundance in a high-nutrient low chlorophyll region, and is not due to temperature restriction or exclusion from a (deeply-penetrating) surface mixed layer (see below).

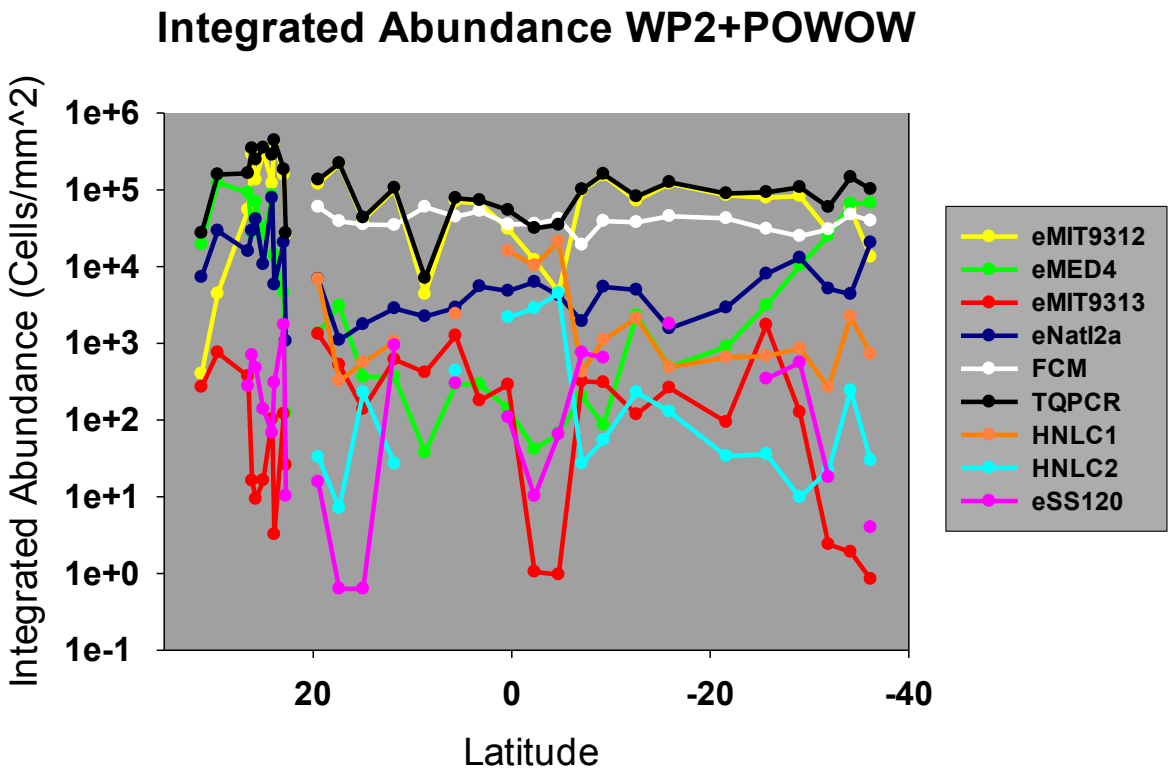


Figure 2.2 Integrated abundance of the euphotic zone (200m depth) of *Prochlorococcus* ecotypes versus latitude for the WP2 (right of the break) and POWOW (left of the break) cruises of the Pacific Basin. Legend bottom right colors correspond to respective ecotypes. Total QPCR- line represents sum of all QPCR integrated through water column. FCM- Integrated abundance of *Prochlorococcus* fraction via flow cytometry.

Individual station highlights-

For each station sampled through the Pacific CTD (conductivity, temperature, and density) casts provided ancillary physical data to compliment sampling from rosette bottles. This physical data is useful to overlay with our abundance calculations ascertained via QPCR to visually align these data and look for trends that may emerge and change with abiotic factors. PAR irradiance denotes the photosynthetically active range of light available for photosynthesis between 400-700nm. The following depth profiles highlight some of those trends and anomalies that were observed along the WP2 and POWOW transects which together comprised the Pacific basin.

WP2 Station 3- the deeply mixed warm oligotrophic ocean

At 19ºN latitude temperatures ranged from around 25ºC at the surface to 19ºC at the base of the euphotic zone at 200m (Fig2.3a, 2.3b). The mixed layer was fairly deep (130 m) compared to the average for the Pacific basin (Chapter3, Table 3.1) and the deep chlorophyll maximum (DCM) was located just below the mixed layer. Typical for this hydrographic profile, the high light ecotype eMIT9312 dominated the upper euphotic zone, exceeding 10^5 cells ml^{-1}, and declined in abundance below the mixed layer, concomitant with a drop in temperature as well as light (data not shown). Within the mixed layer, minority populations of the eMED4, HNLC1, HNLC2, and NATL2a ecotypes were also present. Below the mixed layer, the eNATL2A population peaked at the DCM, and the eMIT9313 ecotype peaked below that at about 150 m. At these peak abundances these ecotypes are co-dominant with the eMIT9312 ecotype. The eSS120 ecotype was not detectable at this station. Interestingly, the HNLC ecotype patterns did not vary considerably with depth, unlike the other four ecotypes present.

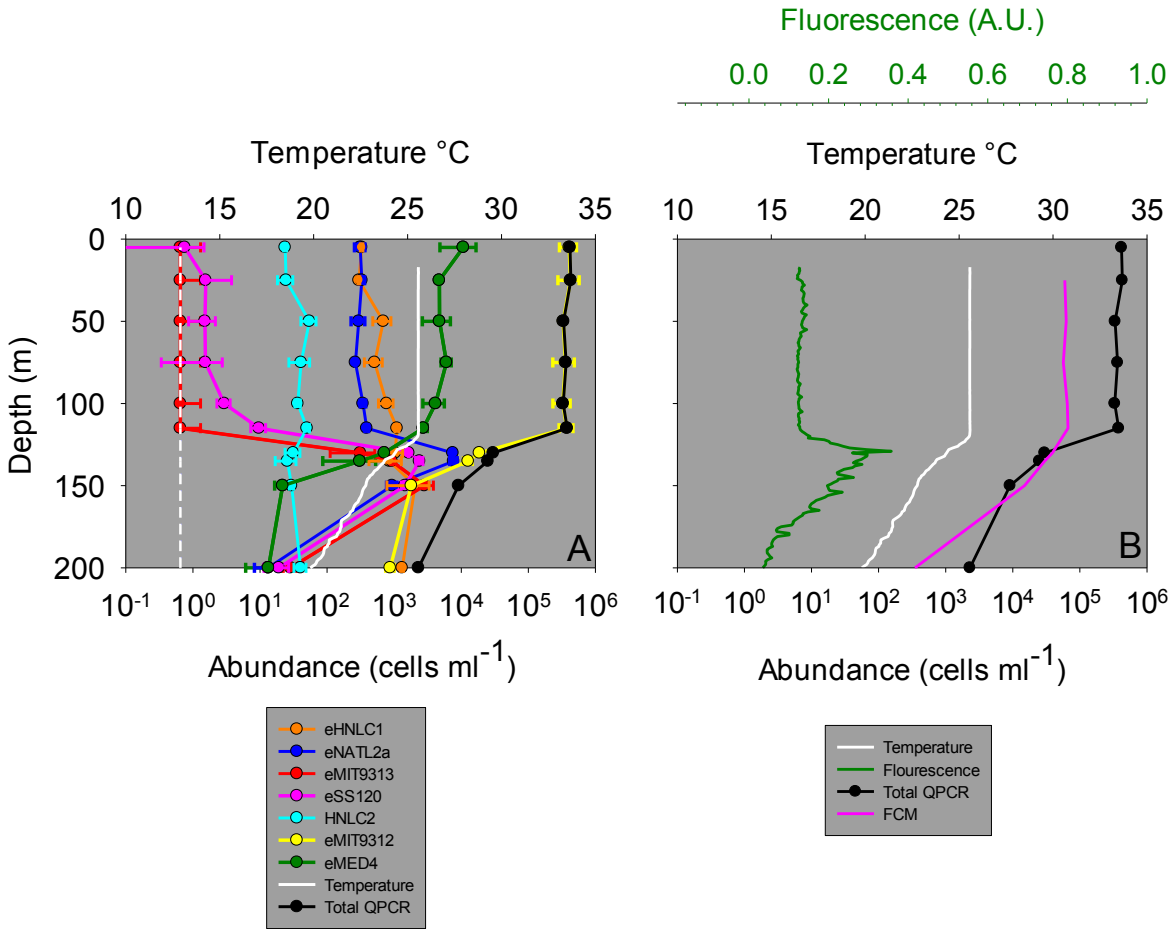


Fig 2.3a- Depth profile of Station 3 from WP2 transect depicting mean *Prochlorococcus* abundance by ecotype for corresponding depth. Total QPCR represents sum of all ecotype QPCR counts. Temperature plotted as continuous data from CTD on top x-axis (white line)

Fig 2.3b- Depth profile of Station 3 from WP2 transects depicting mean total *Prochlorococcus* by QPCR and by flow cytometry. Temperature plotted as continuous data from CTD on top x-axis with light dependent chlorophyll fluorescence.

WP2 Station 12 - HNLC takeover

Station 12 situated just south of the equator at 4ºS latitude provided an exception to the rule of domination of warm euphotic zones by eMIT9312 (Fig 2.4a, 2.4b). Temperatures ranged 30ºC at the surface to 15ºC at the termination of the euphotic zone. This station was characterized by a mixed layer at 59m and a broad DCM that peaked at 100m. The predominant member of the community was the HNLC1 ecotype, while eMIT9312 was still present in fairly high abundance, and matched in relative abundance by the HNLC2 clade. HNLC1 members exceeded mean abundance of eMIT9312 in shallow surface layers and progressively took over a larger proportion of total population with depth to just past the DCM at 125m. HNLC2 clade members also were elevated at this station and tracked closely to that of declining eMIT9312 populations vs. depth. eMED4 ecotypes remained at low cell concentrations throughout the depth profile (10^2 cells mL^{-1}) and began to drop sharply with decreasing light at the DCM. eSS120 had a small abundance increase at the 150m depth mark to around (10^2 cells mL^{-1}) at the termination of the DCM and eMIT9313 had almost no detectable abundance in upper euphotic zone regions, with a small population (10^2cells mL^{-1}) observed at the 200m mark. At approximately the mid-point of the DCM between 100-125m eNATLA2a began an increase in abundance eventually taking over the population as the dominant *Prochlorococcus* ecotype at 150m.

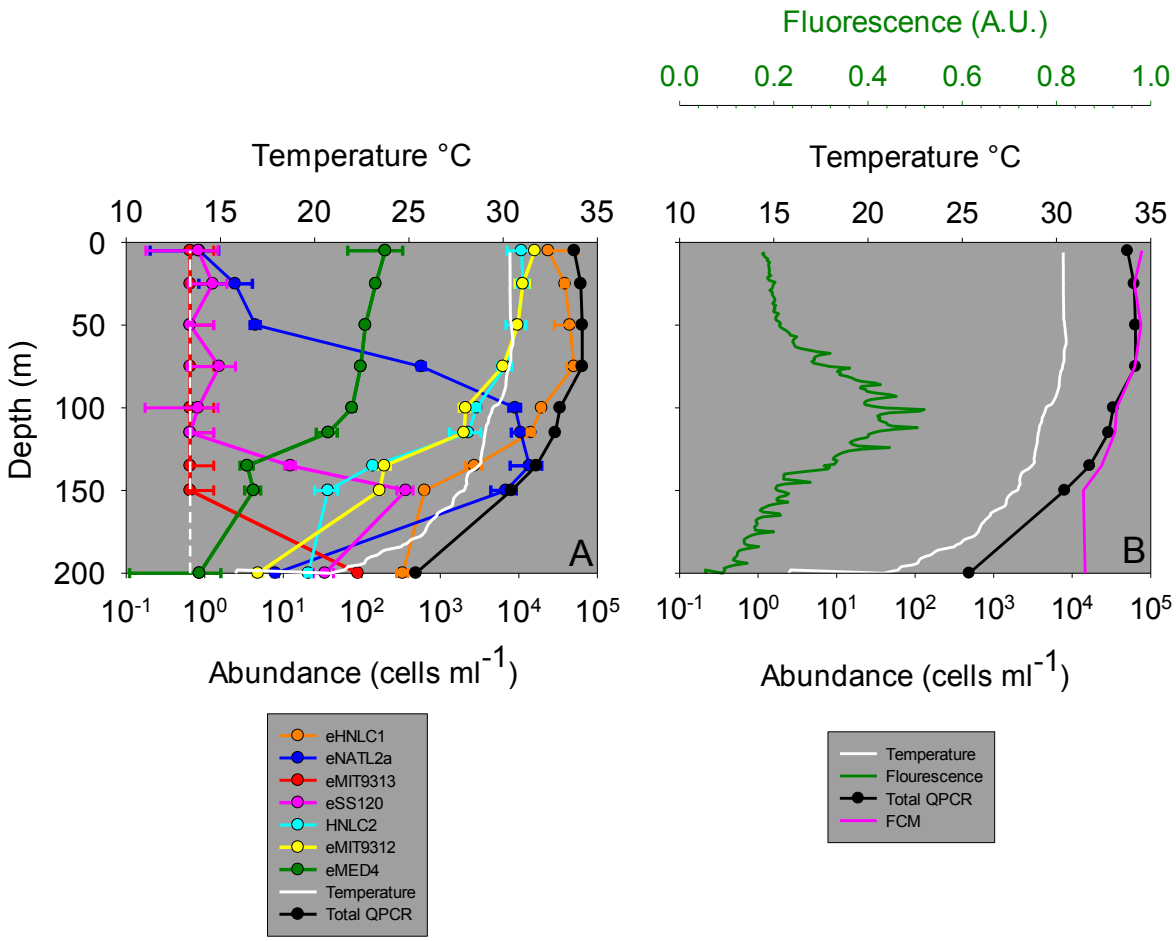


Fig 2.4a- Depth profile of Station 12 from WP2 transect depicting mean *Prochlorococcus* abundance by ecotype for corresponding depth. Total QPCR represents sum of all ecotype QPCR counts. Temperature plotted as continuous data from CTD on top x-axis. **Fig 2.4b-** Depth profile of Station 12 from WP2 transects depicting mean total *Prochlorococcus* by QPCR and by flow cytometry. Temperature plotted as continuous data from CTD on top x-axis with light dependent chlorophyll fluorescence.

WP2 Station 14- The Western Pacific Warm Pool-

This depth profile examines the warmest temperatures encountered on the Pacific basin (Fig 2.5a, 2.5b). With a mixed layer temperature of 30.4 ºC extending down 35m. The base of the euphotic was relatively warm compared to stations immediately north and south, eclipsing 21ºC at the 200m mark. eMIT9312 dominated the mixed layer by 3 orders of magnitude, and interestingly its population size at this station was the highest (700,000 cells mL^{-1}) measured across the Pacific transect. The dominance of eMIT9312 was maintained until the DCM 75m, where eNATL2a peaked and was co-dominant with eMIT9312. We also observed a peak of eSS120 in the midpoint of the DCM and a peak of eMIT9313 low light ecotypes at the lower fraction of the DCM. The HNLC1 clade maintained a uniform population (10^3 cells mL^{-1}) with depth above the DCM; below which they displayed a decline in line with other HL ecotypes. eMED4 and HNLC2 populations were present in very low detection in similar fashion through the DCM at 10^1-10^2 cells mL^{-1}.

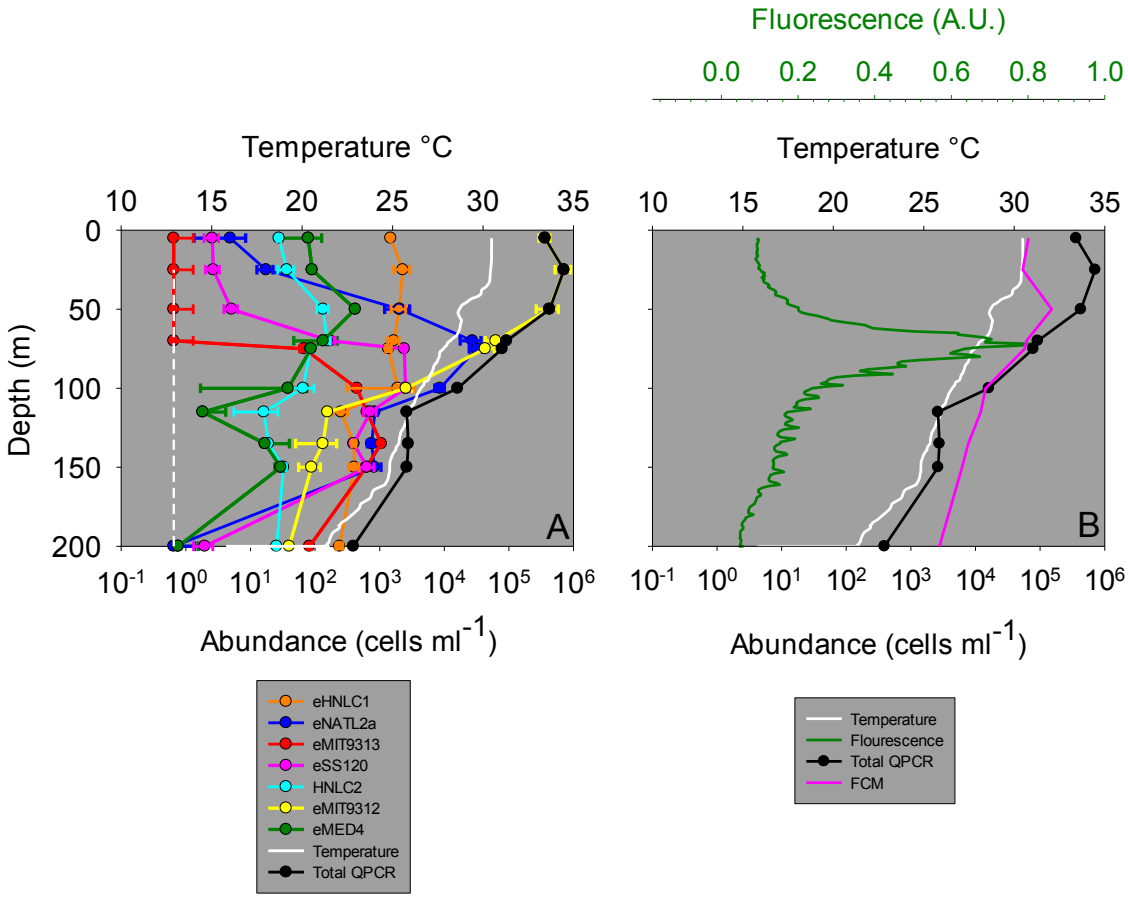


Fig 2.5a- Depth profile of Station 14 from WP2 transect depicting mean *Prochlorococcus* abundance by ecotype for corresponding depth. Total QPCR represents sum of all ecotype QPCR counts. Temperature plotted as continuous data from CTD on top x-axis. **Fig 2.5b-** Depth profile of Station 14 from WP2 transects depicting mean total *Prochlorococcus* by QPCR and by flow cytometry. Temperature plotted as continuous data from CTD on top x-axis with light dependent chlorophyll fluorescence.

WP2 Station 24 -Southernmost WP2 station & eMED4 takeover-

Station 24 represented the southernmost station on the Pacific basin sampling at 36ºS latitude (Fig 2.1, 2.2). This station was characterized by a highly stratified and shallow mixed layer at only 25m and a DCM at 58m. This station represented some of the coldest surface temperatures (20ºC) we observed in the surface layers. Surface waters at this station were dominated primarily by eMED4 ecotypes and represented the highest relative abundance of this ecotype observed during the WP2 cruise, at values above10^5 cells mL^{-1} The shallow DCM and resultant rapid drop in PAR allowed for a takeover of the main population majority by the eNALT2a ecotype. eMIT9313 and eSS120 relative abundances were undetectable or at the limit of detection for the duration of the depth profile, suggesting that cold temperatures combined with a shallow DCM and mixed layer may not favor their preferred niche requirements. Contrary to what was believed to be geographically restrained ecotypes [28], HNLC clades were observed at the southernmost station of the WP2 cruise. The HNLC2 clade remained near the limit of detection throughout the depth profile. Interestingly the HNLC1 clade's abundance of 10^2-10^3 cells mL^{-1} did not vary with respect to depth through the entire 200m euphotic zone profile.

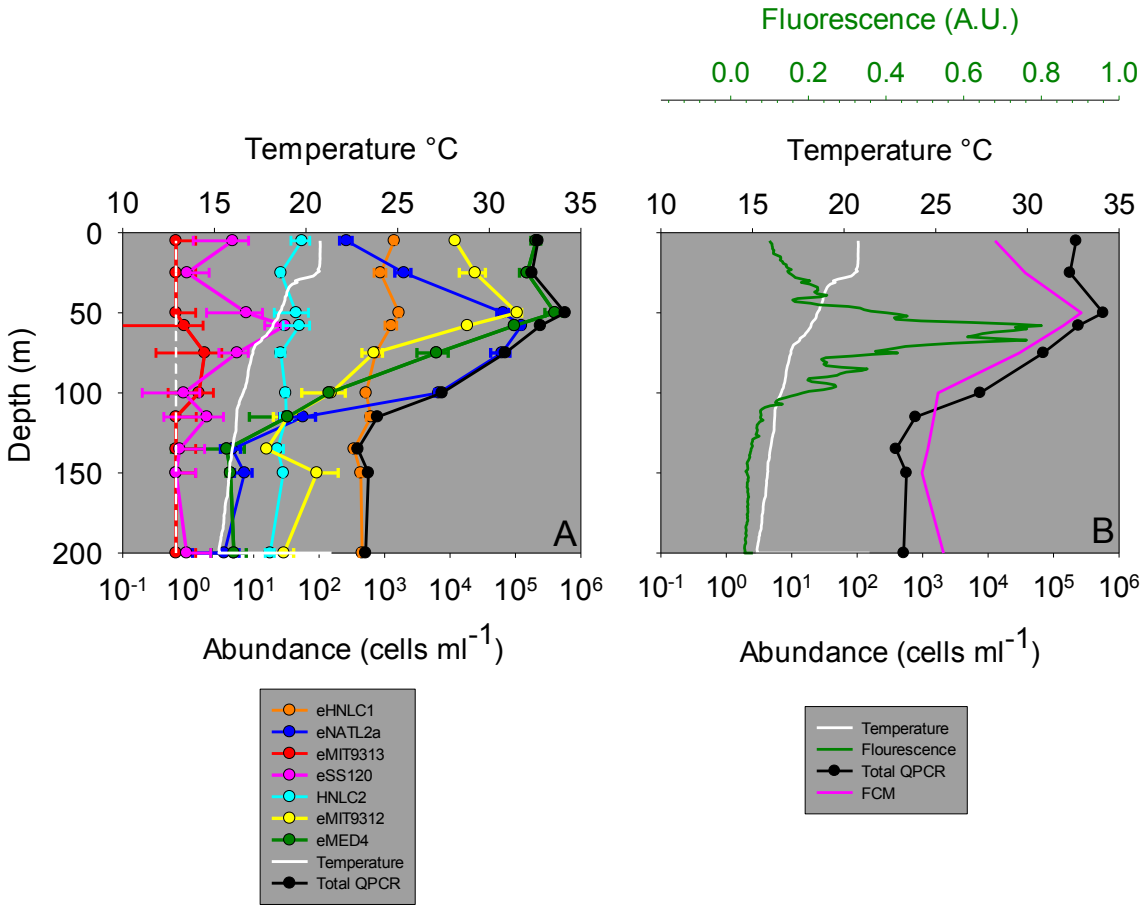


Fig 2.6a- Depth profile of Station 24 from WP2 transect depicting mean *Prochlorococcus* abundance by ecotype for corresponding depth. Total QPCR represents sum of all ecotype QPCR counts. Temperature plotted as continuous data from CTD on top x-axis.

Fig 2.6b- Depth profile of Station 24 from WP2 transects depicting mean total *Prochlorococcus* by QPCR and by flow cytometry. Temperature plotted as continuous data from CTD on top x-axis with light dependent chlorophyll fluorescence.

POWOW Station 10- eMIT9312/eMED4 transition-

Station 10 exhibited a highly stratified mixed layer terminus at 117m depth and a DCM at 120m (Fig 2.7a, 2.7b). The surface temperature for this station was 18.8 ºC and at this station exhibited a steep drop off in temperature below the DCM to 10ºC at 200m. The station is of particular importance because the surface temperatures we observed were correlated with an almost 1:1 ratio of the eMED4 and eMIT9312 populations. eNATL2a populations peaked at the deep terminus of the DCM and dominated the lower euphotic zone. At 125m we observed a peak in eSS120 and eMIT9313 populations, which contrasted with populations that were near or beyond the limit of detection at the southernmost station of the WP2 transect in cooler waters. In general total *Prochlorococcus* abundances at this station down to the 100 m mark were high in the 10^5 cells/mL^{-1} range.

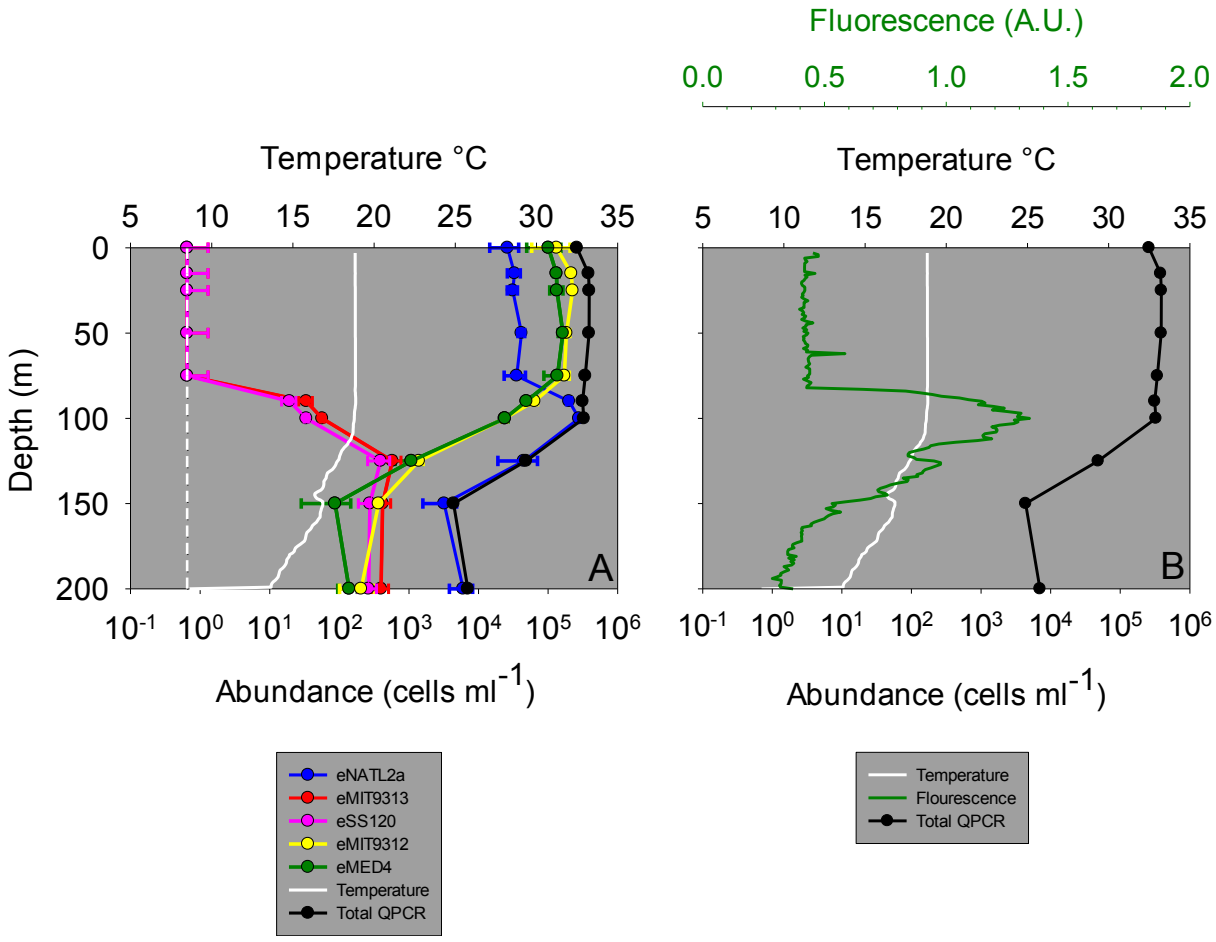


Fig 2.7a- Depth profile of Station 10 from POWOW transect depicting mean *Prochlorococcus* abundance by ecotype for corresponding depth. Total QPCR represents sum of all ecotype QPCR. Temperature plotted as continuous data from CTD on top x-axis.

Fig 2.7b- Depth profile of Station 10 from POWOW transect depicting mean total *Prochlorococcus* by QPCR. Temperature plotted as continuous data from CTD on top x-axis with light dependent chlorophyll fluorescence.

POWOW1 Station 12 –Northern takeover by eMED4-

At station 12 of the POWOW cruise, eMED4 dominated the mixed layer *Prochlorococcus* population (Fig 2.8a,2.8b). This station had the coldest surface temperatures observed along the Pacific basin transect at 13.3ºC, and may be influenced by the nearby (cold) California current. The DCM was located at 70 m and the mixed layers terminated at 108m depth. Temperatures of the lower euphotic zone below the mixed layer dropped rapidly to 6ºC at 200 m. In surface mixed layer, the second most abundant ecotype was eNATL2a, with eMIT9312 present but only third most abundant. In contrast to the southernmost station of the Pacific transect where it was undetectable (Fig 2.6a,2.6b), eMIT9313 populations peaked at over 10^3 cells ml^{-1} just below the DCM (Fig2.8b). All ecotypes experienced a precipitous decline with depth below the mixed layer, concurrent with temperature.

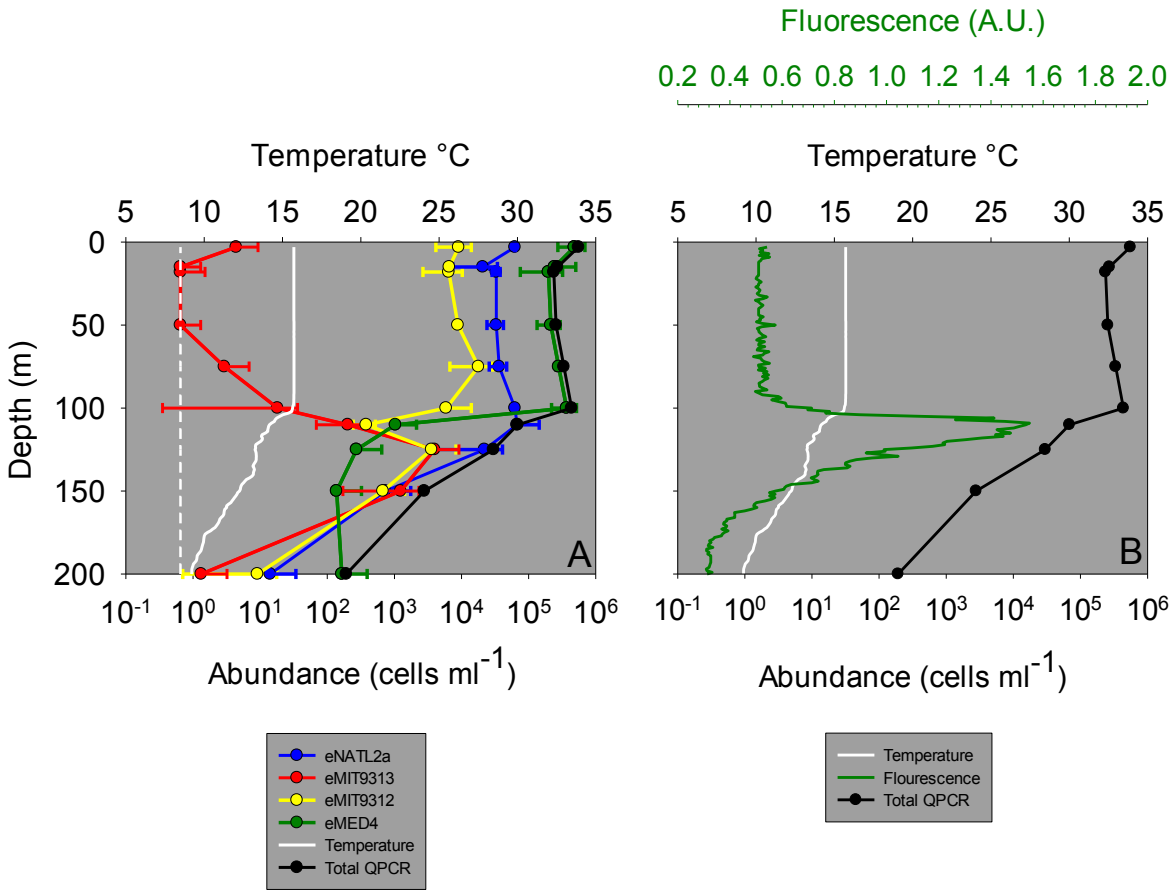


Fig 2.8a- Depth profile of Station 12 from POWOW transect depicting mean *Prochlorococcus* abundance by ecotype for corresponding depth. Total QPCR represents sum of all ecotype QPCR counts. Temperature plotted as continuous data from CTD on top x-axis.

Fig 2.8b- Depth profile of Station 12 from POWOW transect depicting mean total *Prochlorococcus* by QPCR. Temperature plotted as continuous data from CTD on top x-axis with relative chlorophyll-based fluorescence.

Discussion

Overall the trends in *Prochlorococcus* ecotype abundance across the latitudinal Pacific Ocean transect reflected those from an earlier Atlantic Ocean transect [15]. Thus, for the North Atlantic, South Atlantic, North Pacific, and South Pacific a consistent pattern emerges: the eMIT9312 ecotype numerically dominates the lower latitudes, while the eMED4 ecotype dominates the high latitudes, at the limits of the habitat range of the *Prochlorococcus* genus. At these high latitudes, an increase in eMED4 is almost always coincident with an increase in eNATL2A. The eMIT9313 and eSS120 ecotypes never occupy the mixed layer in any significant numbers, whereas eNATL2A occupies the mixed layers in higher numbers at the high latitudes. This difference in eNATL2a's ability to grow within the mixed layer is consistent with the physiological evidence that the eNATL2A ecotype is unique amongst the LL ecotypes in its ability to survive brief exposures to high light [19], as would occur in deeply-mixed surface mixed layers. Hence, any sub-ecotypic differences in genetic content observed between the Atlantic and Pacific Ocean [36] does not appear to impact the structure of the *Prochlorococcus* population structure at the resolution of the ecotype.

The Western Pacific Warm Pool appears to provide a strong selective force in shaping *Prochlorococcus* populations. The very high temperature of surface waters near and around the warm pool region strongly favored eMIT9312 over all other ecotypes; the 3 orders of magnitude difference in closest ecotype by relative abundance is perhaps one of the best examples of eMIT9312's special ability to thrive in exceedingly warm waters. Whether this is the result of reduced competition from other ecotypes unable to fill this niche or the extremely specialized adaptation of the eMIT9312 to warmer temperatures, it is important to note that abundances here of eMIT9312 as well as total *Prochlorococcus* population, were among some of the highest values measured, tied only with populations reported in the Arabian Sea [37]. In the Arabian Sea study, the *Prochlorococcus* population (unidentified at the ecotypic level) was observed during a spring intermonsoon period, with surface

temperatures only 25 ºC, so there is no clear pattern with high *Prochlorococcus* abundances and extreme mixed layer temperature.

We identified a region of the Pacific Ocean just south of the equator that is dominated by the HNLC specialists, consistent with prior studies [28] . Influxes of micronutrients and the resultant decreased bioavailable iron, which has been hypothesized to aid in the growth of eMIT9312[28], may have given the HNLC clades a competitive growth advantage over the population that normally dominates these warmer regions. HNLCs exhibited nearly an order of magnitude in dominance over eMIT9312 at a maximum of 75m. These clades discovered in equatorial regions of the Pacific Ocean with replete micronutrients, specifically high inorganic phosphorous levels, and low iron bioavailability[28] [20]. Equatorial upwelling often occurs a few degrees north and south of the equator and this station and it's reasonable to hypothesize this unique micronutrient niche may have aided in the few stations where HNLC clades represented a significant portion of the population. However, this slice of the Pacific is very narrow, and any advantage the HNLCs may have had disappeared rapidly with latitude as the HNLC clades as they were replaced by the eMIT9312 populations in the stations north and south of this region.

Now that we have shown that the expanse of the HNLC range is more extensive than first thought, future work should include assessment of these populations via quantitative methods to expand upon how far reaching the range for this ecotype may reach. This will include analysis of the HNLC concentrations of the POWOW1 study. Additionally, if members of the HNLC clades can be isolated from the environment, their physiology may also provide insight into their high abundance in the HNLC regions and low but persistent presence in the other regions of the Pacific Ocean.

Close examination of the eMIT9313 integrated abundance and abundance data from depth profiles at stations 11 and 12 revealed an intriguing near-absence of eMIT9313 from the euphotic zones. The mixed layers were not especially deep, so that should not restrict eMIT9313 from this location. Likewise, it was not especially warm or cold in the lower euphotic at these stations, and should be permissive for eMIT9313 growth. If HNLC clades' elevated abundance in these regions were to be used as a metric for potential nutrient replete conditions, perhaps the combination of equatorial upwelling as well as the influx of

these micronutrients could have led to a reduction in these populations, conceivably by competition from other phytoplankton. The two inverse patterns of the HNLC and eMIT9313 ecotypes in this region warrants further observation and study of the dynamics that drive their respective resident populations.

The surface mixed layer of the oligotrophic oceans across many transects seems to harbor the highest numerical fraction of *Prochlorococcus* in the water column through all depth profiles. Further examination of the biotic and abiotic factors that shape these relationships and abundance should be examined, particularly the influence of temperature. In addition to factors influencing population structures, it is becoming increasingly evident that deep sequencing analysis of the dominant populations at these higher south and north latitudes (currently being performed by our collaborators at Duke University) could contribute to the knowledge base as to whether these populations are truly the same, or possess slight genetic variabilities that allow them to extend their ranges. Recent studies of existing *Prochlorococcus* field populations have revealed an underlying diversity whose breadth was previously unknown [28, 38].

Methods

1. Collection of samples for QPCR-

Pacific samples were collected from January 3rd to February 9th 2007 aboard the R/V Kilo Moana on the Western Pacific Warm Pool (WP2) meridional transect from Hawaii to the Tasman Sea, and from February 29th to March 11th 2012 aboard the R/V Thomas Thompson from Hawaii to San Diego. Sample collection was performed according to prior studies [14], [15]. Essentially, selected depths collected from Niskin bottles in the CTD device lowered through the water column were collected in 500mL opaque bottles. Water was moved into a wet lab and for each depth biological quadruplicate samples of 100mL were aliquotted into Pall polysulfone filter funnels and filtered through a .2mm pore size 25mm diameter polycarbonate filter. Filters were chased with 3mL of preservation solution and immediately transferred into -80 ºC storage until thawing for QPCR. Thawed samples were reconstituted in 650µL 10 µM Tris HCl at pH8 and processed via vortexing in a bead

beater at 4800rpm for 2min. 500 ml of the released cells were transferred to clean 1.5mL microcentrifuge tubes and heat lysed at 95 ºC for 15 min. DNA extracted by heat lysis and stored at -80 ºC until needed.

2. QPCR quantification-

Sample preparation and quantitative PCR (QPCR) for the four *Prochlorococcus* ecotypes with cell culture standards was performed as described in Zinser et al. (2006). For the eMED4, eMIT9312, and eMIT9313 ecotypes, the primer sets described in Johnson et al, 2010 were used. For the eSS120 ecotype, the new primer set that co-amplifies eMIT9211 was used [19]. For eNATL2A, a new primer set that amplifies a greater representation of the lineage (Coe and Chisholm, unpubl.) was used. QPCR analysis of duplicate filters per sample was performed with the Takara SYBR Premix Ex Taq QPCR kit (RR420A) for WP2 samples and Qiagen Quantitect Sybr green kit (204145) for POWOW samples in an Opticon II (BioRad) instrument. Measurements of template concentrations were deemed valid if they met two criteria: (i) their threshold cycle (C_T) values were lower than that of the negative controls, which lacked template DNA, and (ii) the melt curve analysis of the products showed an absence of non-specific amplifications. Sample template concentrations lower than the most dilute standard (~10 cell ml^{-1}) were also reported in the data, as long as they had lower C_T values than the negative controls, and with the caveat that they are extrapolated values beyond the standard set. The theoretical limit of detection in this assay is 0.65 cells mL^{-1}, or 1 cell per PCR reaction. To facilitate graphical presentation and comparisons between samples, all samples with values less than this limit of detection were assigned a value of 0.65 cells mL^{-1}, as in prior studies [14, 18]. Integrated abundances were calculated as in Johnson 2006[15].

3. QPCR of the HNLC ecotypes-

Standards for the HNLC1 (or HLIII) and HNLC2 or (HLIV) ecotypes were generated by re-amplification and cloning of PCR amplicons of the ITS regions, using the primers F:5'CCGAAGTCGTTACTYAA CCC and R: 5'TCATCGCCTCTGTGTGCC ([28]). The original PCR amplicons were kindly provided by Rex Malmstrom (MIT). The

PCR amplification consisted of 4 minutes at 94 ºC; 30 cycles of 1 minute at 94 ºC, 1 minute at 52 º C, and 6 minutes at 72 ºC; and 10 minutes at 72 ºC. The resulting amplicons were cloned into Top10 cells following manufacturers protocol (Invitrogen), plated on LB containing 50 mg/mL kanamycin, and incubated overnight at 37 ºC. Colonies were restreaked to ensure clonal populations. Several colonies were picked and tested for amplification using QPCR protocol Clone HL4.1 could be utilized as a standard for HLIV (HNLC2) primer set, while HL3.1 could be used for HLIII (HNLC1). Clones were grown overnight in LB kan media after which plasmid was extracted using Qiagen miniprep kit following manufacturers protocol. The plasmid was sequenced, and primer binding was verified *in silico* using Geneious.

For use as standards, plasmids were treated with PstI digestion to linearize the DNA as per Zinser *et al.* 2006 [14]. Following digestion linearization was confirmed by gel electrophoresis. Linearized plasmid was quantified with Hoechst dye, and concentrations were converted to cells ml^{-1} using the following formula: Log_{10} cell abundance (in cells ml^{-1}) = log_{10} plasmid concentration (in **µ**g $liter^{-1}$) + 6.2 [14]. QPCR with the Takara Sybr Green kit was performed on the Opticon 2 instrument with 15 minutes at 95 ºC; 40 cycles of 45 seconds at 95 ºC, 45 seconds at 50 º C, and 20 seconds at 72 ºC; and 5 minutes at 72 ºC followed by a melting curve analysis from 45 to 95 ºC, measuring fluorescence every 1 ºC. Primers [28] were, for HNLC2: ITSf: 5' -CCGAAGTCGTTACTYYAACCC-3' and HL IVr: 5GTCGTTACTYYAACCC-3'; for HNLC1: HL IIIf: 5'-CGATCGGAACCTCTGAT TTTCGA-3' and HL IIIr: 5' -TAACAGGAAGCTAGATTCTCCCA-3.

Chapter 3

Variable coexistence of *Prochlorococcus* ecotypes in the ocean surface mixed layer along temperature gradients

Publication note:

This section is a version of an article being prepared for submission to the journal of Environmental Microbiology under the same title by Jeremy W. Chandler[1], Yajuan Lin[2], P. Jackson Gainer[1], Zackary I. Johnson[2], and Erik R. Zinser[1*]

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My contribution to this work consisted of field collection of samples for Pacific transect data, Q-PCR for ecotypes, and data analysis of those results.

Abstract

Quantitative PCR analyses confirmed the numerical domination by the eMIT9312 or the eMED4 ecotype, regardless of the presence of other known *Prochlorococcus* ecotypes (e.g. eNATL2A ecotype.) However, while shifts in ecotype dominance along meridional transects were functions of temperature, the less fit ecotypes were not outcompeted to exclusion; rather, a distinct log-linear relationship between relative abundance was observed. These data show that the two dominant ecotypes and the temperature observed, such that for every 2.5 ºC change, the ratio of the two ecotypes shifted by approximately one order of magnitude. The composition of these populations in oligotrophic waters across both the Atlantic and Pacific Ocean basins appears to represent a continuum and stabilized coexistence of these two dominant ecotypes. The contribution of the eMIT9312 or the eMED4 ecotype to total *Prochlorococcus* abundance within the mixed layer was found to be a function of each of these ecotype's relative abundances to the other, while for the eNATL2A ecotype, predominately a minority in the mixed layer, its contribution correlated less well with its abundance relative to eMED4 or eMIT9312. Finally, instances where the ratio of the eMED4 and eMIT9312 abundances did not correlate well with temperature were

identified, and are likely due to the changes in water temperatures outpacing the changes in community structure.

Introduction

Charles Darwin:

"Competition is greatest between those of greatest overall similarity [39]"

Studies identifying high degrees of genetic heterogeneity amongst closely-related microbes in natural samples [40], [16], [7] suggest that the frequency of selective sweeps, and the influence of natural selection and competitive fitness on the construction of the microbial community may be lower than expected from culture-based studies [41], [18], but see [42], [43], [44]. Local environmental fluctuations [45], frequency-dependency [42] and the impact of multiple environmental variables impinging on overall fitness have been invoked as mechanisms to account for the co-existence of microbes of overlapping niches that would otherwise compete to the exclusion of all but the most fit. A key challenge for microbiology is to align the vast extent genetic diversity into a relevant ecological context, by identifying the degree of genetic divergence at which ecologically-coherent lineages can be defined [46], [47]. Despite the high degree of genetic heterogeneity that may exist within a lineage, it is becoming clear that closely-related lineages can have distinctive distribution patterns, and can be classified as different ecotypes.

A challenge equal to that of discovering these ecotypes is the identification of environmental factors - abiotic and biotic - that provide the ecotypes with their distinctive distributions, and by that, their contributions to their respective ecosystems [48]. Diversity of microhabitats within an ecosystem can drive the emergence of ecotypes with differential associations for organic particles, zooplankton surfaces, and the liquid phase [47]. Perhaps the strongest forces for the differentiation into ecotypes are the abiotic environmental gradients that can operate on the scale of millimeters (in the case of biofilms, [49]) to thousands of kilometers (in the case of oceanic meridional temperature gradients, [15]). Gradients of pH [50] and light [13], [18] may contribute to the ecotype development, but the

most common driver of ecotypic distribution is temperature. The role of temperature as a selective driving force for sympatric speciation is well established for the metazoan eukaryotes [51], [52], and appears to be a feature shared by the microbes as well. Ecotype-specific adaptations to different temperatures or to different degrees of temperature fluctuation have been observed for a number of microbes, including *Bacillus simplex* in Evolution Canyon, Israel [53], thermophilic cyanobacteria in several North American hotsprings [54], [55], [56], and as we describe below, *Prochlorococcus* in the open ocean [15], [18].

Since its initial characterization in 1988, several studies have revealed that the marine cyanobacterium *Prochlorococcus* is the numerically dominant phytoplankter in tropical and subtropical oceans [57], [58], [37], [59], [60], [61], [62]. In general it is ubiquitously distributed from N40° to S40° in the euphotic zone of the oligotrophic ocean, reaching depths of approximately 200 m or more. Cell concentrations of surface or subsurface maxima typically exceed 10^5 cells ml^{-1} and are generally more abundant near the surface with decreasing concentrations at depth [3]. In seasonally-dynamic regions of the oligotrophic subtopics, *Prochlorococcus* abundance can be more variable, and during spring bloom events can be outnumbered by other phytoplankton, especially *Synechococcus* [37], [3, 63], [19].

While *Prochlorococcus* is ubiquitously present in the oligotrophic ocean, its genetic composition varies significantly with depth and latitude. In general, the upper euphotic zone, where *Prochlorococcus* is most abundant, is dominated by two closely-related ecotypes, eMED4 ("e" for ecotype, "MED4" for the type strain of the lineage) and eMIT9312 [13, 15, 29]. These two ecotypes share a growth advantage in high light, in relation to the four low-light adapted ecotypes of *Prochlorococcus*, eNATL2A, eMIT9313, eSS120, and eMIT9211, which dominate the deeper euphotic zone [64, 65] (note, low light ecotype eMIT9211 has recently been incorporated into the eSS120 ecotype [19]). In seasonally-stratified regions of the subtropics, deep mixing events facilitate an invasion by the low-light adapted ecotype eNATL2A into the mixed layer [19], which has been attributed to its unique ability amongst the low-light adapted ecotypes to survive brief exposures to irradiances higher than permissible for growth [18], [19].

The two high-light adapted ecotypes appear to partition in the upper euphotic zone by latitude: the eMIT9312 ecotype numerically dominates the middle N30°-S30° band of the Atlantic Ocean, while eMED4 dominates the higher latitudes of N/S 30°-40°, at the limits of *Prochlorococcus'* habitat range [15]. Temperature was the major driver of the observed latitudinal niche partitioning [15], and temperature also describes partitioning in other oceanic regions such as the Indian and South Pacific Ocean [66], [18, 67], [19]. Importantly, the influence of temperature on the abundance of the two ecotypes may be a direct result of temperature's relative influence on their growth. Consistent with their relative distributions in nature, representative strains of eMED4 outgrow eMIT9312 strains at low temperature, while the opposite is true at high temperature [15]. Thus, it appears that different thermal adaptations have allowed the two high-light eMED4 and eMIT9312 lineages to "divide the spoils" and collectively dominate the majority of the ocean's surface phytoplankton community.

That the two high-light lineages are highly related - sharing >99% identity in the 16S rDNA sequence [11] – indicates that this adaptive divergence happened relatively recently on an evolutionary timescale. Thus, relatively few genetic differences between the ecotypes may be responsible for their distinct ecologies. Notably, only a single gene (a homolog of the succinate dehydrogenase subunit-encoding gene *sdhA*) is present in all genomes of one ecotype (eMIT9312) but absent in all genomes of the other (eMED4), suggesting that the ecotype-defining cell properties (e.g. growth optimization for temperature) is likely to result from different allelic states of genes present in both ecotypes (i.e. orthologs) [7].

In this study we closely examined the *Prochlorococcus* ecotype composition within surface mixed layer, adding to our Atlantic Ocean data set ([15]) new data from a meridional transect through the North and South Pacific Ocean. (WP2-2007 and POWOW 2012). In particular, we addressed the habitat ranges of the individual ecotypes, to ascertain if populations exist as a continuum along environmental gradients (especially, temperature), or if they are discretely partitioned, perhaps by threshold values of environmental parameters. Our data indicate that temperature arrays the ratio of eMED4 and eMIT9312 ecotype abundances in a log-linear fashion. Finally, we identify instances when the ratio of ecotypes

diverges from the log-linear relationship with temperature, and suggest how these anomalies could manifest from a temporal lag in population dynamics relative to temperature changes.

Results and discussion

Mixed layer abundances in the Pacific and Atlantic meridional transects

Surface mixed layer samples were collected from large meridional transects in the Pacific (this study WP2-2007) and Atlantic Oceans [15], and three smaller transects in the western North Atlantic [14], [18] (Chapter 2,Fig 2.1a, 2.1b). Stations covered temperate, subtropical and tropical oligotrophic regions, and features including the Gulf Stream in the Atlantic Ocean, the Western Pacific Warm Pool and Tasman Sea in the Pacific Ocean. The Atlantic (AMT13, Sept. 2003) and Pacific (WP2, Jan.-Feb. 2007) transects covered a large range in mixed layer temperature. The mixed layer depth ranged from 10 m to 193 m for all samples (mean = 64 ± 47 m) (Table 3.1) with temperatures varying from 5.6 to 30.5 °C. The mean temperature for the Atlantic and Pacific Ocean transect mixed layers was a fairly warm 23 ºC (Table 3.1), indicating that our dataset has some bias towards oversampling the eMIT9312-dominated waters, relative to the eMED4-dominated waters (with ~19 ºC as the transition point between these regions, see below)

At all stations sampled in the Atlantic ([15], [14], [18]) and Pacific (this study) Oceans, surface mixed layers were dominated by either the eMED4 or eMIT9312 HL ecotype. (Fig 3.1) As the HLNC ecotypes of *Prochlorococcus* contribute significantly to total abundance in the mixed layer only at discrete regions of a narrow temperature range in the Pacific Ocean (Chapter 2, Fig 2.2, Fig 3.1), and no HNLC data are available for the Atlantic Ocean transect, they were excluded from this study. Of the LL ecotypes examined, only eNATL2A was found in abundances exceeding 1000 cells ml^{-1} in the mixed layer, but was never numerically dominant in the surface mixed layer. The majority of the Pacific transect was dominated by the eMIT9312 ecotype, which had a mean mixed layer abundance ~10^5 cells ml^{-1}, and at most stations was approximately 2 orders of magnitude more abundant than that of eMED4 and eNATL2A populations combined. At the Western Pacific Warm Pool station at 9 °S the eMIT9312 ecotype was at 700,000 cells ml^{-1}, which to our knowledge

is the highest recorded concentration of *Prochlorococcus* in the Pacific Ocean, being matched only by populations in the Arabian Sea [37]. South of approximately 30 °S, the eMED4 ecotype abundance increased steadily, and exceeded eMIT9312 abundance in the southernmost station at 36 °S. The steady increase in eMED4 abundance was coincident with a steady decline in mixed layer temperature, a relationship we explore in greater detail in sections that follow.

Table 3.1 Average temperatures and mixed layer depths in both Atlantic and Pacific Ocean Basins examined for this study.

	Avg Mixed Layer Temp	SD Temp	Avg Mixed Layer Depth	SD Depth
All Stations	23°C	±6	64m	±47
Pacific Basin	24°C	±11	72m	±45
Atlantic Basin	22°C	±6	59m	±49
WP2	27°C	±3	54m	±33
POWOW	19°C	±3	112m	±42

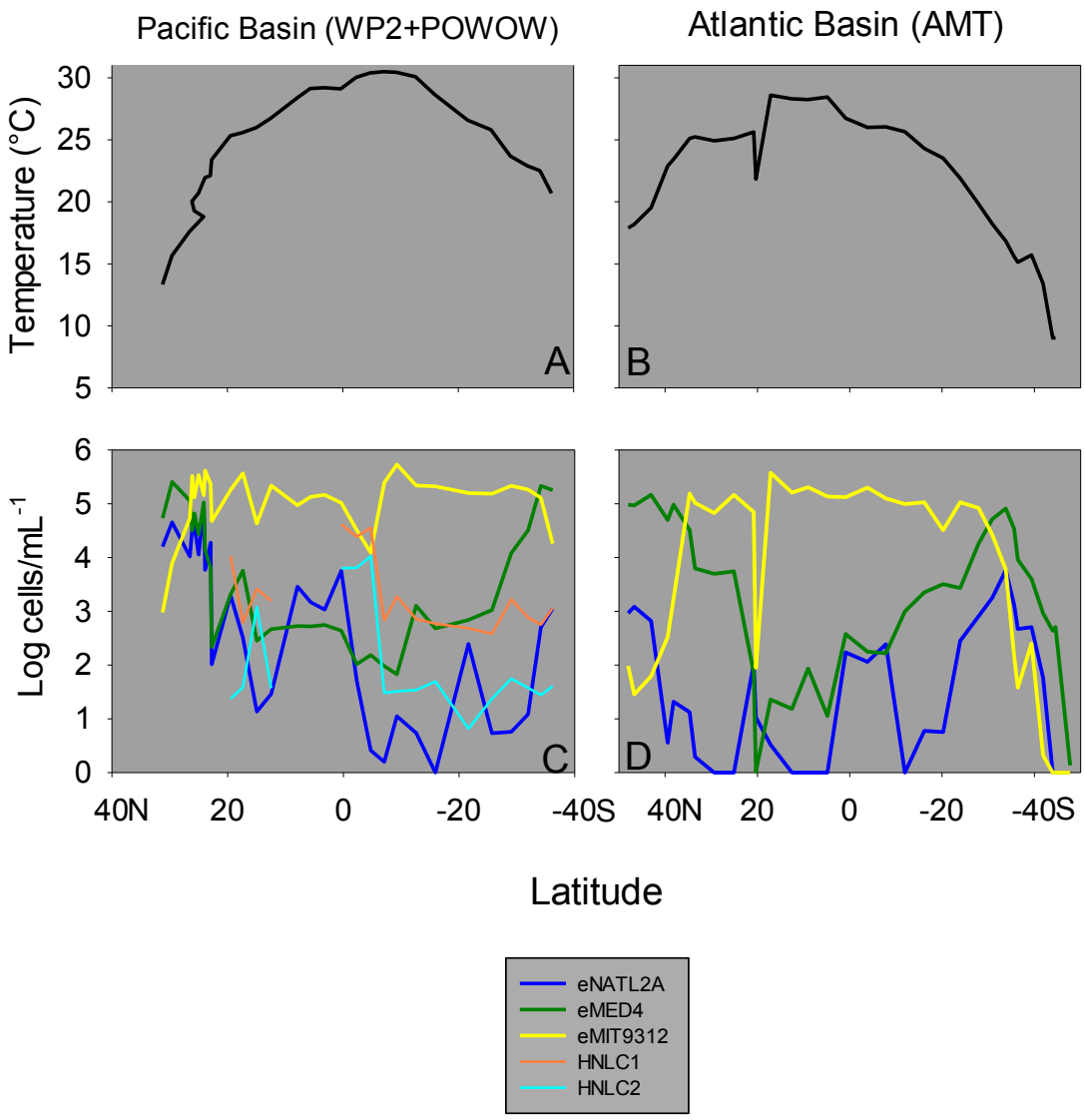


Fig. 3.1- Mean temperature (A,B) and relative ecotype abundance (C,D) in the surface mixed layer as a function of latitude for transect WP2 & POWOW (A,C) and AMT13 (B,D) (Johnson et al. 2006). QPCR abundance (C,D) for the three most abundant ecotypes, eMED4 eMIT9312 and eNATL2A are shown.

Population structure along Pacific Ocean transect was generally consistent with that observed along the Atlantic transect from our prior study [15]. The Atlantic transect reached higher latitudes in the northern and southern hemispheres, and in each hemisphere similar transitions from an eMIT9312- to eMED4-dominated mixed layer occurred at approximately 30-35° latitude (Fig. 3.1 and Johnson et al, 2006). These transitions in ecotype dominance were likewise coincident with declines in temperature, and in the southernmost stations, the mixed layer contained eMED4 exclusively or no ecotypes of *Prochlorococcus* at all.

Ecotype mixed layer abundances as a function of total Prochlorococcus abundance

In our combined Atlantic + Pacific Ocean data set (see Fig 2.1 for stations), only three ecotypes were observed at detectable concentrations in the mixed layer: eMIT9312, eMED4, and eNATL2A (Fig 3.1). We next examined how the relative abundance of the individual ecotypes relates to its fractional contribution to total *Prochlorococcus* abundance (eMIT9312 + eMED4 + eNATL2A). When eMIT9312 < eMED4, abundance of eMIT9312 relative to eMED4 was essentially the same as its abundance relative to total *Prochlorococcus* (Fig 3.2 A). When eMIT9312 > eMED4, abundance of eMIT9312 was essentially equal to the total *Prochlorococcus* abundance. In similar fashion, when eMED4 < eMIT9312, abundance of eMED4 relative to eMIT9312 was essentially the same as its abundance relative to total *Prochlorococcus* (Fig 3.2B). And, when eMED4 > eMIT9312, abundance of eMED4 was essentially equal to the total *Prochlorococcus* abundance.

These results have two important implications. First, for the majority of samples, the mixed layer appears to be overwhelmingly dominated by either eMIT9312 or eMED4; it is only when the eMIT9312:eMED4 ratio approaches 1:1 is the total population not equivalent to either the eMIT9312 or eMED4 population (Fig 3.2A,B). The second implication is that the way each of these ecotypes relates to the total population of *Prochlorococcus* in the mixed layer is a function of how it relates to the other ecotype. Essentially, knowledge of the relative abundance of these two ecotypes gives good predictions of how each contributes towards the total population in both Pacific and Atlantic sample sets.

To put these observations in perspective, trends were less well defined in relation with the third mixed layer ecotype, eNATL2A. eNATL2A abundance relative to eMIT9312, and especially, to eMED4, had a much weaker relationship to its contribution to total *Prochlorococcus* abundance (Fig 3.2C,D). In other words, knowing how abundance of eNATL2A relates to eMIT9312 or eMED4 gives little predictive value as to its contribution to the total population. The same trend is observed for eMIT9312 and eMED4: knowing how abundance of these relative to eNATL2A gives little predictive values as to their contribution to total (data not shown). One interpretation of this combined analysis is that, in accordance with niche theory [68], [69], abundances of the eMED4 and eMIT9312 ecotypes respond to and accordingly align along an environmental gradient(s), whereas eNATL2A abundance patterns are driven by stochastic events such as dispersion, in accordance to neutral theory [70], [71]), or are set by a different environmental variable(s) (rather than influences dictated by presence or absence of the other two ecotypes) than the one setting the other two ecotypes.

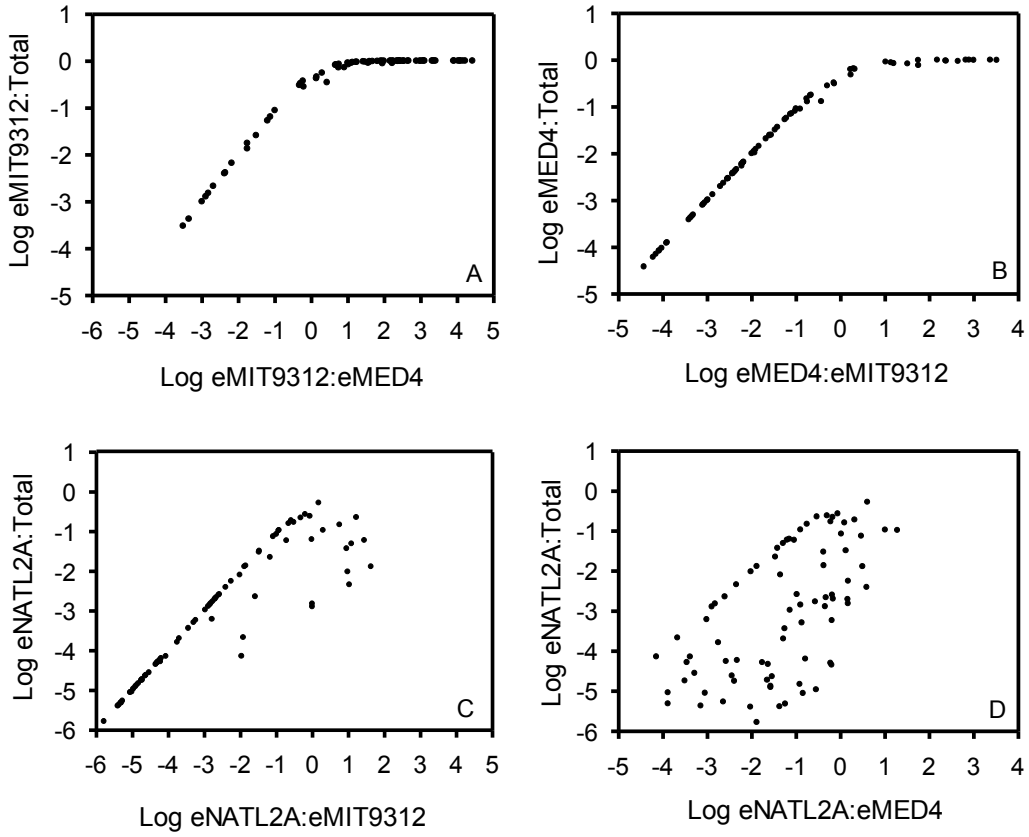


Fig. 3.2- Relative contributions of ecotypes to total *Prochlorococcus* mixed layer abundance as a function of relative abundance to the other individual ecotypes in the Atlantic and Pacific basins. Relationships of (A) eMIT9312 to eMED4 vs. total, (B) eMED4 to eMIT9312 vs. total, (C) eNATL2A to eMIT9312 vs. total, and (D) eNATL2A to eMED4 vs. total are shown.

Our analysis of the relative abundances of *Prochlorococcus* ecotypes in the oligotrophic ocean is not exhaustive, and we recognize several caveats for our interpretations. First, our definition of the total *Prochlorococcus* population is operational, and depends on the ability of our three QPCR primer sets (eMIT9312, eMED4 and eNATL2A) to account for the total population. Disregarding very rare ecotypes (e.g eMIT9313 with counts near the limit of detection, Zinser 2007), it remains formally possible that other, uncharacterized ecotypes may contribute significantly to the total population, and may thus impinge upon the ecotype : total ratios. Only in rare instances in the Atlantic Ocean [15] and Pacific Ocean (data not shown) did total counts by flow cytometry exceed those generated by summing the QPCR counts of each ecotype (although never exceeding an order of magnitude greater). If this discrepancy is not due to experimental sampling error, it may perhaps point to novel ecotypes, or sub-lineages within the known ecotypes (dominating the ecosystem), that do not amplify with the current primer sets, which may be dominating the ecosystem.

Second, there is evidence from a prior study that some regions may be numerically-dominated by low-light ecotypes, at least seasonally. Using dot blot hybridization to quantify *Prochlorococcus* ecotypes (comparable to our QPCR method, see Zinser et al. 2006), Bouman and colleagues found regions of the South Pacific (~30-35°S) where one or more low light adapted ecotypes constituted over 50% of the surface mixed layer population [66]. Our Pacific Ocean transect intersected the Bouman et al. (2006) transect but showed clear dominance of the high light ecotypes in this region (Fig 3.2). This discrepancy may be due to seasonal variation, as our cruise spanned the austral summer, and the earlier cruise spanned during winter. Further analysis of this region, particularly during different seasons, should be particularly interesting to see how the relationships between the eMED4 and eMIT9312 ecotypes may change during periods in which neither is the dominant ecotype. However, despite the possibility that other ecotypes may dominate the oligotrophic ocean in certain regions and/or seasons, the striking relationship between eMED4 and eMIT9312 abundances is clearly indicative that they are responding to environmental variables in ecotype-specific ways that are significantly impinging on their relative contributions to the *Prochlorococcus* population structure in the mixed layer.

Relative abundance of eMIT9312 and eMED4 in mixed layer abundance as a function of environmental variables

A pattern of variation across both the WP2 and AMT transects identified temperature as the principle component across common physical and biological variables catalogued with depth profiles (Yajuan Lin & Zackary Johnson, unpub.). This analysis supported the importance of temperature in the mixed layer and prompted a deeper exploration of the relationship between temperature and the ecotypes in the mixed layer. In concordance with the PCA analysis, eMED4 and eMIT9312 abundances and contribution to total *Prochlorococcus* in the mixed layer exhibited strong, distinctive relationships with temperature. While eMED4 abundances peaked between 17-23 °C, eMIT9312 abundances peaked above 23 °C (Fig. 3.3A,C). Consistently, contributions to total *Prochlorococcus* abundance in the mixed layer was related to temperature in an ecotype-specific manner (Fig 3.3B,D). At low temperatures, eMED4 was the predominant ecotype, while at high temperatures, the predominant ecotype was eMIT9312. For eMED4 at high temperatures, and eMIT9312 at low temperatures, contributions were lower, and more variable. The greater variability of eMED4 and eMIT9312 contributions at their respective suboptimal temperatures is due to the variable contribution of the third ecotype, eNATL2A. In general, at cold temperatures, the eNATL2A ecotype had a significant contribution to total (>1%), although it never assumed the position as most abundant. Whereas, in warmer samples, eNATL2A abundance was highly variable, spanning 5 orders of magnitude. Thus, with decreasing temperatures, the variability of eNATL2A abundance decreased, while its contribution to the total tended to increase.

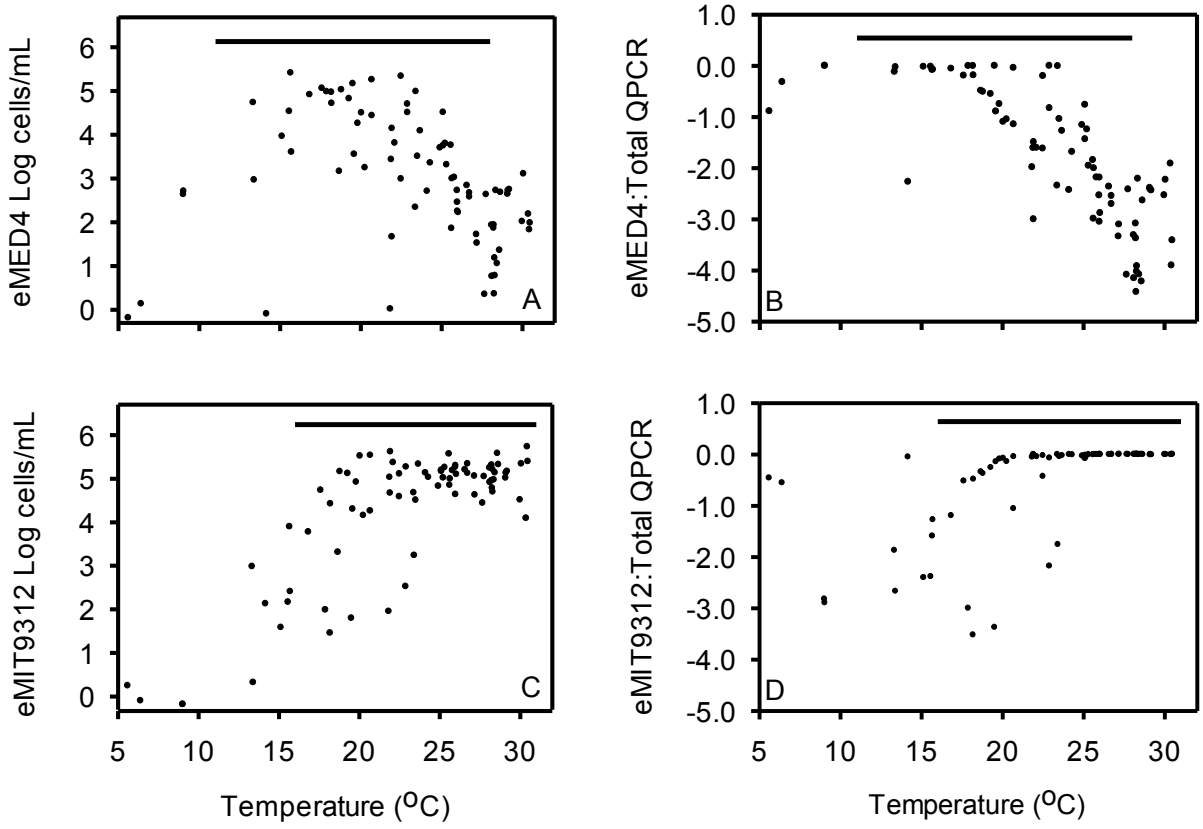


Figure 3.3- Ecotype abundance and contribution to total *Prochlorococcus* as a function of temperature in surface mixed layer. (A) eMED4 and (B) eMIT9312 abundance vs. temperature, (C) eMED4 and (D) eMIT9312 abundance relative to total population vs. temperature are shown. Permissive growth range for representative strains of the ecotypes (Johnson et al. 2006) is denoted by a horizontal bar.

Our results reinforce the concept of zones of the ocean mixed layer defined by numerical dominance by eMED4 or eMIT9312, with sharp transitions between these zones, and temperature as the primary factor establishing these zones [15],[67]. However, closer inspection of the relative abundances of these two ecotypes along a temperature gradient revealed a significant and previously unrecognized relationship (Fig. 3.4). The ratio of the eMIT9312 to eMED4 abundances for the combined Atlantic and Pacific open ocean mixed layer samples exhibits a log-linear relationship with temperature ($r^2 = 0.75$), with roughly one order of magnitude increase in the eMIT9312 : eMED4 ratio per 2.5 ºC increase. Importantly, the temperature at which eMIT9312 and eMED4 are equally abundant is approximately 19 ºC, which matches well with the temperature at which representative strains grow at equal rates [15], consistent with prior assertions that intrinsic optimalities for growth define the zones of dominance for these ecotypes [15], [67]. At temperatures exceeding 19 ºC, eMIT9312 outnumbers eMED4, while the opposite is true at temperatures below 19 ºC. Thus, while the zones of numerical dominance are sharply defined - consistent with prior studies [15], [67] - at roughly 20 ºC, the relative abundances within each zone is variable and temperature-dependent. *Prochlorococcus* population structure in the mixed layer therefore exists as a continuum, with temperature defining the relative contribution to total. This relationship between relative abundance of the eMED4 and eMIT9312 ecotypes and temperature has three important features, whose implications we discuss in turn: (1) the open ocean samples most removed from the linear regression (i.e. the outliers) are from water masses moving northward or southward in currents from warmer or colder source waters, respectively, (2) the log-linear nature of relative abundance as a function of temperature, and (3) within the range of overlapping thermal ranges, neither ecotype appears to be outcompeted to extinction.

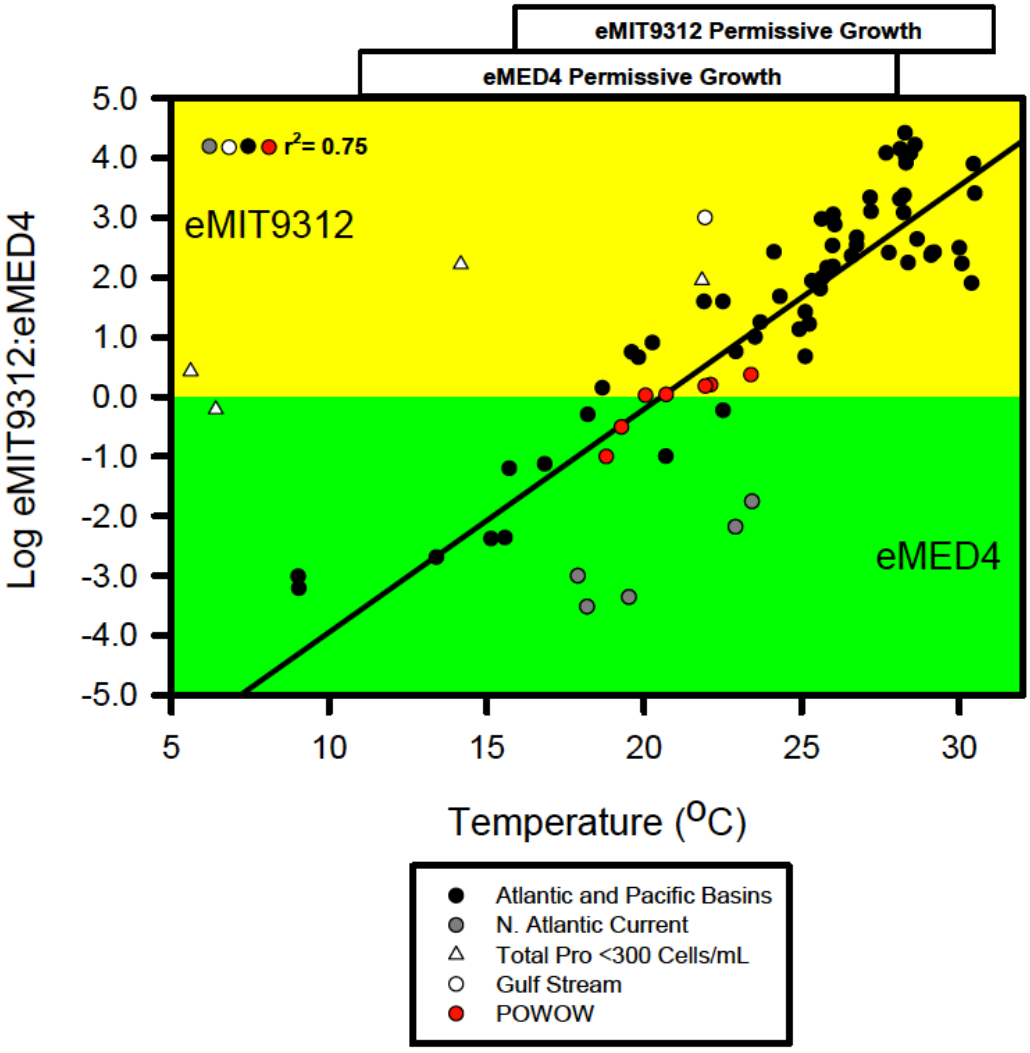


Fig. 3.4- Relative abundance of eMIT9312 and eMED4 in the mixed layer as a function of temperature. Coastal stations with < 300 cells ml^{-1} total *Prochlorococcus* (triangles) and open ocean stations with >300 cells ml^{-1} total *Prochlorococcus* (circles) are shown. The black diagonal line denotes linear regression for all open ocean stations. Stations in the Gulf Stream (open circles) and the eastern N. Atlantic (gray circles), discussed in the text, are distinguished from the other open ocean stations (black circles), POWOW (red circles). Permissive growth ranges for representative strains of the ecotypes (Johnson et al. 2006) are denoted by horizontal bars above the plot.

The relative abundance of the two ecotypes shows a clear trend according to temperature. The positive slope of the log-linear relationship between eMIT9312:eMED4 and temperature correlates with a similar positive slope in relative growth rates of cultured representatives of the two ecotypes, both in isolation (Johnson 06 and this study) and in co-culture. The strong correlation between intrinsic growth rates and relative abundance suggests ecotype distribution is defined by niche differentiation (i.e. relative fitness) [72], rather than by neutral forces (e.g. dispersal limitation, stochastic death) acting on ecologically-equivalent types [73]. Thus, along an isotherm, the relative fitness of the two ecotypes in the mixed layer are constant and predicts, that as isotherms shift due to seasons and ocean circulation, the respective fitness of the ecotypes at fixed locations will vary accordingly.

Despite the influence of selective forces favoring one ecotype versus the other at selected temperatures, neither ecotype is outcompeted to (local) extinction within their overlapping range of permissive temperature (as defined by cultured representatives). This co-existence within the range of mutually-permissible temperature appears to be stable (i.e. permanent); of all the mixed layer samples analyzed for both oceans, only at the non-permissive temperatures is one or the other ecotype below the limits of detection. It is perhaps not surprising to find that these two *Prochlorococcus* ecotypes co-exist in the surface mixed layer; bacterial diversity is high in the oceans, especially in the tropics and subtropics [74] where these two ecotypes co-habitate. Indeed, Hutchinson coined the phrase "paradox of the plankton" to describe the disconnect between the principle of competitive exclusion [39] and the high level of phytoplankton diversity in large bodies of water [45]. Temperatures where competition to exclusion does not occur may indicate presence of stable coexistence between the populations from the range of habitation (10ºC -30ºC) at fixed ratios. This suggests a compensatory rate of colonization and loss as a function of temperature change along that gradient.

While co-existence prevails largely in nature, factors preventing competition to extinction, particularly for the case of the *Prochlorococcus* ecotypes, are relatively poorly

understood. The principle of competitive exclusion states that that n competitors cannot co-exist indefinitely if competing for less than n resources [39]. Of note, members of the eMED4 ecotype can utilize organic forms of phosphorus, while the eMIT9312 members apparently cannot [75]. It is likely that co-existence in P-limited surface mixed layers might be stabilized by the eMED4 ecotype's ability to circumvent exclusion by superior competition of eMIT9312 for phosphate by utilizing an alternative form of the resource. The relative transport kinetics and binding affinities of the two ecotypes for phosphate are still unknown, especially as they relate to different seawater temperatures, but future studies might shed light on this possible explanation for co-existence.

Apart from resource partitioning, which has been unable to account for the full breadth of microbial diversity, many mechanisms explaining the co-existence of competitors have been invoked, with the overarching hypothesis that one competitor is not best under every environmental condition, and that conditions can vary within the ecosystem in relevant spatial and temporal timescales [76], [45], [77]. These typically involve fitness tradeoffs [77], [78],[79], and function to dampen or negate net fitness differences between the competitors that would otherwise comply with the principle of competitive exclusion. Thus, while the fitness differences between the competitors may drive the mixed community towards the extinction of one or more of the competitors, environmental disturbance, spatiotemporal variation in the ecosystem, and differential responses to environmental parameters can prevent competition from achieving extinction. Hutchinson asserted that the marine phytoplankton community approaches but never achieves an equilibrium state where competitive exclusion is achieved, in large part due to the relatively slow growth (and takeover) kinetics of the competitors [45]. In agreement, experimental and model systems indicate that the frequency of disturbance (e.g. nutrient pulses, light variation) appears to be important for the maintenance of coexistence: too low or too high can lead to extinctions [80], [81], [82], [83], [82]. Vertical mixing [84], [85], toxin production [76], viral Winter[86], [78] and grazing pressure [45], [76], [87], [88] have also been implicated in experimental and theoretical systems where co-existence has been established.

Numerous mathematical models, including Lotka-Volterra competition and resource competition theory have incorporated one or more of these variables to identify conditions

whereby co-existence of competing microorganisms is possible ([77], [76, 89]). The latter model was able to account for the higher level of phytoplankton diversity in the low versus high latitudes of a global ocean simulation ([82]), which correlates well with empirical data ([74]). A recent model by Neill et al ([90]) "…predicts that the relative abundance of two species is a continuous function of their intrinsic parameters with respect to growth, mortality, and resource use efficiency." This last model may resonate well with our *Prochlorococcus* ecotype data, and begs the question: does temperature impinge on these parameters in an ecotype-dependent manner, to account for the observed log-linear relationship in relative ecotype abundance as a function of temperature?

The 4th transition described: the N. Pacific.

The two major transects of the study identified three locations of takeover of numerical dominance in the surface mixed layer: the N. and S. Atlantic, and the S. Pacific: polewards of the 19ºC isotherm the population is dominated by eMED4; towards the equator the population is dominated by eMIT9312. To confirm that same relationship is also true for the N. Pacific Ocean, we collected samples along a transect from Hawaii to San Diego in Spring 2012 (Fig 2.1). As predicted from the other three transition zones, the eMIT9312 populations ceded dominance of abundance in the mixed to that of eMED4 at approximately 19 ºC (Chap 2 Fig 2.2, Fig 2.6, Fig 2.8). Consistent with the Atlantic and the other Pacific stations, the log ratio of eMED4:eMIT9312 correlated strongly with temperature (Fig 3.4).

Ecotypic lag

The anomalously-high eMIT9312:eMED4 ratio (1000:1) for the 2001 Gulf Stream EN351 station (Fig. 3.4, open circle) may be explained by a lag between temperature decline and population re-structuring to the expected ratio for that temperature, 21.9 ºC [18]. The higher than expected ratio is due to the lower than expected value of eMED4 abundance ($1.03x10^2$ cells/ml^{-1}), rather than a higher than expected value of eMIT9312 abundance. Given a conservative estimate of current velocity of 2 m s^{-1}, the body of water sampled at the EN351 station was about 10 days removed from the origin of the Gulf Stream in the Florida

Straits (for this purpose = N25, W80); temperature in this region was significantly warmer, at 25.7 ºC (on 4/1/01) (NOAA Comprehensive Large Array-Data Stewardship System; http://www.class.ngdc.noaa.gov/saa/products/welcome). Thus, during the 10-day transit of this population to the site of sampling, the mixed layer temperature decreased almost 4 ºC. While we did not measure ecotype abundances at the Gulf Stream origin, we did measure abundances 10 days earlier in the Equatorial current (9.98N), which contributes to the Gulf Stream, and has a comparable temperature (26.0 ºC) [18]. The eMIT9312:eMED4 ratio in the Gulf Stream (1000:1) was much more similar to that in the Equatorial current (340:1) than to the Sargasso station at BATS (8:1), even though the latter was geographically closer and had a more similar instantaneous temperature (20.3 ºC). We suspect that, given enough time at the lower temperature, the absolute abundance of the eMIT9312: eMED4 ecotype ratio in this north-bound water mass would shift to levels approaching that in the Sargasso Sea. The second group of anomalous data are from the eastern North Atlantic September 2006 stations, which exhibit lower than expected ratios of eMIT9312:eMED4 for their respective temperatures (Fig. 3.4, gray circles). Currents and current velocity in this region is less resolved than for the much faster Gulf Stream, but ADCP data of surface waters indicated a southerly direction of flow at all of these stations (data not shown), and suggest that these communities were transported from the colder, higher latitudes, where eMIT9312:eMED4 ratios are lower.

We believe that such anomalies may point to an important relationship between relative ecotype abundance and temperature; namely, that for fast-moving surface currents, the instantaneous temperature may be less predictive of ecotype abundances than prior temperatures. We thus denominate the term describing this phenomenon as “ecotypic lag”. Such a lag in population restructuring may ultimately come down to a rate issue: as temperature changes shift the relative fitness of a given ecotype, how quickly does that shift in relative fitness manifest itself in relative abundance within a given population? Laboratory studies with a temperature shift of 1 degree per day from temperatures favoring growth of eMIT9312 have revealed a distinctive competitive advantage in response to these dynamics (See Chapter 4, Fig 4.4). When shifts from 24ºC to 27 ºC were conducted eMIT9312 populations outpaced growth of the MED4 populations.

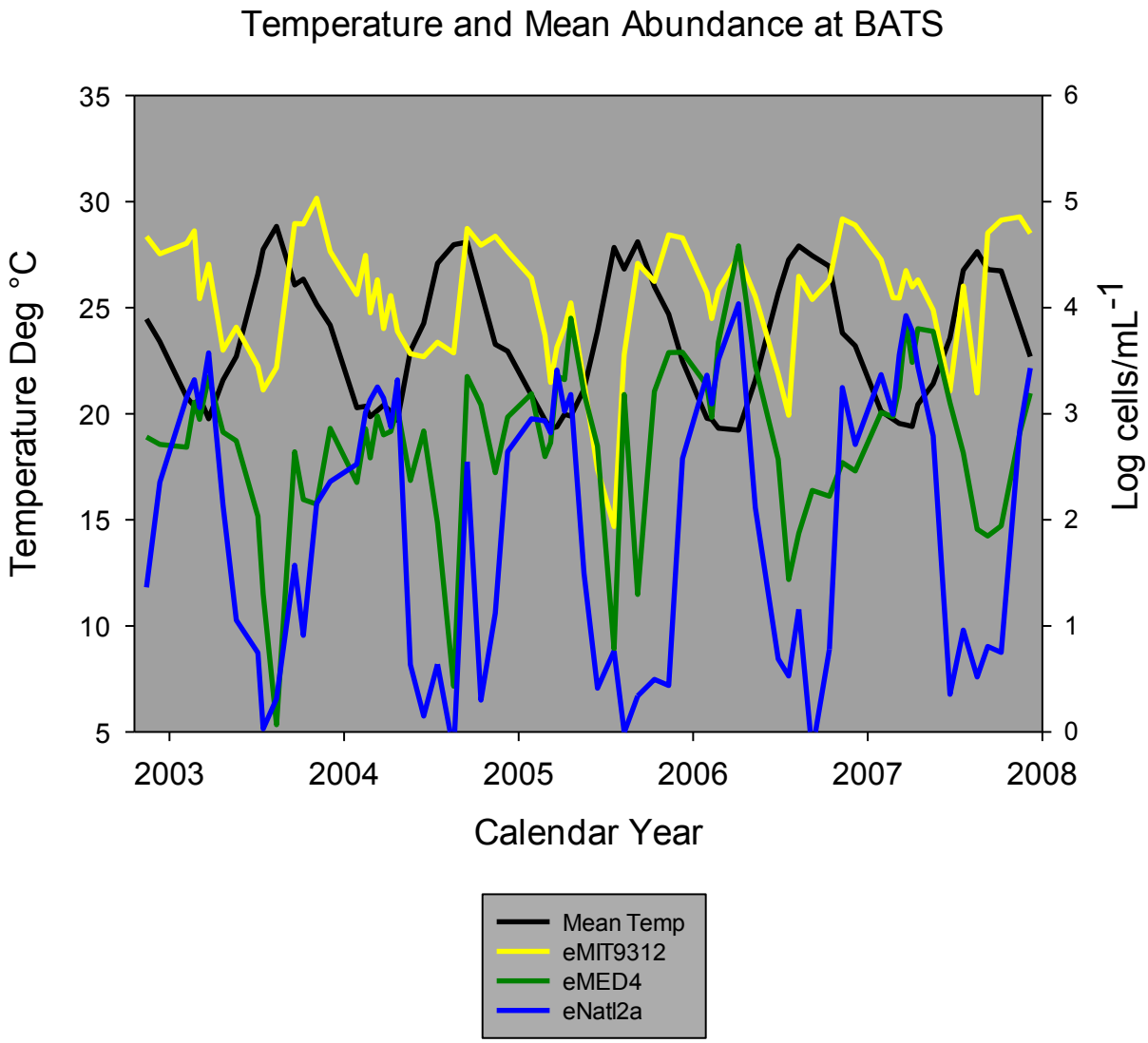


Figure 3.5. Mean temperature and mixed layer abundance of dominant *Prochlorococcus* populations vs. time from winter of 2002-2008. (Adapted from data of Malstrom et al. 2010).

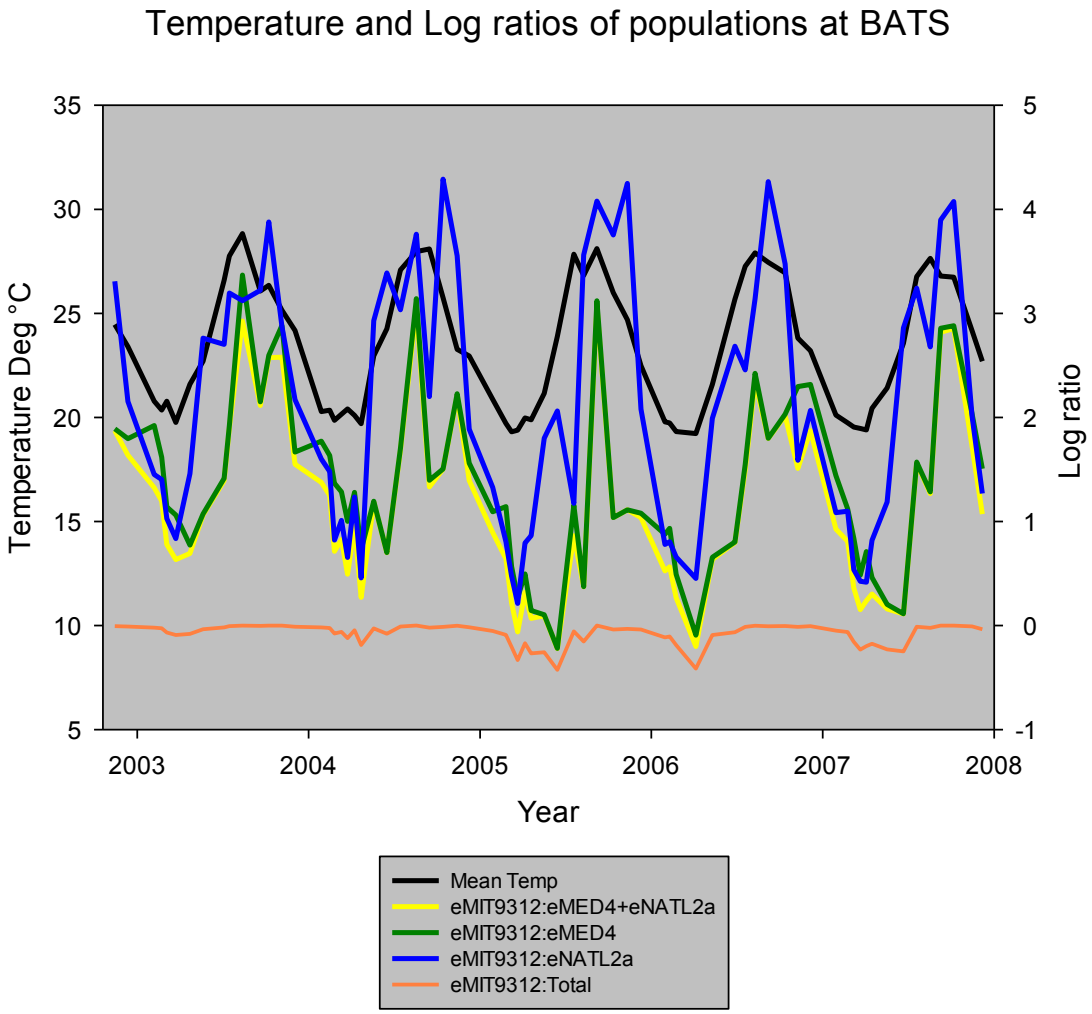


Figure 3.6. Temperature profile and Log transformed ratios of mean mixed layer abundances when compared to dominant eMIT9312 populations at BATS over time in the mixed layer. Data adapted from (Malstrom et al. 2010)

Further evidence of ecotypic lag comes from our new analysis of the results of a published study [19] on the dynamics of the *Prochlorococcus* ecotypes at a station in the Sargasso Sea (Fig 3.5, Fig 3.6, Fig 3.7). Ecotypes were quantified once a month over the course of 5 years at the Bermuda Atlantic Time Series (BATS) station. Malmstrom et al. reported that ecotype abundances showed strong and reproducible seasonal successions, with the eMIT9312 ecotype maintaining dominance throughout the year, but with differential contribution of eMED4 and the other ecotypes depending on the season. We were interested in seeing how the ratio of the ecotypes in the mixed layer vary over the seasons at this station.

Temperature flux in the mixed layer at BATS ranged from 19.2 to 28.8 ºC and was predictable with seasonality (Figure BATS-1). eMIT9312 and eMED4 ecotypes exhibited dominance throughout this time course, with eMIT9312 dominating the majority of the time [19]. The one instance in which MED4 abundance exceeded that of eMIT9312 was at a low point of temperature of 19.2 ºC reached in 2006. Interestingly this data point also coincided with the highest abundance of eNATL2a in the mixed layer as well. The previous warm season over the fall and early winter of 2005 leading into this cooler season was the most active hurricane season in recorded history (NOAA.gov). Given eNATL2A's status as a primarily low light adapted strain with the added potential of more diverse nutrient usage, strong turbulent mixing in addition to a change in nutrient regime may have allowed the eNATL2a population to peak higher than previous years [13, 91].

Dynamics of the three ecotypes in the mixed layer displayed a lag with respect to the seasonal dynamics of temperature. The average time from temperature maxima to abundance maxima for eMIT9312 was approximately 3 months, encompassing up to four orders of magnitude of increase in population mean abundance density change during that time. Populations of eMIT9312 did not increase until temperatures increased to approximately 25 ºC. A lag was also seen between temperature minimum and minimum eMIT9312 abundance. The duration of the drop to eMIT9312 minima was longer than that for the climb to maxima, and averaging almost four months for a four orders of magnitude drop. It is noteworthy that in 2005 the dip in temperature just before the maximum is

mirrored over a month later by a similar dip in eMIT9312 abundance prior to its maximum (Fig. 3.5).

In contrast to eMIT9312, peak abundance of eMED4 in the mixed occurred when temperature was at the annual low (Fig. 3.5). In support of potential niche competitive or takeover interactions we noted that the change in eMED4 concentration over time was almost always negatively correlated with that of eMIT9312. This is especially evident when the eMIT9312 population is in decline: this is consistently a period of gradual increase in eMED4. In mean abundance dynamics of eMED4 populations we observed similar but reversed trends that tracked more closely with temperature than that of eMIT9312. Curiously the apparent ability for the eMED4 populations to respond to the temperature changes are less temporally separated than for eMIT9312 populations. For example, in 2005, the dip in temperature preceding the temperature maximum for the year is concurrent with a sharp spike in eMED4 abundance. It is possible that the response times for changes in abundance for eMED4 after temperature changes are faster than that of accompanying eMIT9312 populations; experiments with laboratory cultures can offer insight into this possibility. Also of note was that for corresponding declines of the three main ecotypes eMIT9312 experienced in general an order of magnitude less drop in mean abundance with the exception of the 2005 season (most active hurricane season on record). Referring back to figure 3.4 it can be seen that the drops in temperature never reached beyond the limits for growth for eMIT9312. This contrasted with the range of eMED4 ecotypes, which would have experienced temperatures beyond the upper limits of their permissive ranges.

Similar to eMED4, eNATL2A abundances exhibited a close track with temperature, but were often interrupted by large swings in magnitude across broad gains and declines. The status of the eNATL2A ecotype as a LL ecotype with the ability to endure short periods of HL intensity further complicates analysis of abundance changes in the eNATL2A ecotype. Broadly from BATS abundance data it seems evident that the phenomenon of ecotypic lag may be differentially experienced by various ecotypes and that the variations observed by the three most successful ecotypes in the surface mixed layer warrants further investigation.

Despite the lag in maximal abundance of eMIT9312 with respect to maximal temperature, the ratio of eMIT9312:eMED4 abundance correlated strongly with temperature

(Figure 3.6). The ratio was highest and lowest at the temperature maxima and minima, respectively. Additionally, the ratio of eMIT9312:eMED4 exhibited a close, inverse relationship to the ratio of eMIT9312:Total abundance, whereas for the eMIT9312:eNATL2A over time, the relationship with eMIT9312:Total is less clear. This confirms a greater impact of community composition for the eMED4 ecotype relative to eNATL2A, and reinforces the notion that domination of the mixed layer is a function of temperature's impact on the relative growth characteristics of the eMIT9312 and eMED4 ecotypes.

Finally, when BATS ratio data from the mixed layer was introduced into our ratio model (Fig 3.7), we see further evidence of how ecotypic lag may influence ratios. When aligned with previous data massed from both Pacific and Atlantic transects, data the broad trend is satisfactorily still supported (r^2=0.63), but with higher deviations likely due to the incursion of warmer currents combined with seasonality at BATS. It would be interesting to compare these seasonal influences to those that occur in higher latitudes, where the temperature range may favor the eMED4 ecotype.

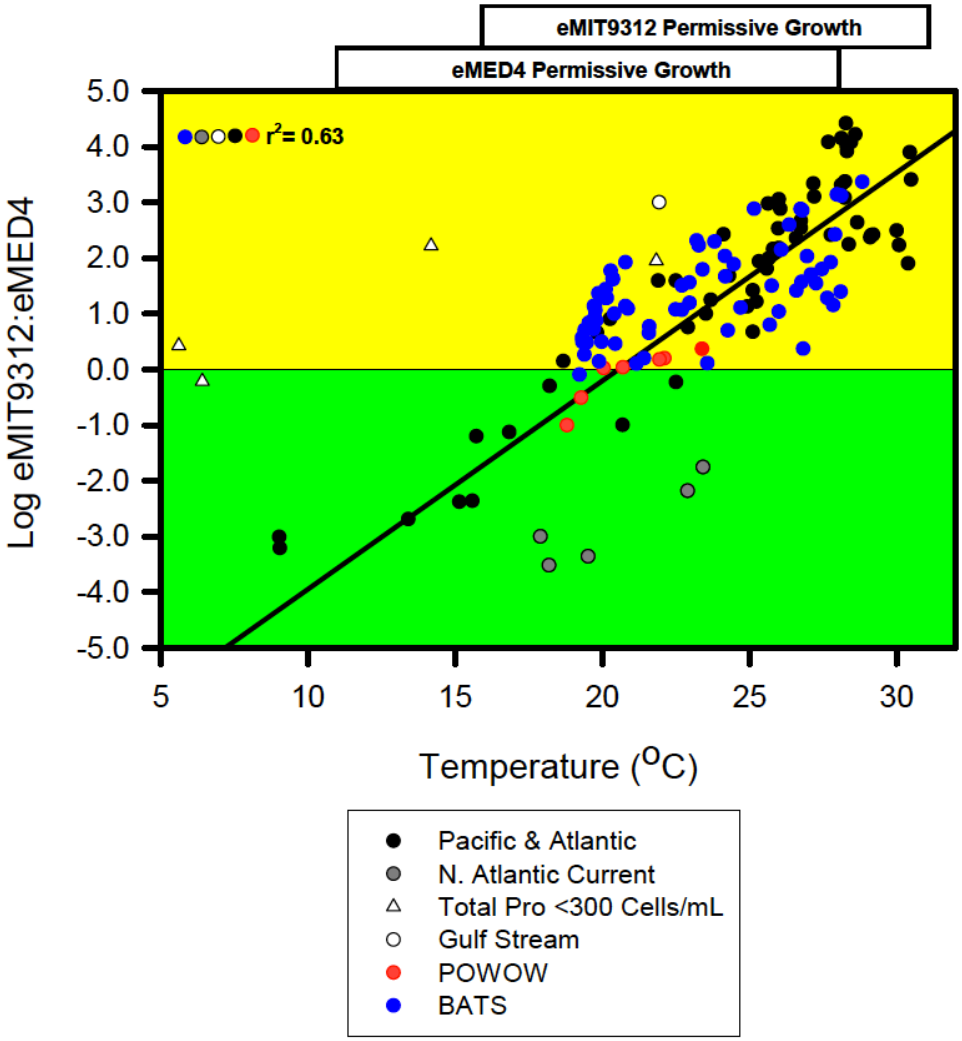


Figure. 3.7- Relative abundance of eMIT9312 and eMED4 in the mixed layer as a function of temperature. Coastal stations with < 300 cells ml^{-1} total *Prochlorococcus* (triangles) and open ocean stations with >300 cells ml^{-1} total *Prochlorococcus* (circles) are shown. The black diagonal line denotes linear regression for all open ocean stations. Stations in the Gulf Stream (open circles) and the eastern N. Atlantic (gray circles), POWOW (red circles), BATS (blue circles) are distinguished from the other open ocean stations (black circles). Permissive growth ranges for representative strains of the ecotypes (Johnson et al. 2006) are denoted by horizontal bars above the plot.

Conclusions

This work extends the prior work in the Atlantic Ocean establishing the ecotype abundances along a latitudinal transect of the Pacific Ocean. Despite the high intra-ecotype diversity [16], [38], suggesting weak selective forces, there are strong inter-ecotype differences in distribution across latitude , indicating that the environment is nonethess imposing selection at the ecotype level. What factors are primarily responsible for these inter-ecotype variabilities are largely unknown, but it is likely not a coincidence that the relative growth rates of cultured representatives [18] reflect their relative distributions in field as they relate to temperature.

This work represents the first formal consideration of the relative abundance of the dominant ecotypes with plausible different contributions to biogeochemistry in relation to niche associated biotic and abiotic influences. Our observation that the ratio of eMIT9312:eMED4 changes an order of magnitude for every 2.5 ºC change, supports that in the Atlantic and Pacific basins temperature trends are a definitive physical determinant affecting niche portioning among these ecotypes. Deviations from these lines in special cases due to hypothesized ecotype lag warrant further investigation into spatial and temporal relocation of resident ecotypes associated with specific temperature regimes.

The population ratio data observed from BATS (Fig 3.7) shows that our model is strong for existing data sets (r^2=0.63), but that our data analysis needs more representatives from cooler waters below the critical threshold of (18 ºC-20 ºC). Key to this data is the uncertainty that lies in the region where we would expect eMIT9312:eMED4 to maintain a 1:1 ratio. As evidenced by BATS data this ratio for the majority of the samples in this region is shifted into the eMIT9312 dominated projections. Abundantly clear from the BATS data is the fact that ecotypic lag is not equally expressed among *Prochlorococcus* isolates. While abundance data shows strong support for ecotypic lag in eMIT9312 populations, it is less clear for the eMED4 and eNATL2A ecotypes. While many factors could contribute to this, one direction to pursue in the future is to assess the influence of temperature shifts on the growth physiology of cultured representatives of these ecotypes. Further research should

also focus on the specific current data with seasonality in the region to try to determine if ecotypic lag is in play due to temperature transitions of water masses and their resident populations of *Prochlorococcus*.

The mechanistic basis of different temperature optima of the two ecotypes is still unknown, however temperature can affect an array of varying systems in microorganisms included but not limited to, cytoplasmic membrane ultrastructure, function, and fluidity [92-95], photosynthetic machinery [96-98] and even reduced efficiency of translation and transcription [99, 100]. Unknown is the potential role of mortality as a result of temperature or potential allelopathic responses to temperature between and among ecotypes in these populations. Our future work will aim to replicate and resolve this relationship in the laboratory with axenic competitions between eMIT9312 and eMED4 in co-culture.

Chapter 4

Direct competitions between axenic eMIT9312 and eMED4 culture representatives reveal distinct competitive advantages

Abstract

The oligotrophic oceans are dominated by coexistence eMIT9312 and eMED4 ecotypes (Chapter2), whose relative abundance varies as a function of temperature (Chapter 3). We sought to develop a competitive assay to test this coexistence in the laboratory environment. Temperature ranges from 19°C to 26°C were examined with axenic representatives of ecotypes eMIT9312 (VOL4) and eMED4 (VOL7). Fitness was assessed through growth rate measurements for large volume batch cultures and calculated competitive index (CI) based on Q-PCR-based abundances. Competitive interactions varied with temperature, with a general trend of VOL4 emerging as the dominant competitor, consistent with the numerical dominance of this ecotype at these temperatures in the ocean. Growth in the dilute, large-volume pure cultures compressed the permissive growth range of both ecotypes, possibly due to oxidative stress acting synergistically with temperature stress. Finally, a gradual, +1 ºC day^{-1} shift in temperature resulted in a concomitant change in fitness of the two ecotypes as predicted by their growth rates in static temperatures. Our study represents a foundational approach to developing an understanding of fitness of individual ecotypes and how that fitness may be influenced by biotic and abiotic factors, specifically temperature and presence of immediate competitors in their respective niches.

Introduction

To date no research has directly addressed how competitive relationships may influence population structures and niche partitioning in *Prochlorococcus*. Analysis of basinal transects through both the Atlantic and Pacific Oceans demonstrated a clear trend of numerical dominance of the *Prochlorococcus* population by the high light adapted ecotypes eMIT9312 and eMED4 in the surface mixed layer (Chapters 2 and 3). Cells in these mixed layers contribute a large fraction of the total *Prochlorococcus* population across the euphotic zone (upper 0-200 m), and the relative contributions of the dominant ecotypes was correlated with temperature, with the eMIT9312 ecotype in higher abundance at higher temperatures, and eMED4 in higher abundances at lower temperatures (Chapter 3). Whether temperature

impacts these contributions directly via affecting cell physiology, or indirectly via affecting nutrient availability, grazing rates, etc., is unknown, and warrants further investigation in a controlled laboratory setting.

In a prior study, the growth patterns of representative strains of the eMIT9312 and eMED4 ecotypes with respect to temperature were consistent with their ecology: while both ecotypes had the same growth optimum (24 °C), the eMED4 strains grew at higher rates at cold temperature than the eMIT9312 strains. They could also grow at cold temperatures that the eMIT9312 strains could not. Likewise, the eMIT9312 strains could grow faster than eMED4 at high temperature, and had a higher temperature limit as well [15]. While this study was instrumental in providing the first supporting evidence that temperature impacts the ecology of *Prochlorococcus* by differentially affecting the growth physiology of the ecotypes, there were several limitations. First, as with many culturing experiments with *Prochlorococcus*, the cultures were grown at concentrations several orders of magnitude higher than ever observed in the ocean [15]. Whereas the culturing experiments typically involve concentrations exceeding $1x10^8$ cells mL^{-1}[4, 65, 101, 102], the highest field concentrations of *Prochlorococcus* to date - observed in the Arabian Sea by Campbell et al. [37] and our group in the Western Pacific Warm Pool (Chapter 2) - is only $7.0x10^5$ cells mL^{-1}. Second, the prior work on temperature physiology was performed with non-axenic cultures[15]. These cultures had a mixture of heterotrophic bacteria, likely to be different for each strain (see Morris et al, 2008), and represented an uncontrolled variable in the study. Finally, the strains were grown separately, thus any ability of one strain to directly or indirectly impact the growth of another was not detectable in that study.

Understanding more of how these relationships operate at a very basic level - and identifying potential allelopathic interactions - is imperative to increased understanding of relative fitness of the ecotypes as they relate to important environmental parameters such as temperature. With the development of axenic cultures of representative strains we were afforded the opportunity to examine direct competition among *Prochlorococcus* ecotypes, without the potential influence of any outside community members.

We sought to examine and assess growth patterns and inter ecotype competition between dominant ecotypes eMIT9312 and eMED4, at key temperatures identified in our

field study (Chapters 2 and 3). Cells were grown at ecologically relevant concentrations, and we adapted our QPCR methods [14] to monitor the concentrations of the competing strains. Here we detail the results of those experiments and implications for potential competitive relationships in the field. We also report an initial study describing the different responses of the ecotypes, in competition, to shifts in temperature, which may have implications for climate change.

Results

1) Development of the growth assay

Due to the large volume required for the QPCR assessment of the low-density cultures (50 ml / sample), large-volume cultivation was required. Initial attempts to culture dilute axenic *Prochlorococcus* in polycarbonate bottles yielded erratic growth. This could have been due to the release of toxic bisphenolic compounds (BPA) from polycarbonate vessel structure as a result of vigorous acid washing or repeated autoclaving of the plastic [103-105]. Additionally, research has shown elevated generation of hydrogen peroxide in polycarbonate vessels in the presence of light (Lanying Ma, unpub.), and the presence of BPA has also been shown to induce reactive oxygen species (ROS) defenses in mammalian systems [105]. *Prochlorococcus* has been previously shown in the presence of reactive oxygen species to have highly variable growth patterns at concentrations of $3.5x10^5$ cells mL^{-1}, thus at ecologically relevant targeted concentrations of $5.0x10^4$ cells mL^{-1}, we might expect to see poor transfer survivability and culture growth if ROS stress was elevated [8].

2) Growth in pure culture

VOL4 and VOL7 demonstrated lower growth rates across all temperatures when compared to the growth data from Johnson et al. 2006 (Figure 4.1, 4.2). This was not unexpected as the latter study involved cultures at much higher cell densities that were not axenic and a higher light regime (66 µmol photons m^{-2} s^{-1} on a 14:10 light:dark cycle, compared to 40 µmol photons m^{-2} s^{-1} on a 12:12 light :dark cycle for this study).

In monoculture, both ecotypes exhibited maximal growth optima at 24 °C, consistent with the prior study [15]. In sharp contrast to the prior study, however, there was for both strains tested a precipitous drop in growth rate for both 19 °C and 26 °C from 22 °C and 24 °C respectively. Growth in both cases dropped notably from the closest 2 °C gap and 24 °C optima for both strains. Extrapolating the projected growth reduction as a function of distance from the 24 °C optimum, a negative growth rate is indicated for both the axenic and competitive treatments beyond 19 °C colder and 26 °C warmer. This may in part explain our repeated failure to obtain measureable growth in both 16 °C and 28 °C (data not shown).

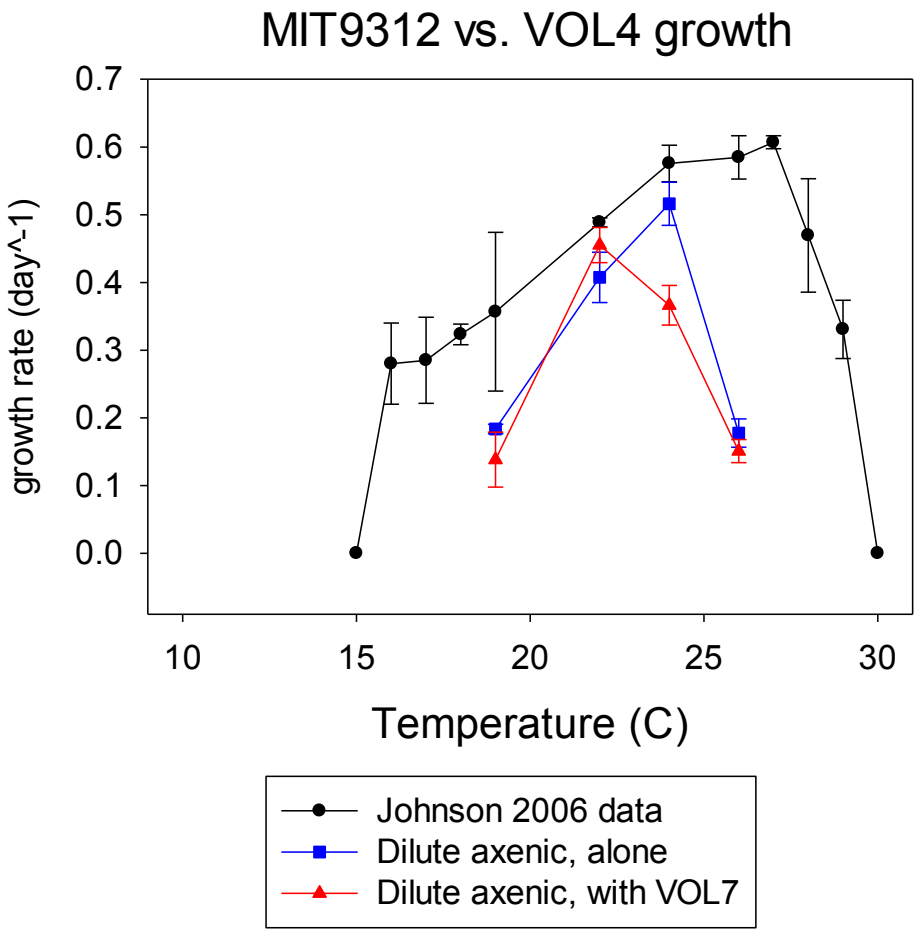


Figure 4.1- Growth rates of VOL4 in treatments alone (blue) and in direct competition with (red) VOL7 in ecologically relevant concentrations as compared to growth rates observed by eMIT9312 culture representatives in Johnson et al. (2006) across a range of temperatures.

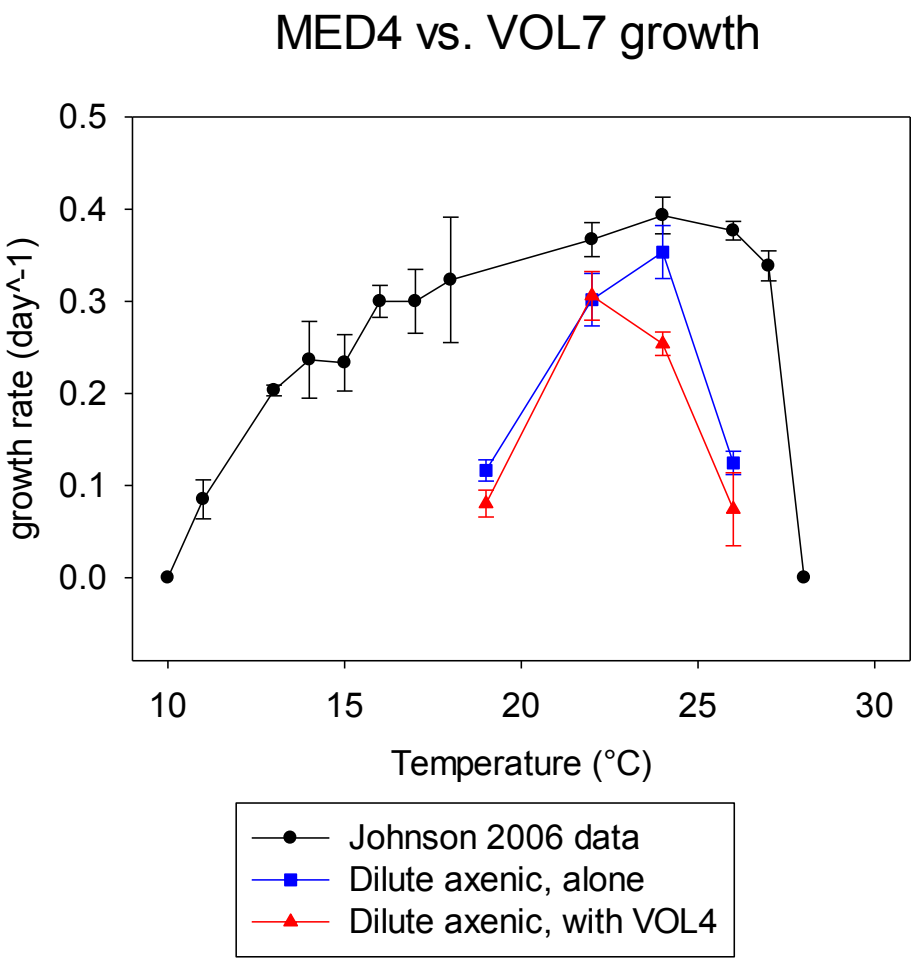


Figure 4.2- Growth rates of VOL7 in treatments alone (blue) and in direct competition with (red) VOL4 in ecologically relevant concentrations as compared to growth rates observed by eMED4 culture representatives in Johnson et al. (2006) across a range of temperatures.

3) Growth in mixed culture: competitions

The unexpected decline in growth rate for both strains as a function of distance from optimum temperature observed in monoculture was largely reproduced in the co-culture experiments. However, in contrast to growth in isolation, growth optima for both strains in competition was shifted from 24°C to 22 °C. Notwithstanding this sharp temperature-dependent decline in growth rate, comparisons of growth between mono-cultures and mixed culture competitions yielded some significant results, as described below.

Consistent with the prior study of concentrated non-axenic cultures [15], the overall trend was that the high temperature-adapted VOL4 strain outcompeted the low temperature-adapted VOL7 strain at all temperatures assayed (Fig 4.3). VOL4 exhibited varying degrees of competitive index (CI), but all were substantially distanced from 1 denoting its larger competitive abilities over VOL7. The strongest competitive index (advantage) for VOL4 was seen at 24 ºC with a CI of 3.25 (a value of 1 represents equal fitness), and was seen to drop off on either side of this value with CI values approaching 1.

Interestingly, growth in co-culture with VOL7 decreased growth rate of VOL4 relative to the monoculture control at 24 ºC, but increased it at 22 ºC. (Table 4.1) VOL7 growth rates were decreased in both of the upper temperatures ranges tested at 24ºC and 26ºC No significant changes in growth rate compared to controls (changes in fitness) were observed for either strain upon co-culturing at 19 ºC.

Table 4.1- Comparison of growth rates of mixed vs. monocultures for respective ecotypes at ecologically relevant concentrations for competition series. ⇧ Denotes higher growth rates in mixed cultures when compared mono culture.⇩ Denotes lower growth rates in mixed cultures when compared to monoculture. Number in parenthesis represents average reduction or increase in growth from mixed to monoculture.

T (°C)	Strain	Growth rate day^{-1} in mix vs. monoculture	p-value
19	VOL4	↓ (-0.045)	0.864
	VOL7	↓ (-0.036)	0.344
22	VOL4	↑ (0.048)	*0.024
	VOL7	↑ (0.004)	0.608
24	VOL4	↓ (-0.150)	**0.001
	VOL7	↓ (-0.100)	**0.006
26	VOL4	↓ (-0.027)	0.54
	VOL7	↓ (-0.050)	**0.04

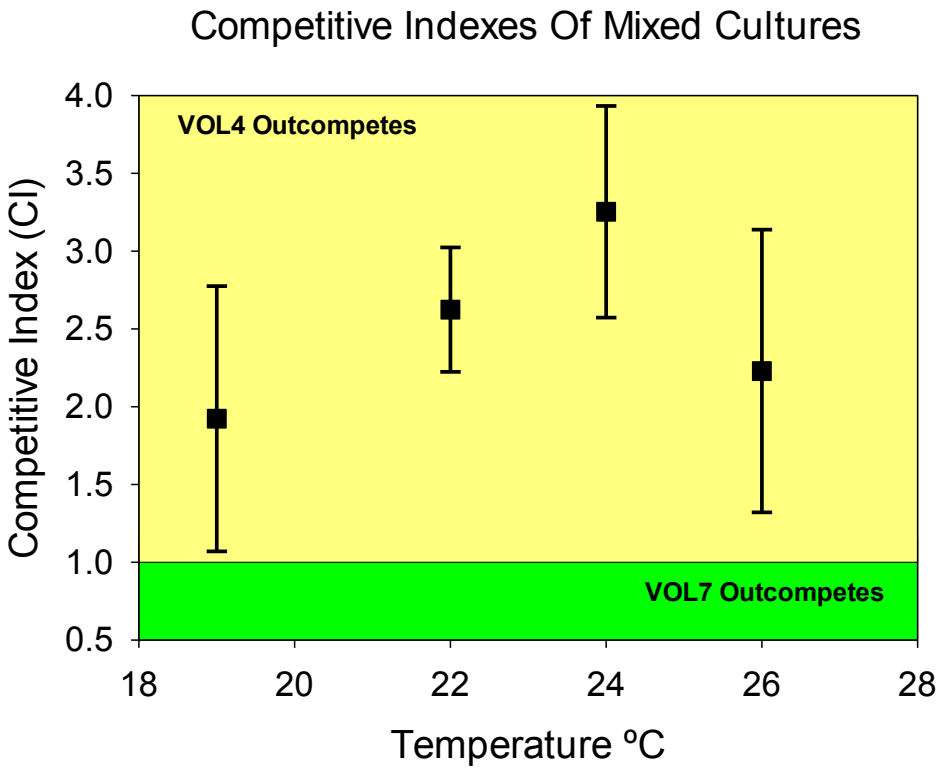


Figure 4.3- Competitive indexes of axenic VOL4 and VOL7 in coculture at ecologically relevant levels. Values above 1.0 line indicate VOL4 outcompetes and is the superior competitor and values below the 1.0 line indicateVOL7 outcompetes VOL4.

4) Competitions during a temperature shift

In a pilot study for a field project, we observed that ratios of VOL4:VOL7 in cultures could be influenced by upward temperature shifts. Low inoculum axenic competitors at approximately $5.0x10^4$ cells mL^{-1} were exposed to a temperature increase of +1ºC per day ranging from 24ºC to 27ºC. Both strains grew during the shift, but the initial ratio of .89 for VOL4:VOL7 increased to a ratio of 1.45 by day 4 (Fig 4.4). Thus, the high-temperature adapted strain VOL4 was able to take over as the majority during this gradual shift to higher temperature.

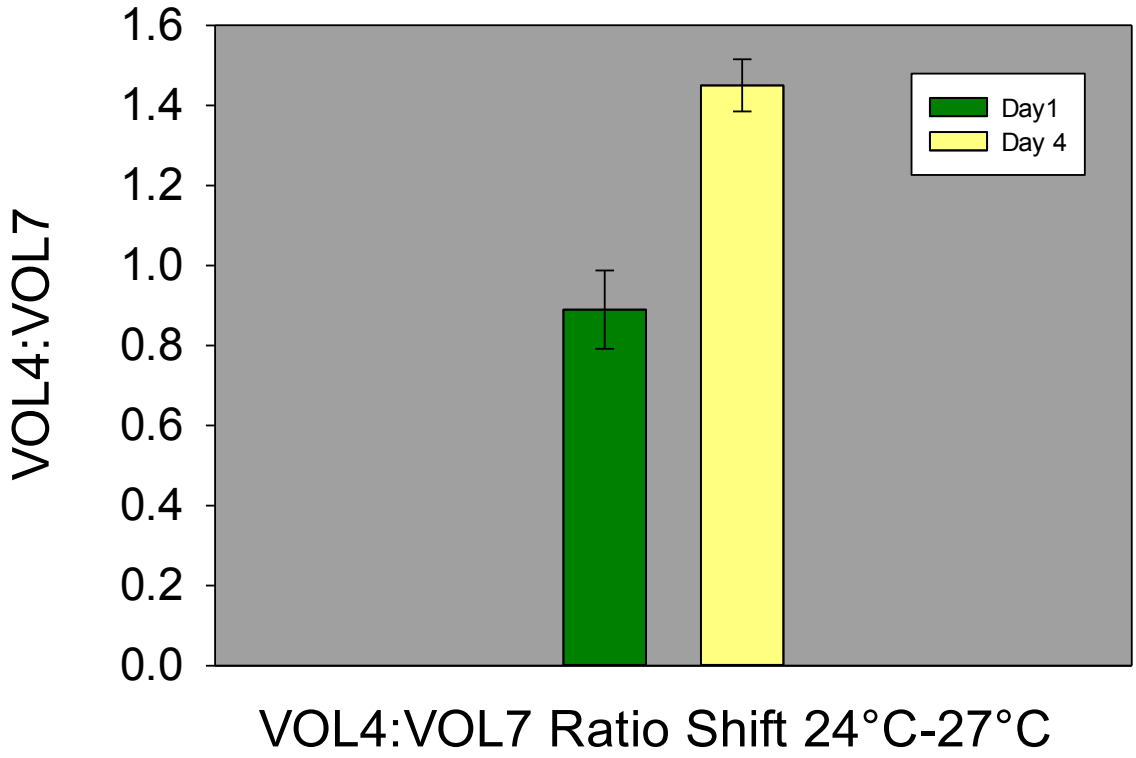


Figure 4.4- Change in ratio of VOL4:VOL7 over a four day period with an increase of 1°C per day. The ratio above the 1.0 mark on day 4 indicated that VOL4 overtook VOL7 numerically as a result of the temperature shift.

Discussion

This work describes the first investigation of the fitness of strains from the two dominant ecotypes of *Prochlorococcus*. Overall, the trends in relative fitness as a function of temperature were consistent with 1) field abundance data and 2) examinations of non-axenic cultures grown in monoculture at high cell concentration. From field data (Chapter 2 and 3), we expected the competitions to be dominated in terms of growth rate/day by VOL4 at the temperatures examined (19-26 ºC). The largest competitive difference as demonstrated through differences in growth rate between competitors in mixed competitions, was interestingly observed at 24 ºC. This is supported by prior studies indicating that maximal growth potentials for both parent strains of VOL4 (eMIT9312 at 0.62 day^{-1}) and VOL7 (eMED4 at 0.40 day^{-1}) are often seen in this temperature range, especially for that of eMIT9312 [18]. The characteristic ability to select a clear winner as a product of superior growth rate further reinforces that the influence of temperature on cell physiology can modulate a competitive relationship between the two ecotypes.

Development of the fitness assay, involving dilute, uncontaminated cultures grown in glass containers and quantified by QPCR, represents a positive step forward in the investigation of relative fitness of the two ecotypes. This development has also uncovered a new challenge for future experimentation on this topic, which is the management of an apparent synergistic effect between growth without co-cultures of bacteria and growth at non-optimal temperatures.

The nature of this synergy is unknown, however one potential source is the synergistic effects of temperature and oxidative stress. Steps were taken to reduce the concentration of ROS such as hydrogen peroxide in the medium, including 1) avoiding autoclaving, 2) replacing HEPES buffer with TAPS [106], and 3) switching from plastic to glass. Despite these measures, there is a possibility that the ambient HOOH in the medium, which was not quantified in this study, may have been high enough to act synergistically with temperature to reduce the growth capabilities of these strains. Such effects have been identified in another study (Ma and Zinser, manuscript in preparation), and may be consequence of damage to the photosynthetic apparatus and/or membranes [[23, 98, 107]]. In the future, it

would be useful to measure the peroxide in the medium. It will also be worth investigating formally whether the addition of heterotrophic bacteria to the cultures in this system is able to expand the temperature range towards that observed in the non-axenic cultures of Johnson et al. 2006.

Calculations of competitive index values for all competitions at all temperatures revealed an expected trend of dominance of VOL4 over VOL7. (Fig 4.3) Intriguingly these competitive index values somewhat mimicked temperature performance curves (Fig 4.1 and 4.2). Assessing competition through growth rate, in that the largest significance in growth rate at 24 ºC was also established by the competitive index value at 24 ºC. The competitive index values also proved very useful when attempting to assess competitive capability of competitions at less predictable temperatures where synergistic effects could mask competitive capacity through reduced or low growth rates. Even though VOL4 showed higher competitive index values throughout the temperature range, as temperatures approached 19 ºC the trend was clearly beginning to progress towards a CI value of 1. The respective decline from 0.30 at 24 ºC through 0.42 and 0.58 at 22 ºC and 19 ºC respectively showed that the balance was beginning to approach 1 where VOL7 and VOL4 would be equalized competitors. The increase in temperature to 26ºC and CI value of 0.47 also suggests while VOL4 still maintains its competitive advantage, there is an associated fitness cost with temperatures above optimal (24 ºC) for growth.

Although only a preliminary result at this stage, it is encouraging that the ratio of ecotypic strain abundance changed in favor of the high-temperature adapted strain when the temperature was gradually shifted from optimal (for both competitors) towards higher temperatures in the co-culture experiments. It would be worth testing additional temperature shift scenarios, including optimal to colder temperature, to examine the change in competitive fitness during transitions across the temperature range that both ecotypes can grow and field evidence indicates can co-exist (Chapter 3). While obviously a +1 °C day^{-1} increase in temperature is highly unusual in the open ocean, and likewise less reflective of the modeled increase in surface temperature during climate change the next 100+ years [108-110], it nonetheless can serve as a framework to begin to constrain future models for how

ecotypic shifts within the *Prochlorococcus* population might occur, and how such changes may impact carbon cycling, oxygen production, and other biogeochemical cycles.

Methods

1) Media and culture conditions

Competitive interactions were assayed for axenic *Prochlorococcus* strains VOL4 (streptomycin-resistant MIT9312) and VOL7 (streptomycin-resistant MED4) at 19,22,24, and 26 °C. Both ecotypes were grown alone in axenic monocultures (control) and in direct competition with one another in tandem for each experimental temperature range tested and included biological quadruplicates. All competitions were carried out in the range of 10^4-10^6 cells ml^{-1} which mirrors average ranges and maximal cells densities seen in field populations of the Atlantic and Pacific Ocean surface mixed layers. At no time was the population allowed to exceed 10^6 cells ml^{-1}. Cultures were grown in 400mL of AMP-J artificial seawater medium which sterilized by vacuum filtration through a 0.2 µm pore sized polycarbonate filter [65]. Cultures were grown in 500 ml Corning Pyrex™ wide mouth borosilicate glass bottles (CLS1397500). These bottles were acid washed with 1N HCL and autoclaved. Standard threaded screw caps were replaced post autoclaving by autoclaved clear glass petri dishes as lids (KIMAX® borosilicate glass petri dish 100x 20mm) to allow light to pass through and no shading to occur. Light cycles of 12:12 light:dark regime under cool white fluorescent lamps for all replicates at all temperatures. Light levels were maintained across all experiments at 40 µmol photons m^{-2} s^{-1} as measured by integrated light meter (QSL-2100, Biospherical Instruments).

Cell cultures were transferred in large volume transfers of 1:5 dilutions from late log phase cultures for initial acclimation to temperatures. These transfers were continued through three successive large volume transfers and then adjusted to transfers of 10^5 cells mL^{-1} for three successive transfers prior to the start of the experiment maintaining log phase growth. At the beginning of all competitions cells were inoculated at an initial starting concentration of ~$5.0x10^4$ cells mL^{-1}. Prior to the start, and after the conclusion of each competitive experiment, purity tubes [9] to observe and indicate heterotrophic contamination

were conducted in 1/10 ProAC (ProAC medium with only 1.7 g of AC Difco broth liter^{-1}), YTSS medium (4g 1^{-1} yeast extract, 5g 1^{-1} Tryptone, 20g 1^{-1} sea salt and 18 MΩ water, YT3 medium (28.1g 1^{-1} NaCl, 6.9g 1^{-1} $MgSO_4*7H_2O$, 5.49g1^{-1} $MgCl_2*6H_2O$, 0.67g L^{-1} KCl, 1.47g 1^{-1} $CaCl_2$, 2 mL 0.5 M TAPS pH 8.0, 10g 1^{-1} Tryptone, 5g 1^{-1} yeast extract, with milli Q water and .2um filter sterilized (Supor filter), and a minimal medium (PLAG [75% Sargasso seawater supplemented with 0.05% each; wt/vol or vol/vol sodium *p*yruvate, sodium *l*actate, sodium *a*cetate, and *g*lycerol; 800 μM NH_4Cl; 50 μM NaH_2PO_4; 1× Pro99 trace metal mix; and 1× Va vitamin mix)[8]. No purity tubes were positive for contaminants supporting that all cultures maintained their axenic nature throughout the study.

2) Quantification of Ecotypes

Cell were quantified via flow cytometry (Guava-HT6), using standard methods [27, 58, 111, 112], or via quantitative PCR (QPCR) adapted from Zinser et al (2006). 50mL aliquots for QPCR analysis were taken every other day for a period of 8 days.

3) Calculation of Competitive Index

Competitive index values for each competition were calculated and adapted from Bertin et al. 2011 [113]. Fitness index was calculated based on the formula :

(number of individuals recovered VOL7/ number of individuals recovered VOL4)/
(number of individuals inoculated VOL7/number of individuals innoculatedVOL4) = CI (competitive index)

Whereby if:
1. CI<1 then VOL4 outcompeted VOL7
2. CI≈1 then neither ecotype possesses a competitive advantage over the other
3. CI>1 then VOL7 outcompeted VOL4

Chapter 5

Discussion and Future directions

Broad swaths of both the Atlantic and Pacific Oceans are dominated by two ecotypes, eMIT9312 and eMED4, in the surface mixed layers where *Prochlorococcus* abundances are highest. Within these oligotrophic surface mixed layers from our wide-ranging data set covering both basins we observed an average mean abundance of 1.44×10^5 cells/mL^{-1}. This enormous standing crop of organisms just by numerical standards give them global significance in nutrient cycling, given their function of converting light energy into chemical energy [6].

Our analysis of depth profiles derived from the three transects encompassing latitudinal cross sections of known the Pacific and Atlantic basins has further reinforced the nature of predictable niche partitioning by light and temperature in *Prochlorococcus* ecotypes as previously supported in the literature [14, 15, 18]. We showed that population structures among these profiles of the Pacific (WP2 and POWOW cruises) are similar to trends observed in the Atlantic, yet at points of extremes we observed new unexploited trends in diversity which warrant further future investigation. Of particular interest were trends that evolved around the newly discovered HL adapted ecotypes, the HNLC1 and HNLC2 clades. Our evidence from observed field abundances for these clades supports the prevailing theory that they are specialists evolved for conditions seen in regions of equatorial upwelling where nutrients conditions are often iron limited and micronutrient replete [19]. Unreported however, is the circumstance that their range extends beyond that of equatorial regions and even experienced small increases in the regions surround the southernmost portions of the WP2 transect. It is plausible that regions of upwelling seen in the southern most stations of the transect provided the high levels of inorganic P that they have been observed to thrive in.[20]

Of the low light adapted ecotypes analyzed eMIT9313 and eSS120 exhibited periodic and sporadic deep takeover events in the DCM (deep chlorophyll max) regions of depth profiles where conditions were most favorable. Clear from these profiles was that while light seemed to play an important role in niche partitioning in the Pacific similar to results noted in previous studies [18], other biotic and abiotic factors need to be further explored to better understand deep *Prochlorococcus* ecotype diversity. Perhaps these populations are underappreciated because in sheer numerical terms of the *Prochlorococcus,* they are minority

contributors to the total abundance in the water column when compared to their high light adapted counter parts. In line with theories of genomic streamlining in *Prochlorococcus*, these extant low light adapted members may help provide direction when studying relationships to ancient phylogenetically related ancestors of today's *Prochlorococcus* populations.[40, 114] Given their diverse gene content in relation to that of more recently evolved high light lineages, the increasing focus on studying core and flexible genomic characteristics could provide useful information for modeling predictions of future evolutionary change and the direction of the species.[6, 114-117].

Through the depth profiles in the Pacific it was also clear that the persistent presence eNATL2A as the majority member of the minority fraction deeper euphotic regions provides them to be treated as a special case in *Prochlorococcus* diversity studies.. It has been documented that eNATL2A populations are tolerant to exposure in high light conditions over short durations [18, 118] and as evidenced by our analysis and observations of their continual low level presence in the upper euphotic zone across the Pacific transects. Of particular interest for our overall analysis of the depth profiles for the transect was that in addition to a documented intermediate susceptibility to high light conditions, they also exhibited a large range of tolerance in temperature optima, not observed for other *Prochlorococcus* ecotypes in the euphotic zone. For example in both southern latitudes of the WP2 transects and northern latitudes of the POWOW transect we observed abundances in surface layers at times approaching 10^5 cells/mL^{-1}. These observations across the broad Pacific transect lead us to believe that eNATL2A populations themselves warrant further investigation in concert with abiotic and biotic factors. Perhaps eNATL2A may represent an ecotype that has forgone numerical dominance at the hands of becoming more generalist in favor of greater "ecotype plasticity." Given their common location in the water column around boundary layers, the ability to endure large temperatures swings in thermoclines and isoclines, as well as large variability in light intensity due to mixing and shading affords them the place as a special case in most considerations of diversity in the water column.

Of the most dominant ecotypes present in the euphotic zone through 200m depth investigations, eMED4 and eMIT9312 by far dominated the water column and were the predominant ecotypes, with the one exception noted above in the case of the HNLC clades.

Our analysis of integrated abundance for both the WP2 and POWOW transects show a robust relationship in which eMIT9312 dominates warmer waters and latitudes and quickly cedes this dominance to eMED4 in regions of cooler water. Crossover in populations between eMIT9312 and eMED4 occurred in temperatures ranging from 18°C-20°C in both the Atlantic and Pacific Oceans in the high north and south latitudes, stressing the importance of temperature as a possible abiotic factor affecting this relationship.

Our abundance data in depth profiles should provide further foundation and another useful data tool when taking *Prochlorococcus* niche specialization into account. These levels of abundance are also likely influenced through competitive interactions among ecotypes and may lead to further evolutionary pressure to increase levels of specialization [45] that exist across *Prochlorococcus* ecotypes. Recently the true breadth of genetic divergence in *Prochlorococcus* was elevated to a new level by discoveries through next generation sequencing technologies indicating the presence of hundreds of subpopulations existing in *Prochlorococcus* in the field [38]. If this discovery holds true the wide-ranging implications are that in lieu of stable and concrete static genetic content and existence for these dominant ecotypes the landscape is perhaps underlying more complex within ecotypes as we know them. This may for example help provide clarifications for example of the eNATL2A's ability to among *Prochlorococcus* ecotypes exhibit a broad range of existence.

Perhaps the largest and unaccounted for variable in our study and many others in microbial ecology of the oceans is that of the role of viruses. Globally the abundance of oceanic viruses exceeds even that of the standing crop of *Prochlorococcus* by orders of magnitude.(10^{30})[2, 119] Viruses are thought to play a large part in nutrient cycles and influence biogeochemistry on a global scale.[2] In addition the development of photosystems in *Prochlorococcus* has been linked to probable viral transduction events in their lineage.[117, 120-122] Viral interactions may also be important when considering genome reduction events in *Prochlorococcus* lineages and theories such as the Black Queen hypothesis[10] which hypothesize gene loss through deleterious selective adaption. It is conceivable that a virus might be able to carry part or all of a "black queen card" (an evolutionarily detrimental gene) or in another term be dealt a "joker/red queen" through transduction events. Maybe ecotypes with less reduced genomes as the most recently

branched lineages were literally dealt a bad hand (genes at a cost) at the *Prochlorococcus* niche table. Future research into trends associated with biotic and abiotic factors associated with diversity of ecotypes should be paired and contrasted with these abundances to better understand the results of viral mortality on these populations.

Our examination of functional relationships among *Prochlorococcus* ecotypes is among the first studies to examine variable factors that influence population structure in the mixed layers between the dominant ecotypes eMED4 and eMIT9312. Chief among our results is that unexpectedly across many environments the relationship of eMIT9312:eMED4 is not only stable, but is also able to be explained in great detail by effects of temperature.(r^2=0.63, Chapter 3 fig. 3.7) The ability to predict a log linear change such that for every 2.5°C there is an order of magnitude increase in this ratio allows us to ascertain a rough estimate of community composition at a given point in oligotrophic waters with one physical variable in the form of temperature. While this variation is not is not immune from invasion effects of ecotypic lag, the ability to apply a predictive model across the surface mixed layer in this manner is an exciting development.

In addition this ratio also shows support for the paradox of stable coexistence.[41] Stable coexistence may have a higher likelihood of being fulfilled in surface mixed layers due to the very nature of the system, in that by definition the mixed layer provides a stable environment where physical variables (temperature, density, salinity, and light levels) do not deviate in large swings. Additionally variable growth rates between ecotypes as a product of mortality, growth optima, or potentially with the surrounding community at given temperatures may exist as a stable coexistence because with all other factors tied in the net effects between eMED4 and eMIT9312 is dispersed by a diverse interaction with the community (biotic and abiotic) and thus small and variable.[123] Support that interactions may in fact be weak was seen in variable growth rate interactions between axenic monocultures and mixed culture experiments between these two ecotypes where at temperatures approaching the hypothesized 1:1 ratio (18°C-20°C)

While some literature on co-cultures exist, to date no study has examined two different ecotypes of *Prochlorococcus* in co-culture. Furthermore, of studies that have been conducted with co-culture, most of these studies have been with the addition of heterotrophic

bacteria either by design or unintentionally as background organisms, the product of clonal isolation of ecotypes in the lab [8, 18, 124, 125]. Some ecotypes may in fact be uncultivable and rely on helper hosts to even be cultivated in laboratory settings [8, 126]. Our study of inter ecotype competitions in the lab was the first of its kind and we are also the first lab to begin to assign loose fitness estimates to isolates in tandem with their aligned niches as they relate to permissive temperature optima.

eMIT9312 was found to outcompete eMED4 in axenic co-culture and our findings solidify and loosely follow our ratio model for population prediction in the environment of the two ecotypes (chapter 3) in accordance with variable temperatures. While there are limitations of such experimentation to be extrapolated into dynamics of field isolates we were encouraged by the fact that we saw reductions in relative fitness at points where field populations indicated a variable coexistence in dominant ecotypes in the 18°C-20°C range. We were also encouraged to see at temperatures approaching maximal growth ranges for the two ecotypes we observed some of our largest effects on competitive index and growth rates compared to controls.

We assigned, in the context of variable temperature regime and ecotype presence, relative fitness rates and competitive indexes for both eMED4 and eMIT9312. Competitive indexes have historically been used for comparison of strains with closely related growth rates [113] and with these values now established for two *Prochlorococcus* ecotypes in pure culture, we have laid the ground work for efforts towards more exhaustive models of ecological competition and coexistence. Understanding basic interrelationships and the ability to assign them mathematically applicable terms at the laboratory level, may be key in future modeling and predictions of overlapping 2 and 3 dimensional niche expansions and invasions for *Prochlorococcus*. For example with known temperature ranges and effects of competition and potentially the aid of helper heterotrophy on that range we may be able to move towards the assignment of a R* value for specific ecotypes of *Prochlorococcus*, or the ability given physical and biological factors for an ecotype to persist and compete in a given environment [79, 127, 128]. As seen in our competition experiments across temperatures ranges where competitive indexes are variable, we might expect the R* value for given ecotype to vary with that temperature as a component of available resources. Competition

combined with temperature may alter R* values in a way that outcomes may be driven by community composition around *Prochlorococcus* populations. With known metabolic capabilities and temperature ranges of ecotypes future work should take the larger microbial and viral community into account as well, especially in temperature ranges where variable coexistence may hinge on minor factors to determine ecotypic dominance. The fact that we observed negative interactions and competition between two ecotypes in our direct competition studies (chapter 4) supports that there is indeed the possibility of direct competition in field populations independent of accompanying community structures. The development of such values could provide the framework towards more formal mathematical models of community not just for *Prochlorococcus* but also the large community they thrive in. With these results we have moved from an observational model of *Prochlorococcus* into the realm of operational models in which variables beyond relative fitness can be determined across ecotypes [129-131].

Future studies with laboratory co-culture experiments should seek to examine long-term competition more closely in continuous culture simulations to truly understand if these interactions in a permissive range of temperature for each respective ecotype may result in the eventual competitive exclusion of one member. Finally competitive interactions between eMIT9312 and eMED4 ecotypes are not contained within controlled circumstances independent of other variable interactions in the environment, namely the presence of multiple ecotypes in the same spatial region. It would be interesting to repeat these experiments with eNATL2A ecotypes to observe effects of competition independently on both ecotypes from a low light adapted strain that see periodic intrusion into the mixed layer and to conduct competitions amongst all three ecotypes to understand the effect of a third ecotype on eMIT9312:eMED4 ratios at varying temperatures. As seen in BATS abundance in natural populations (Fig 3.5, yr.2006) eMIT9312 ecotype decline is also often interspersed by periodic spikes in abundance that fall in line with eMED4 and eNATL2A populations. While some of the core nutrient requirements for *Prochlorococcus* are shared, it is difficult to imagine why the presence of multiple ecotypes competing for a single pool of resources manages to all increase simultaneously without competitive costs or interactions. Perhaps the largest unknown that confounds our ability to predict with any more certainty competitive

relationships is that of growth rates in the field. There are two documented estimates of growth in the field that vary widely [132, 133] however recent work in field estimates of *Prochlorococcus* growth with more specificity towards individual ecotype growth[134] rates in situ may allow us to understand from growth stand points how population dynamics as a function of growth rate are regulated in space through the water column and over time scales.

Ultimately better understanding of interactions in the environment beyond abundance measurements may need to focus on the metabolic potential of the community at large and possible ecotype specific differences in metabolisms. Understanding a metabolic profile of a *Prochlorococcus* community may provide a window into understanding population dynamics as well. For example the recently highlighted HNLC clades found in equatorial regions are high light adapted and tolerant of warmer waters in these regions, but their defining characteristic that has allowed them to carve out their niche where they dominate seems at present to be metabolically based. [17, 20, 28, 135] With these results we have moved from an observational model of *Prochlorococcus* into the realm of operational models in which variables beyond relative fitness can be determined across ecotypes. [129-131]

This work represents the first study of niche partitioning and diversity compared across two oceanic transects from the Atlantic and Pacific Oceans, modeling of connections in high abundance ecotypes in surface mixed layers waters, and finally co-culture of competitive relationships among the dominant ecotypes eMIT9312 and eMED4 in axenic laboratory cultures. Our studies indicated that temperature, is the primary abiotic factor influencing this relationship and as predicted future oceanic temperatures are expected to rise [136] the dynamics related to which ecotype dominates in a region might have profound impacts on ecosystem structure. We have laid the groundwork for future field to laboratory simulation and exploration of competitive relationships among *Prochlorococcus* ecotypes and their underlying diversity.

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Appendix

This appendix contains list of stations and physical data from the Pacific basin transects WPW and POWOW listed in the map in Figure 2.1. All panels denoted "A" represent Q-PCR mean abundances for specified ecotypes in accompanying legend. Panel "B" represents available physical data that may include FCM (abundance by flow cytometer), relative chlorophyll fluorescence, and total Q-PCR enumerations (the sum of all ecotype mean Q-PCR values).

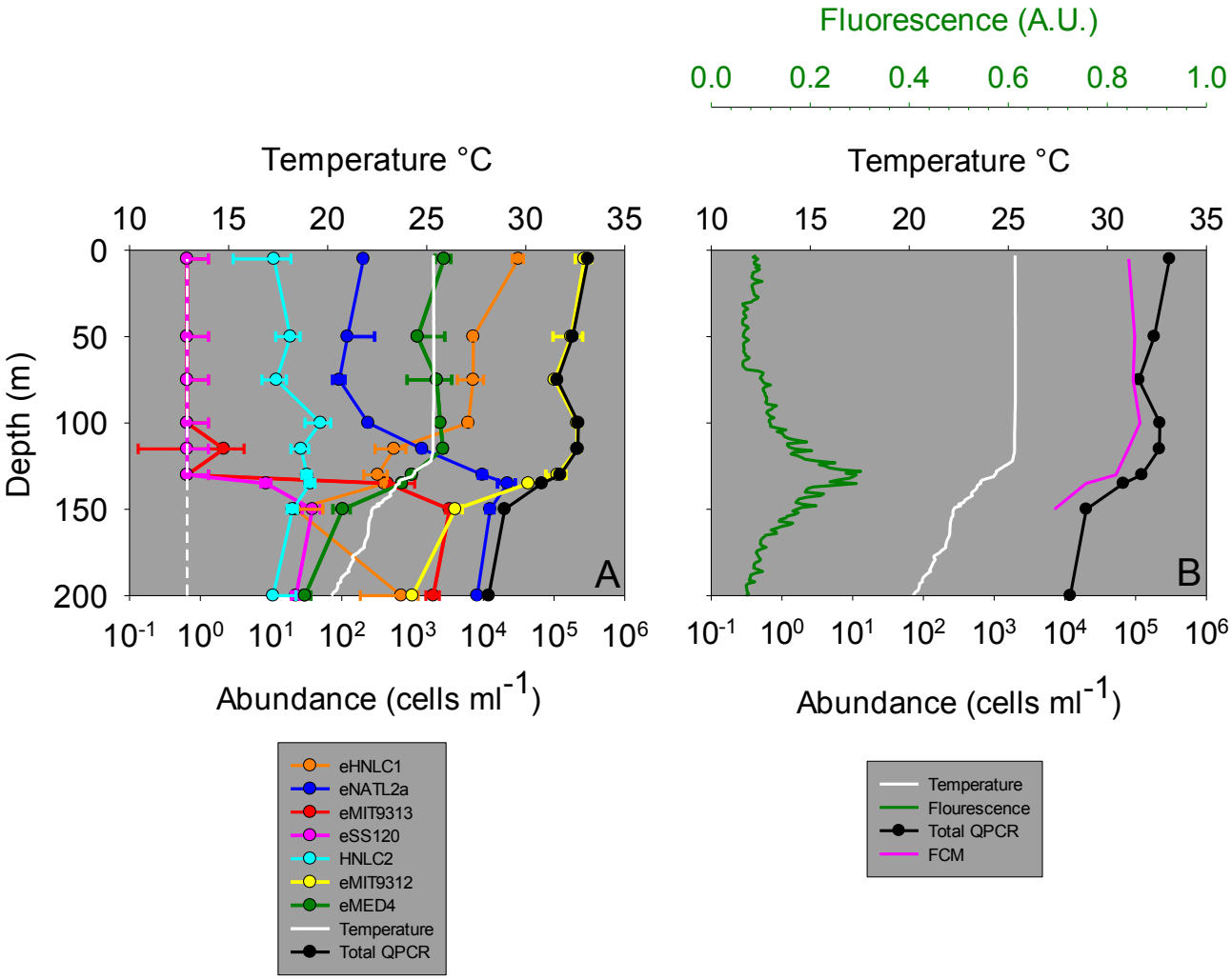
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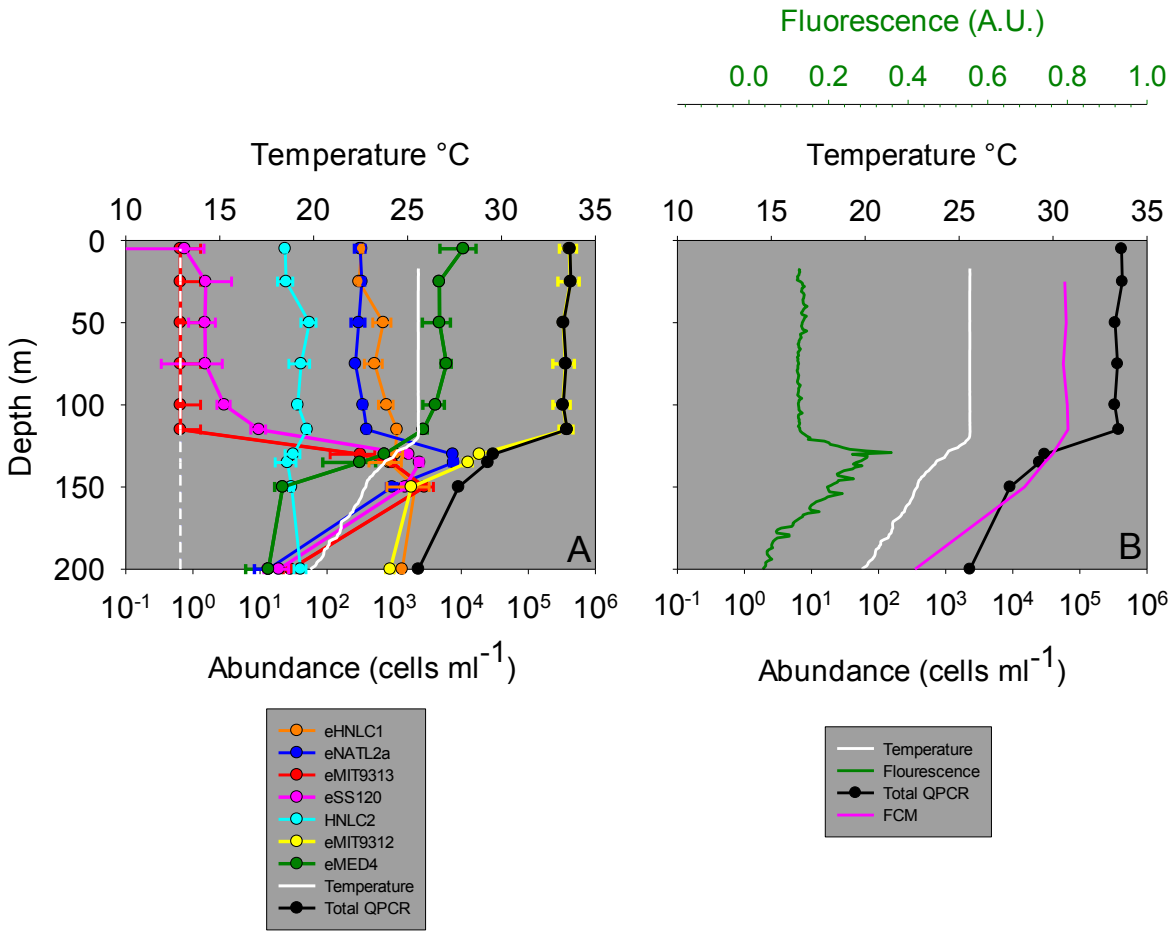
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Station12.....123
Station13.....124

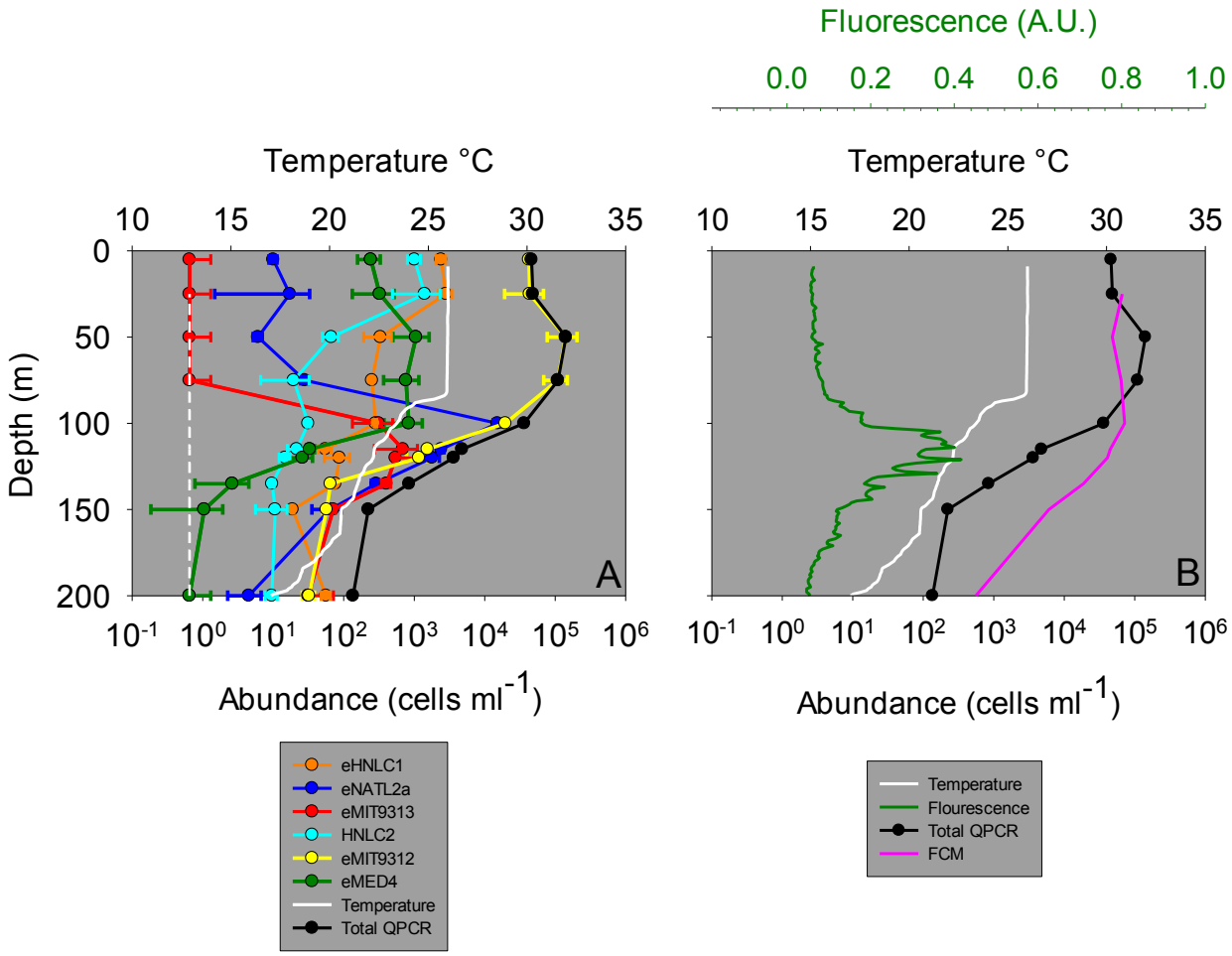
WP2 Station 2



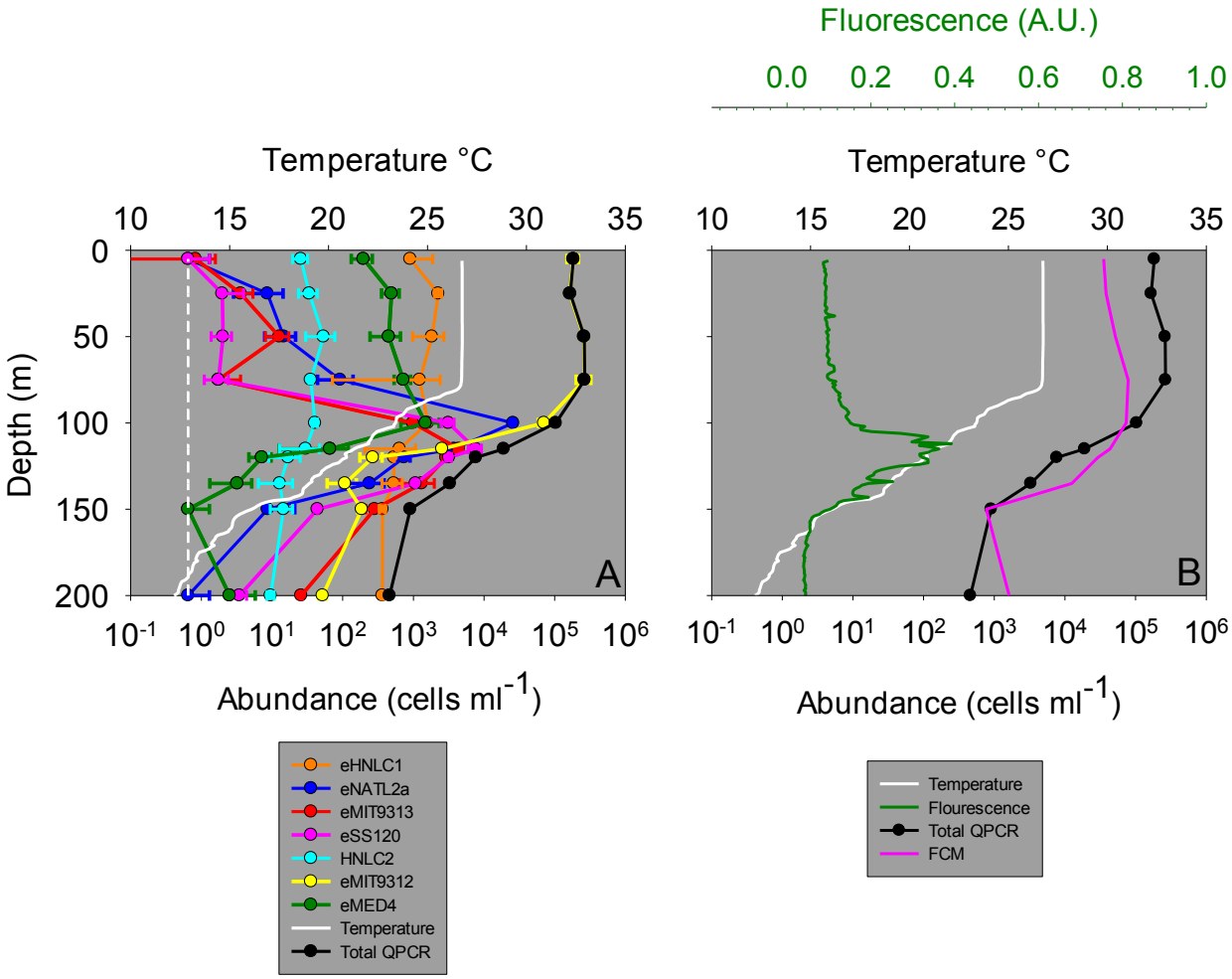
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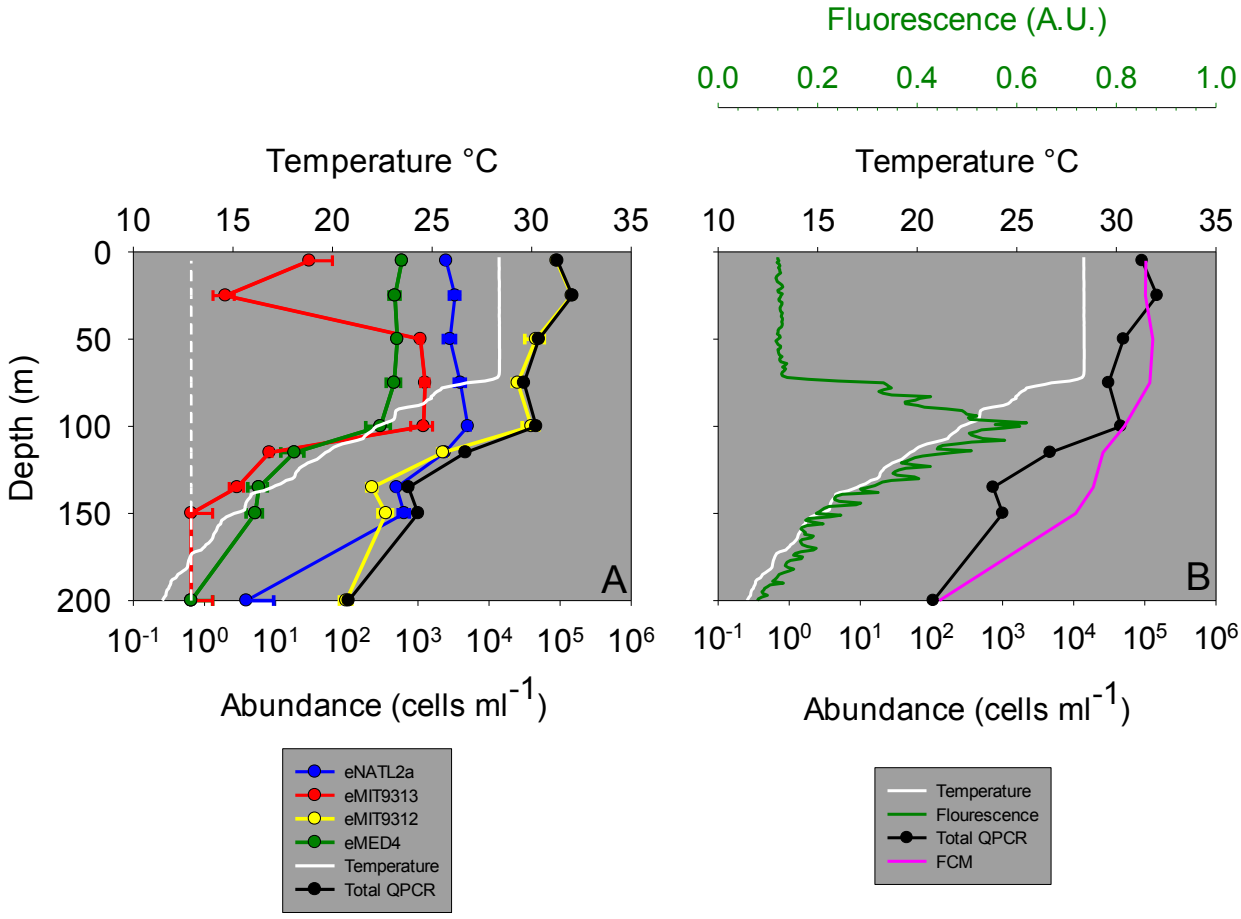
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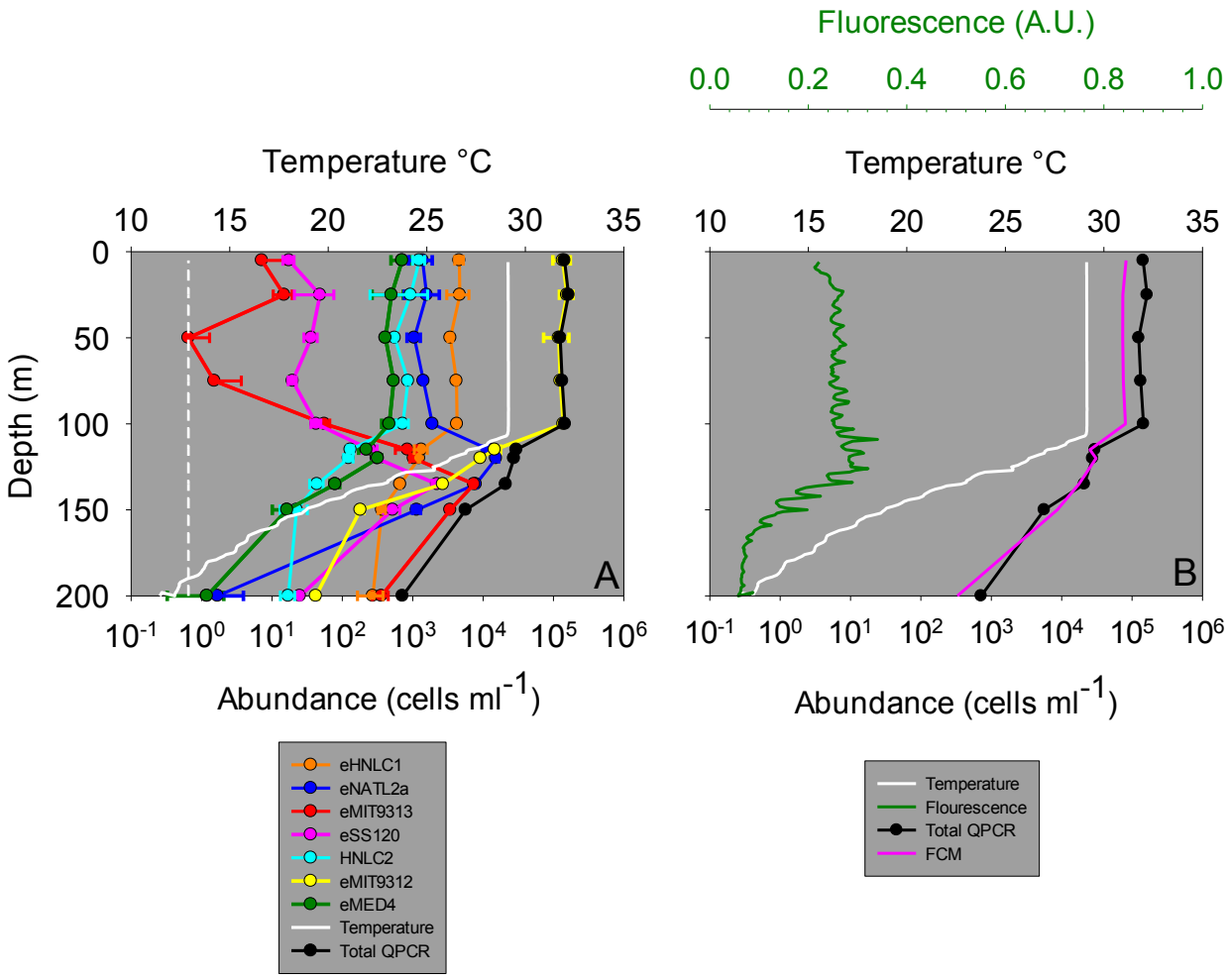
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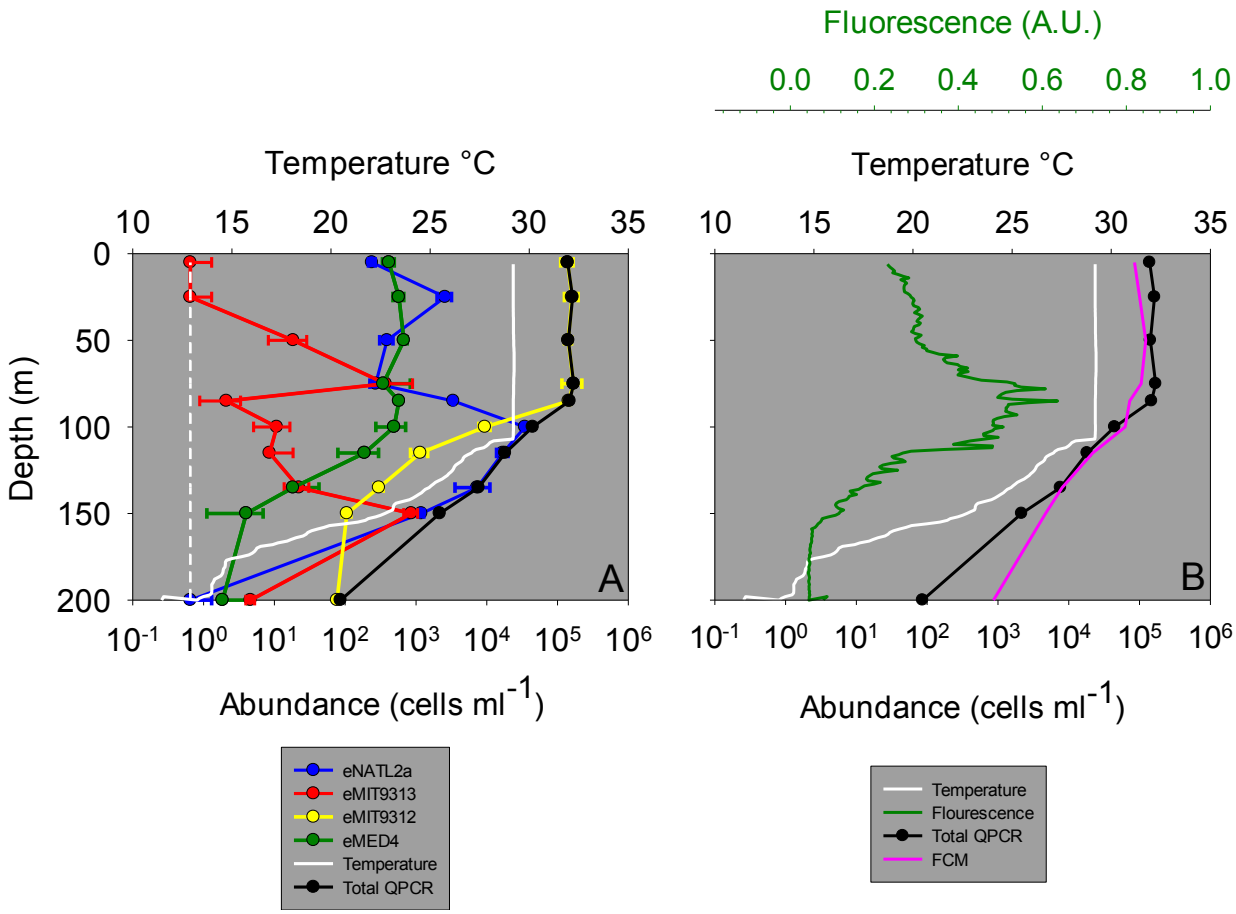
WP2 Station 7



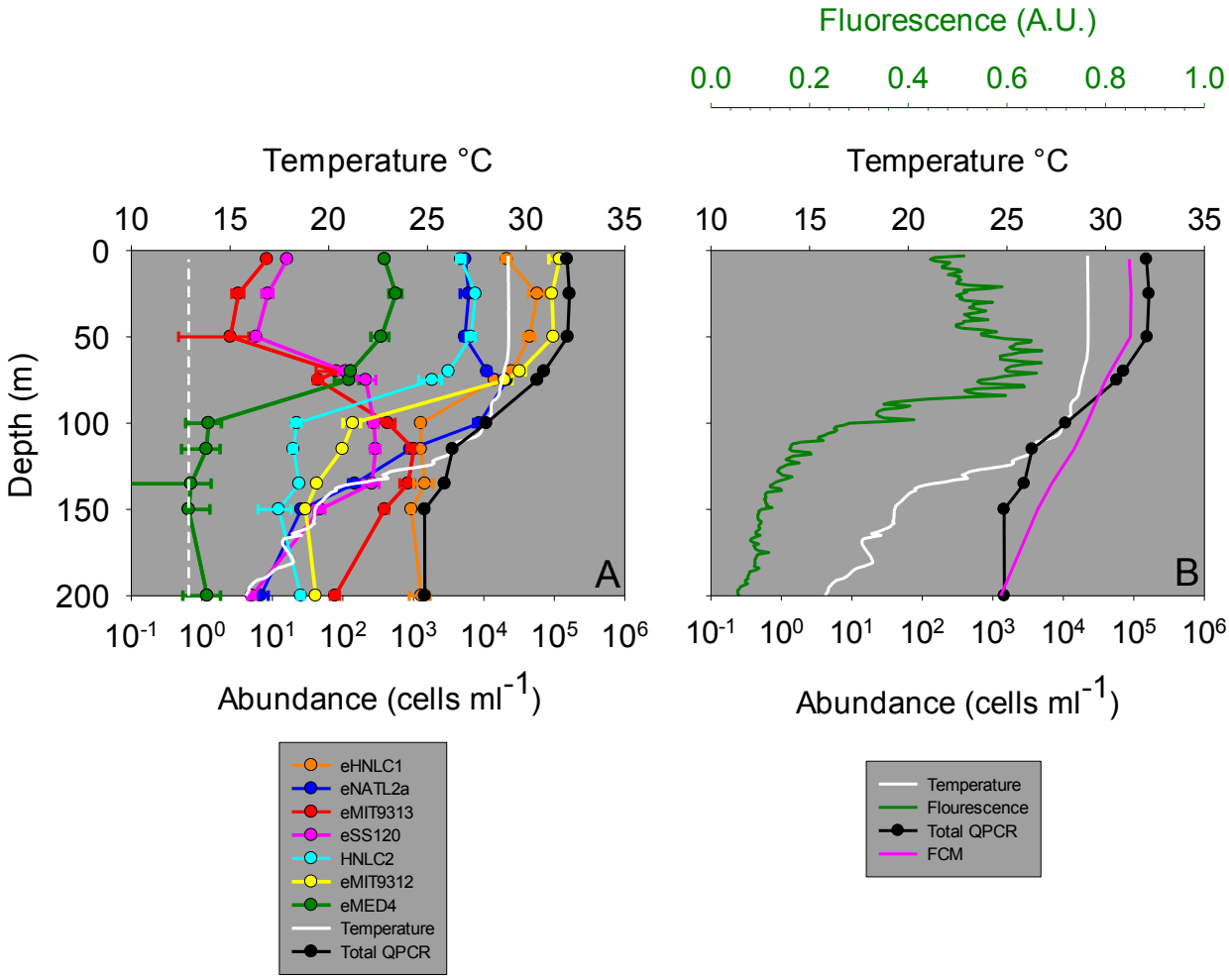
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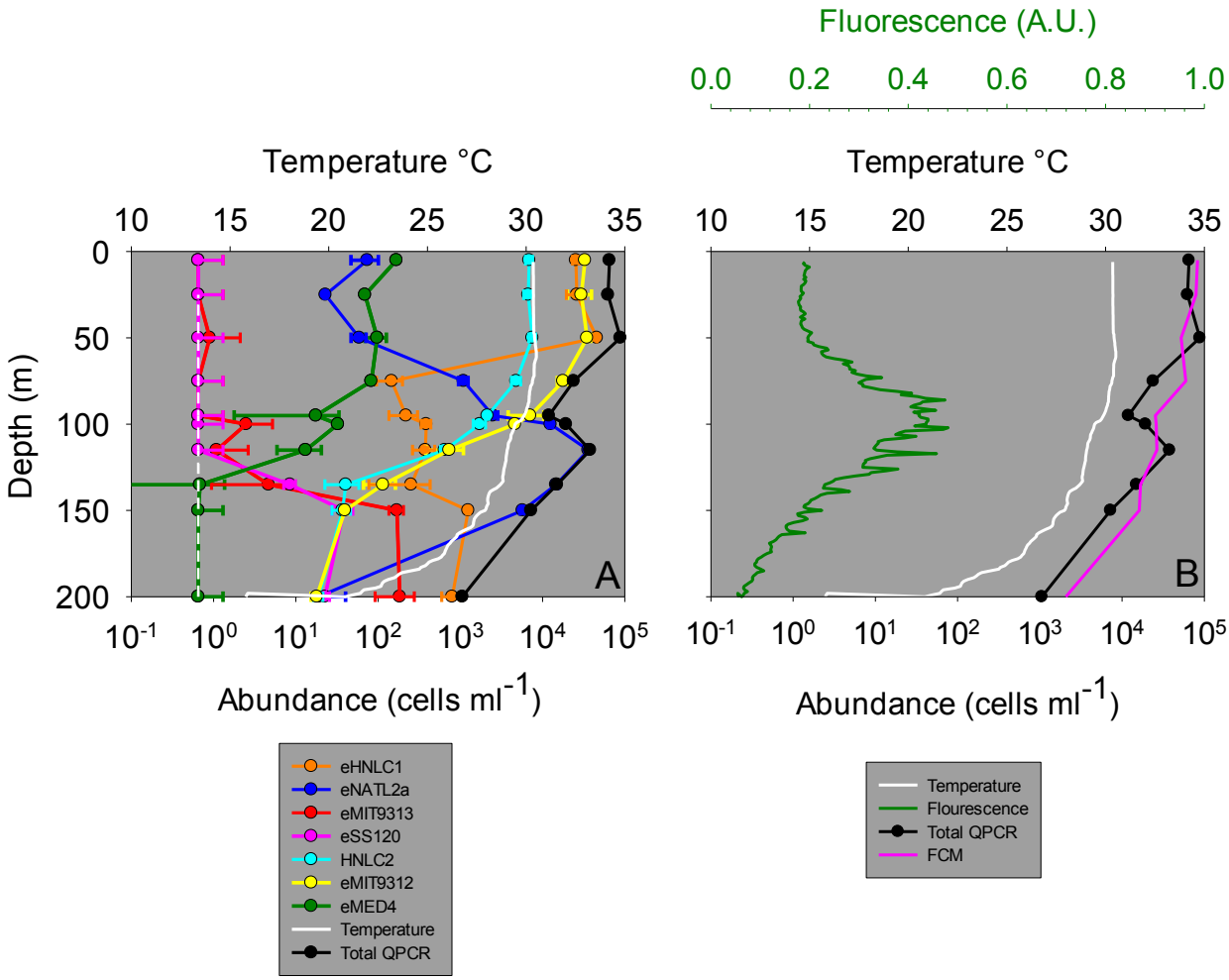
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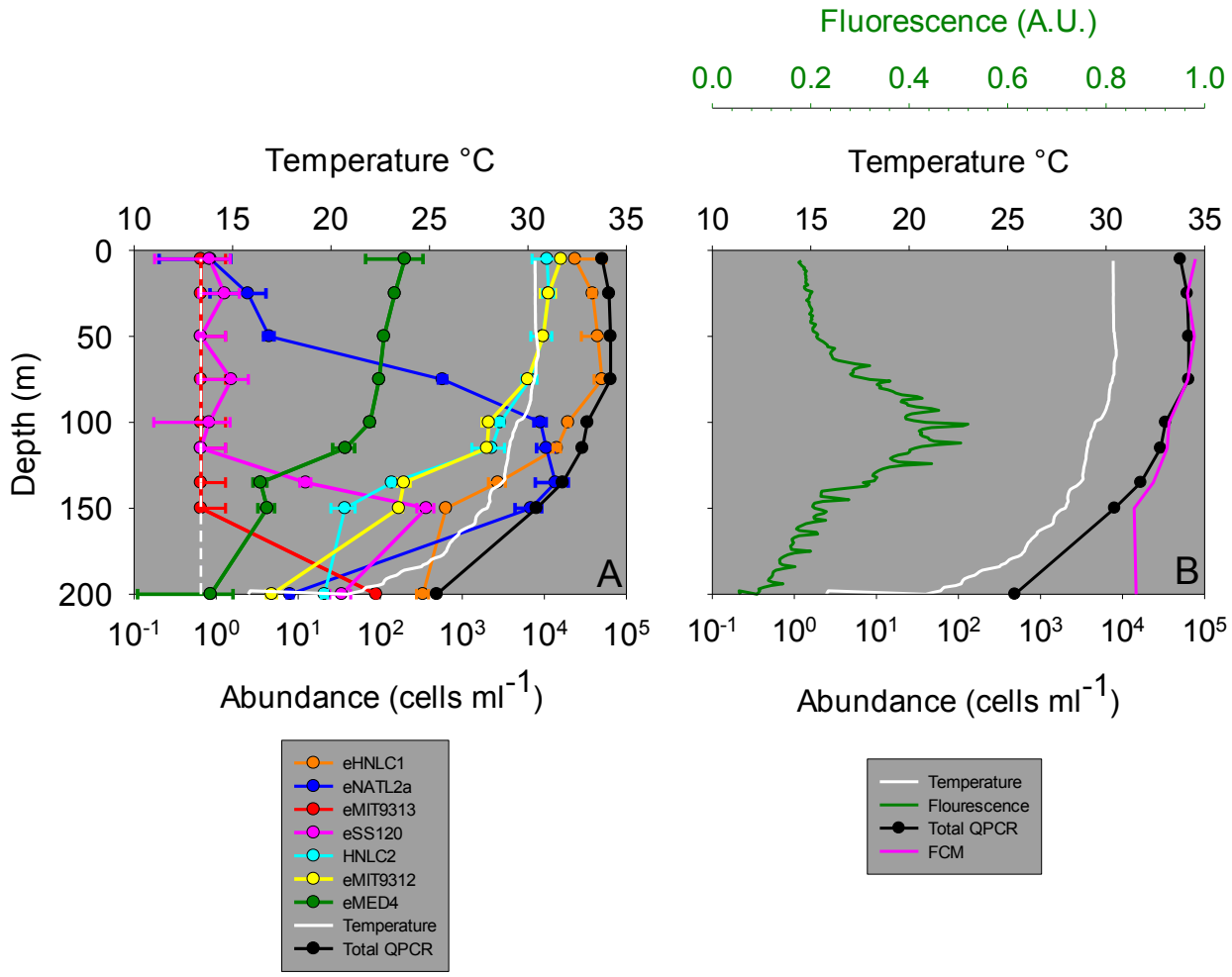
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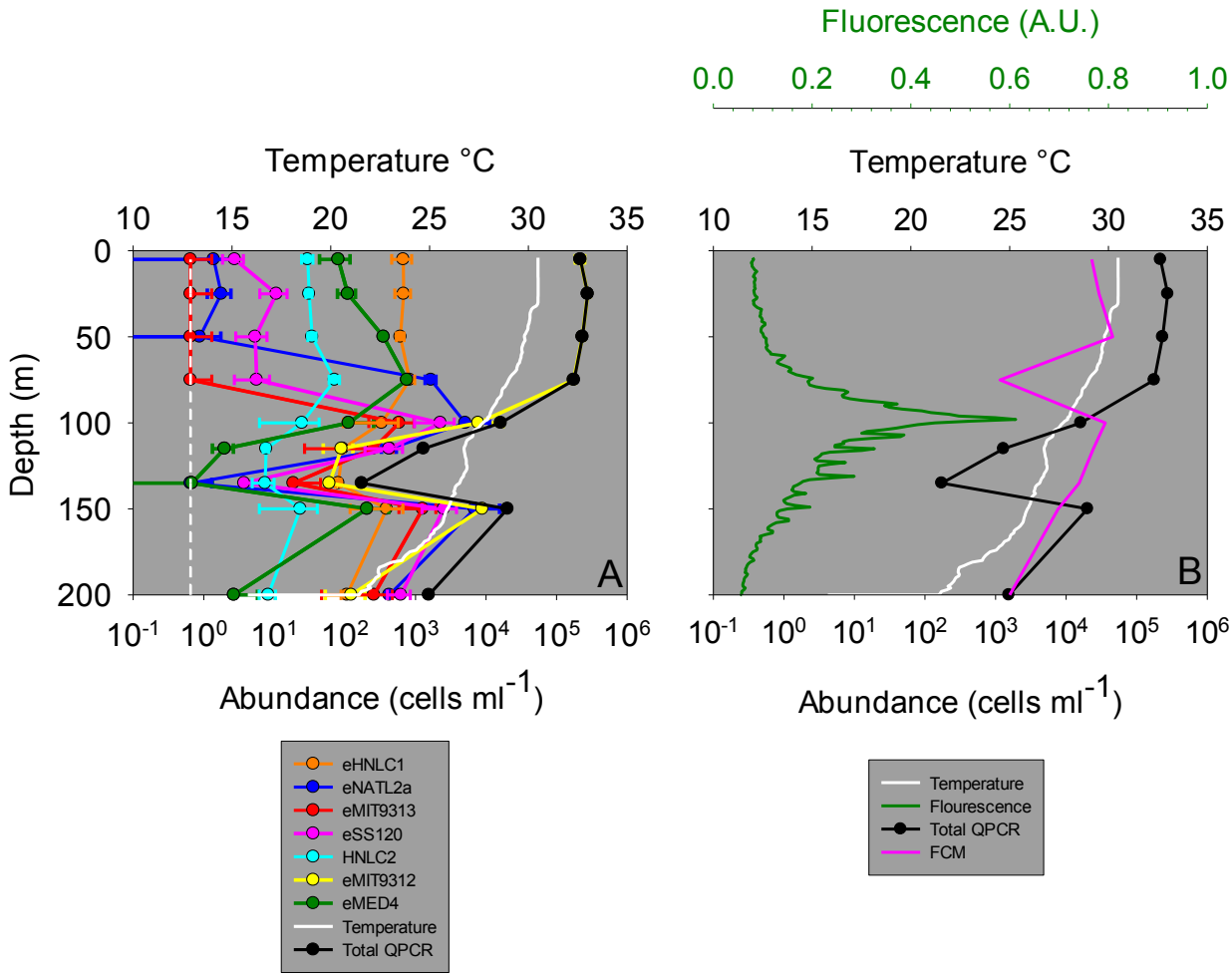
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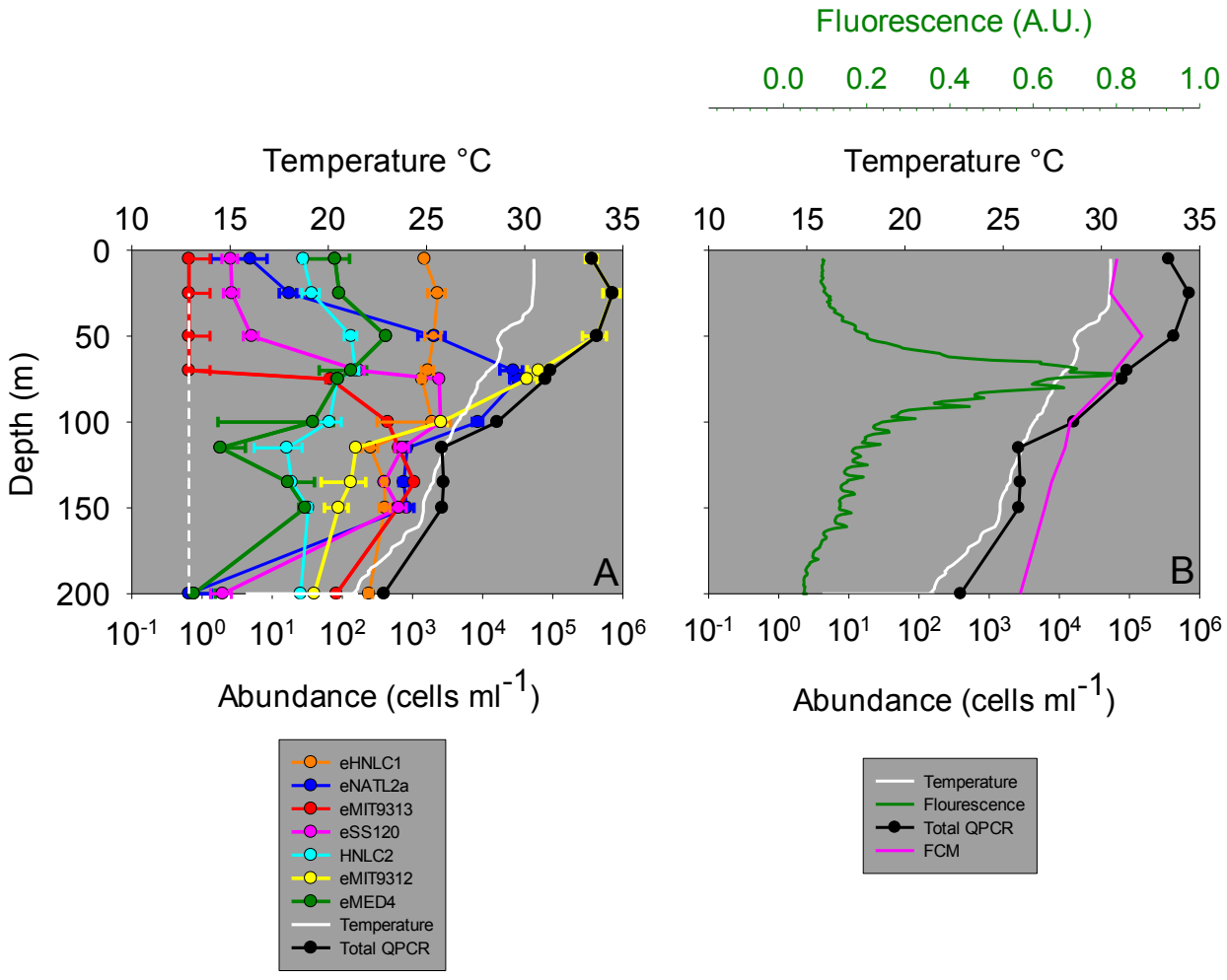
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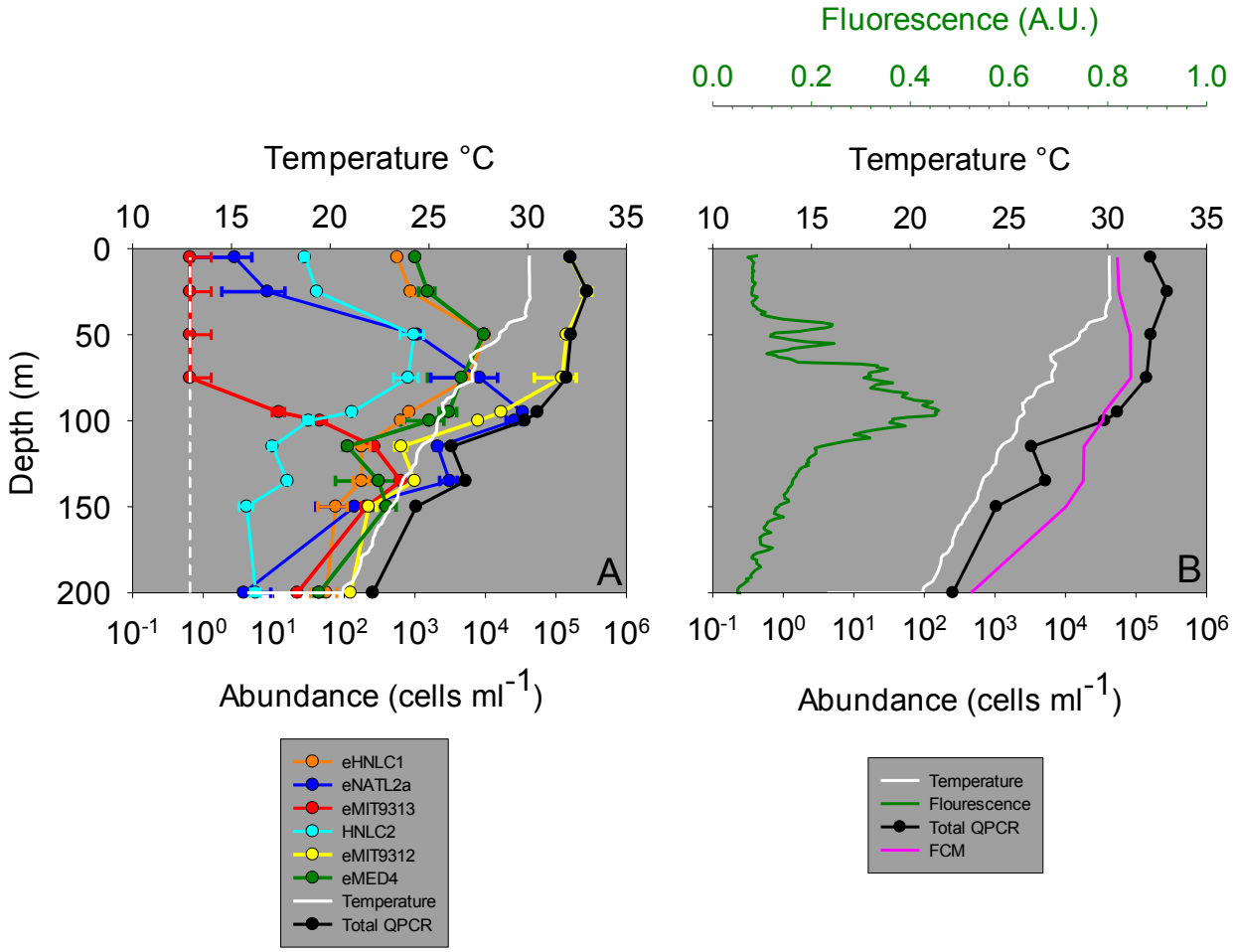
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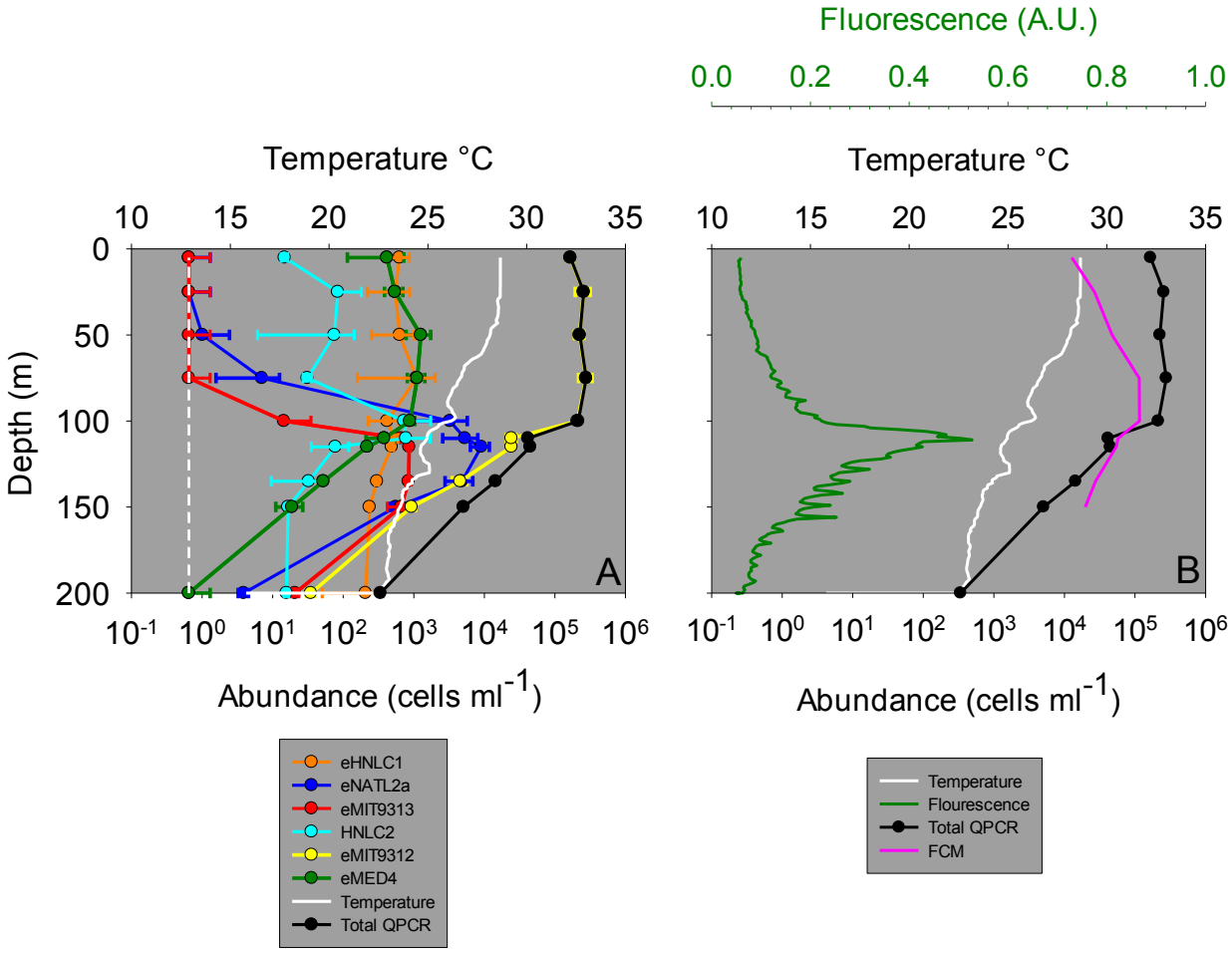
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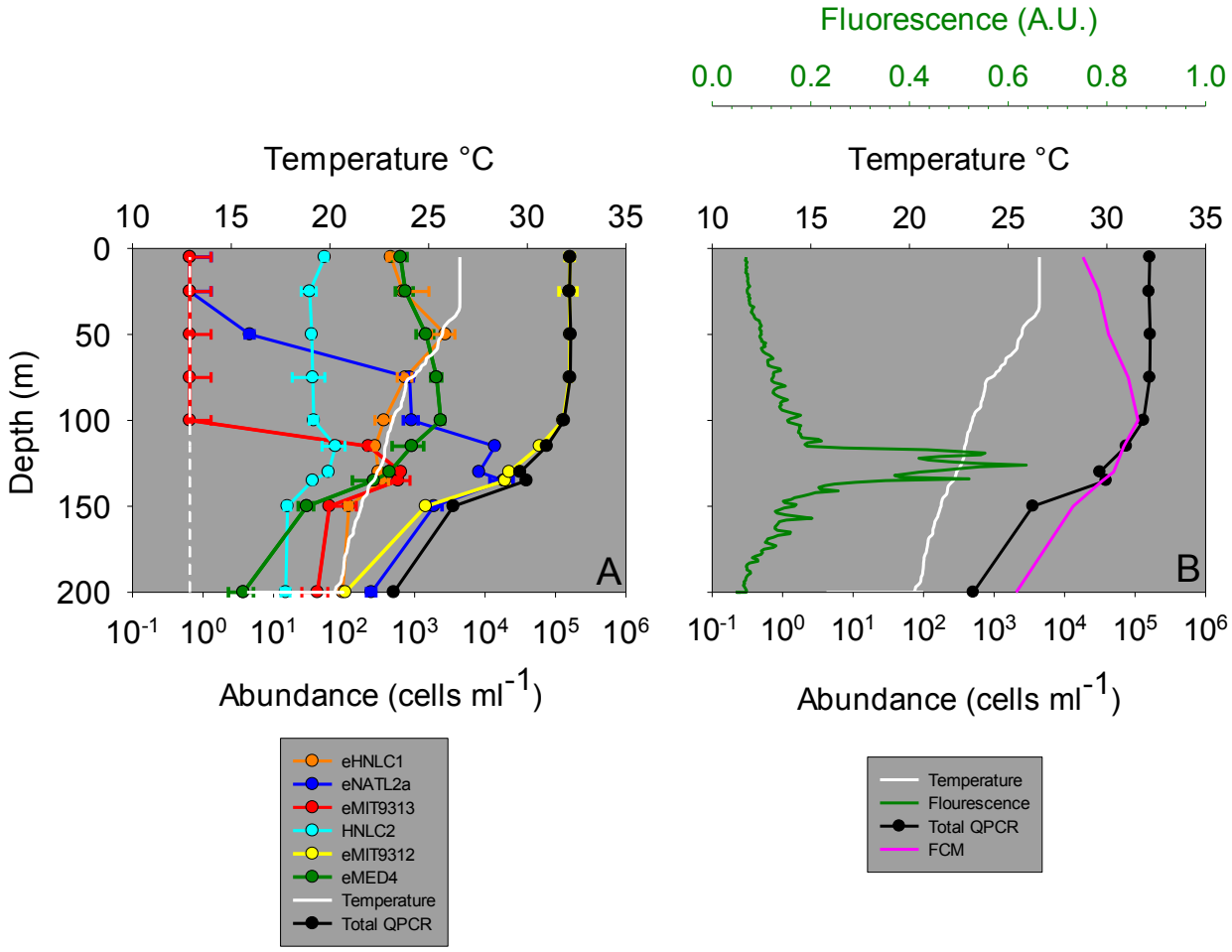
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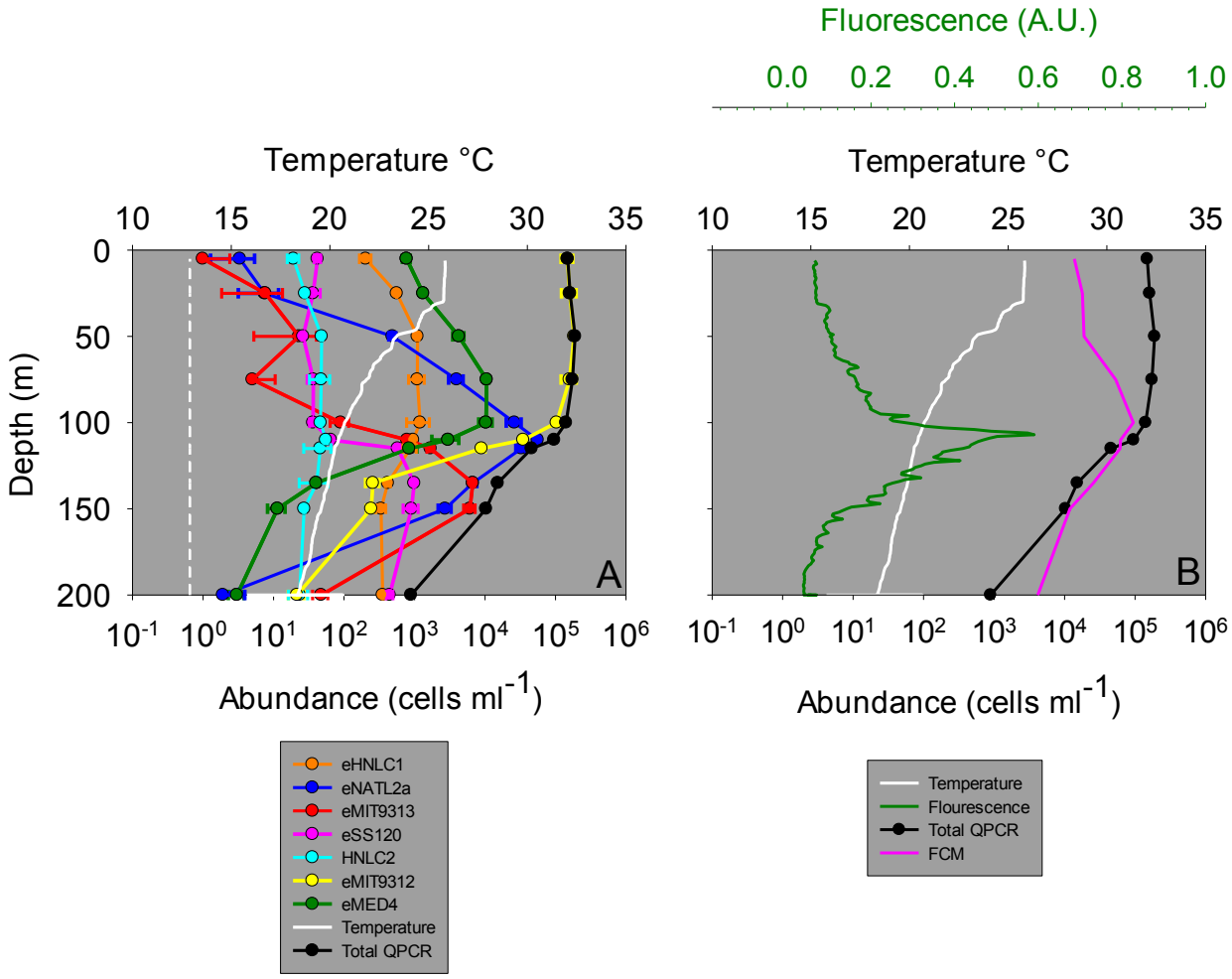
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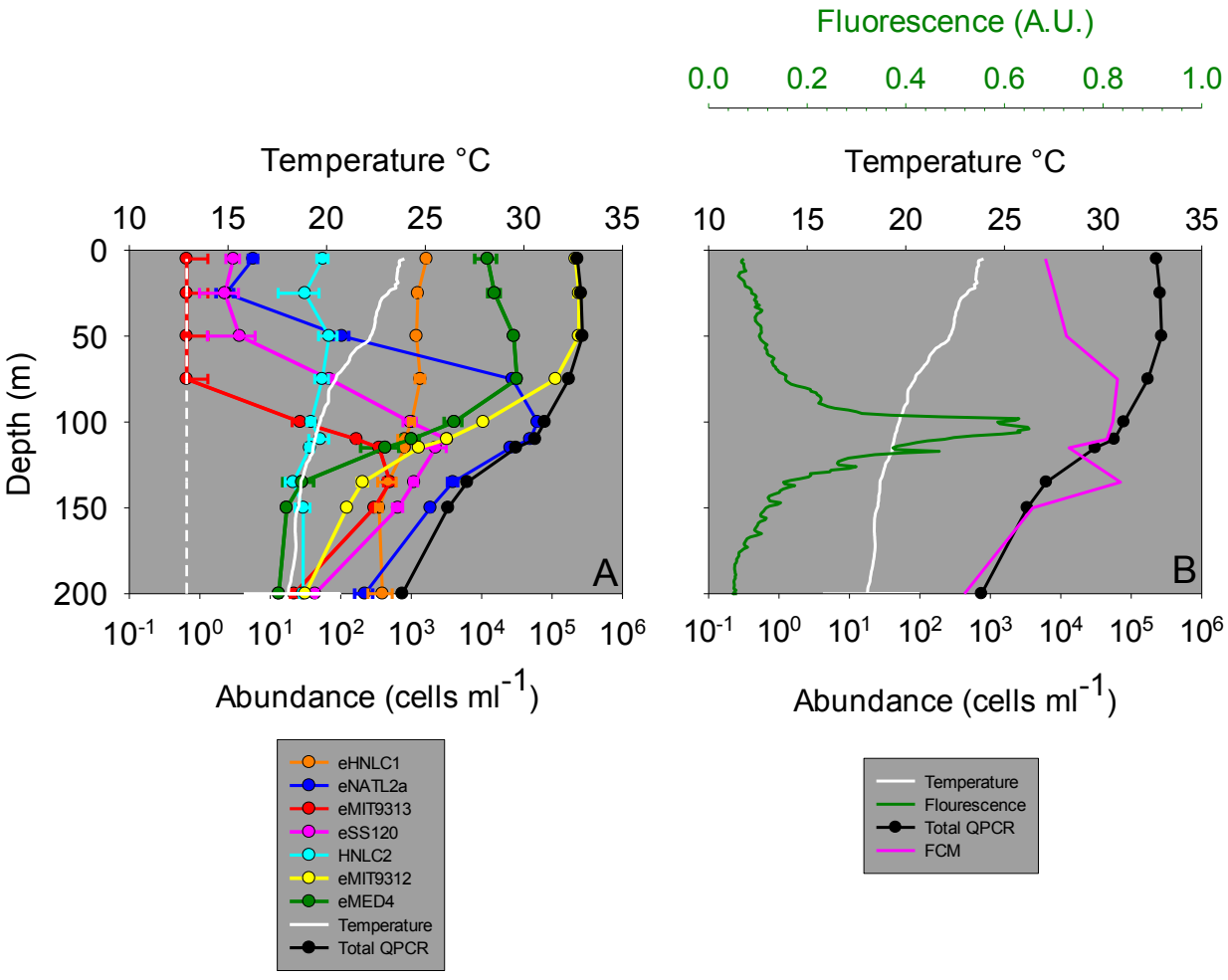
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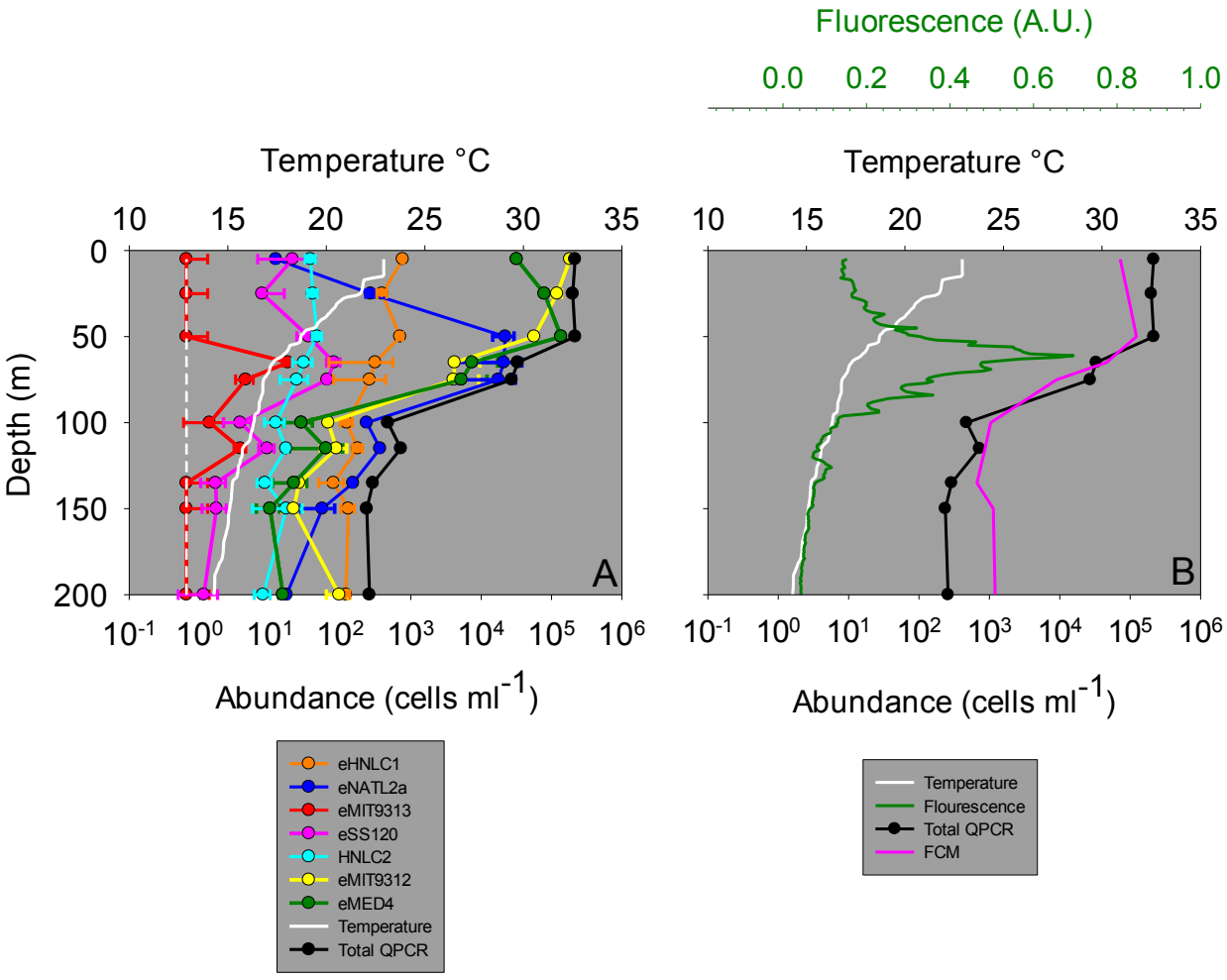
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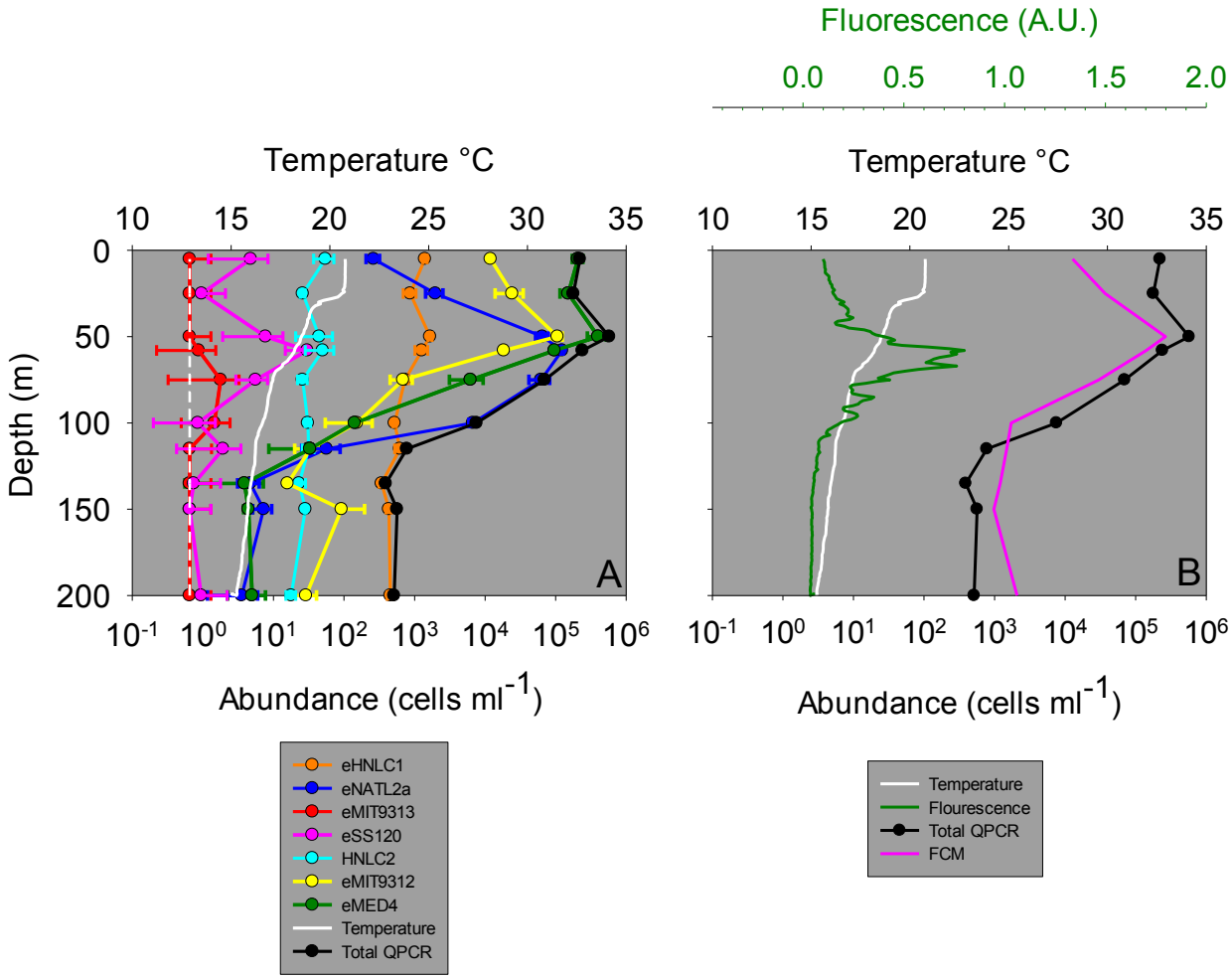
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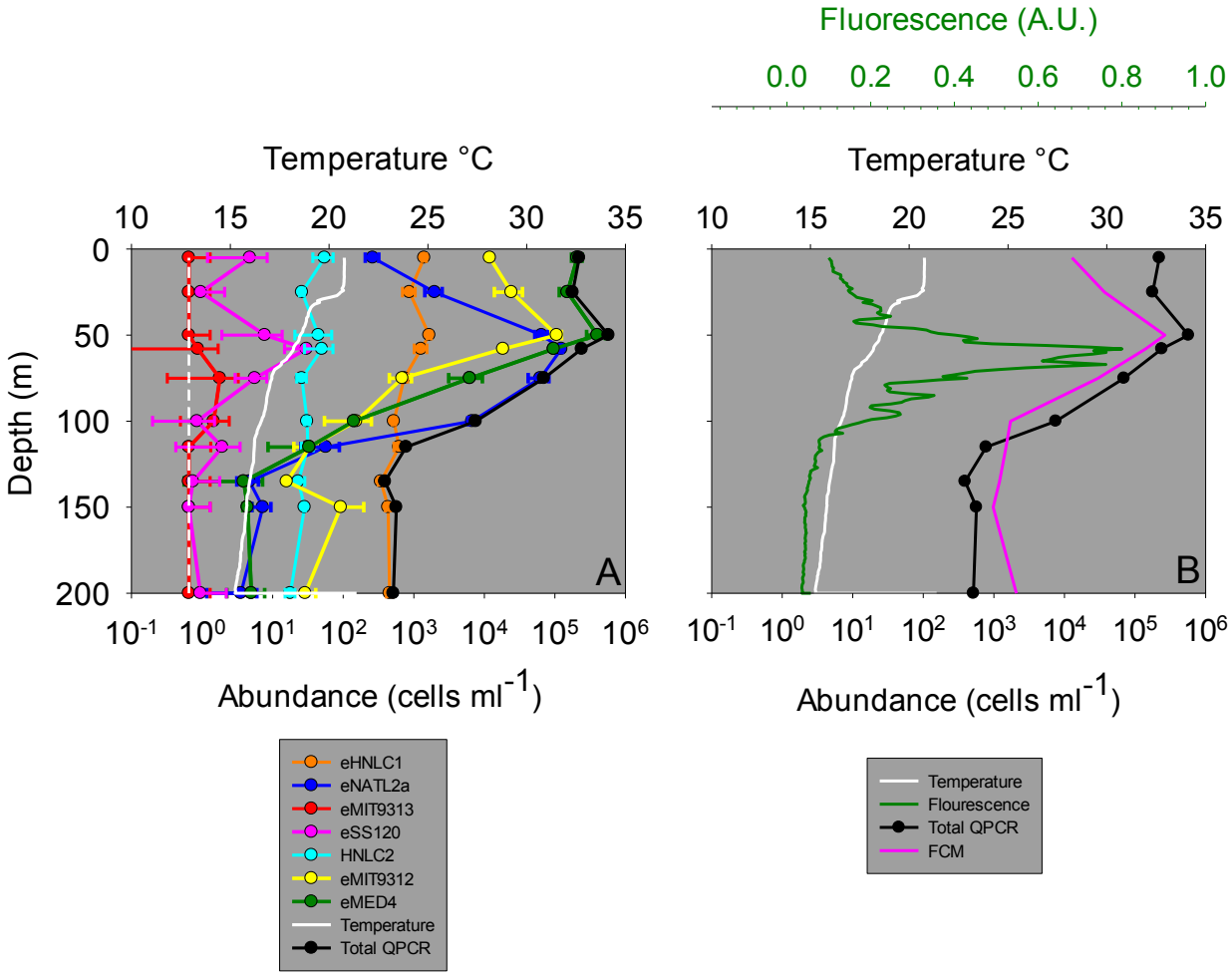
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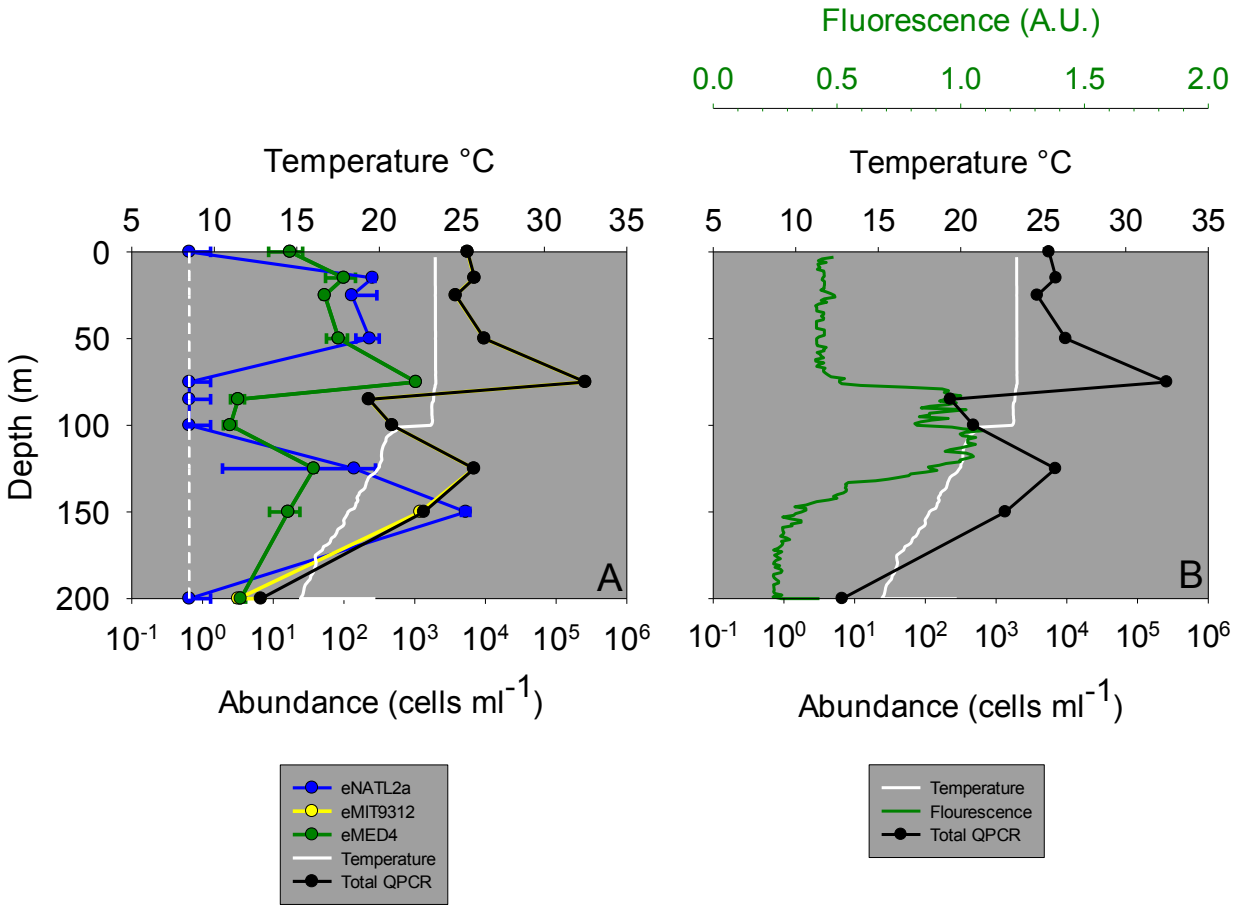
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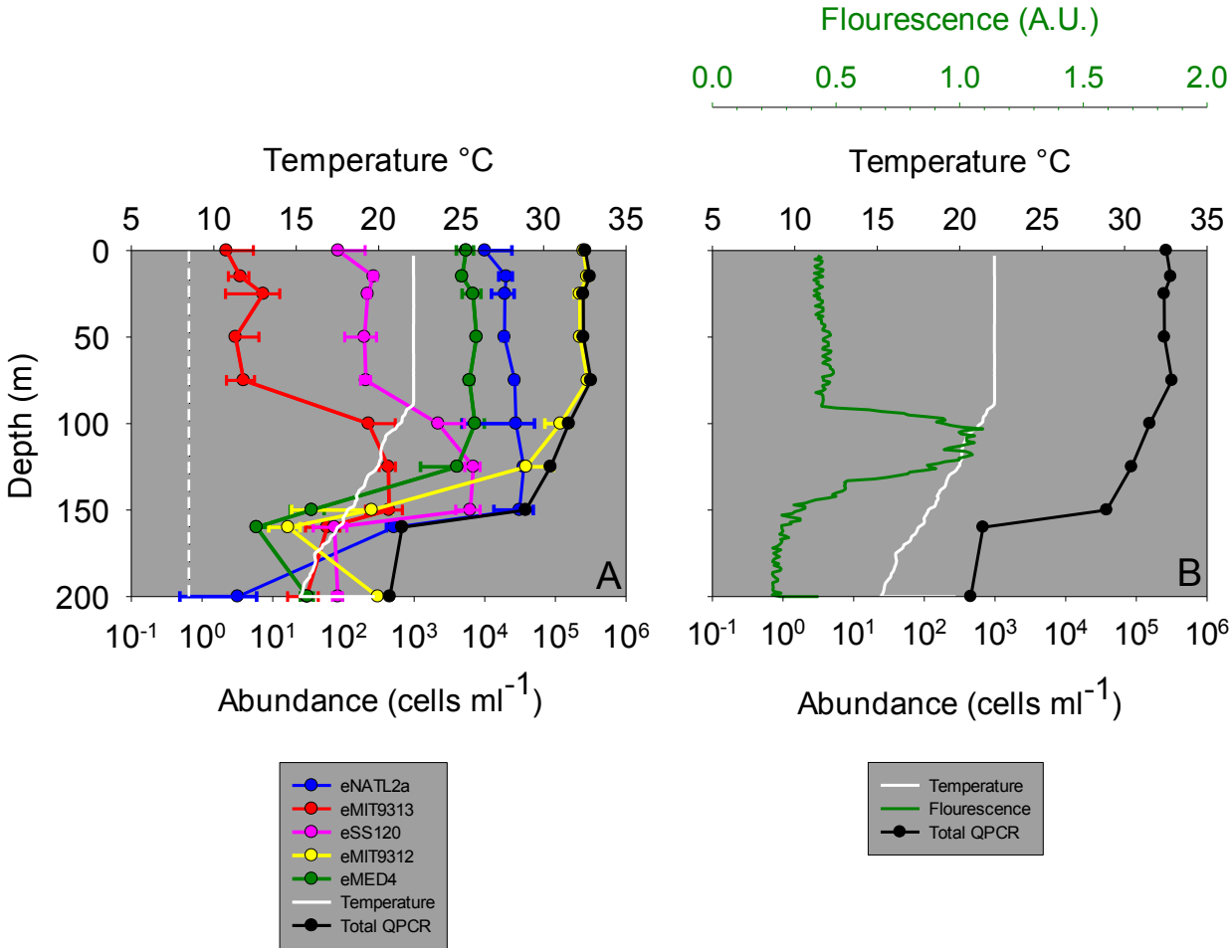
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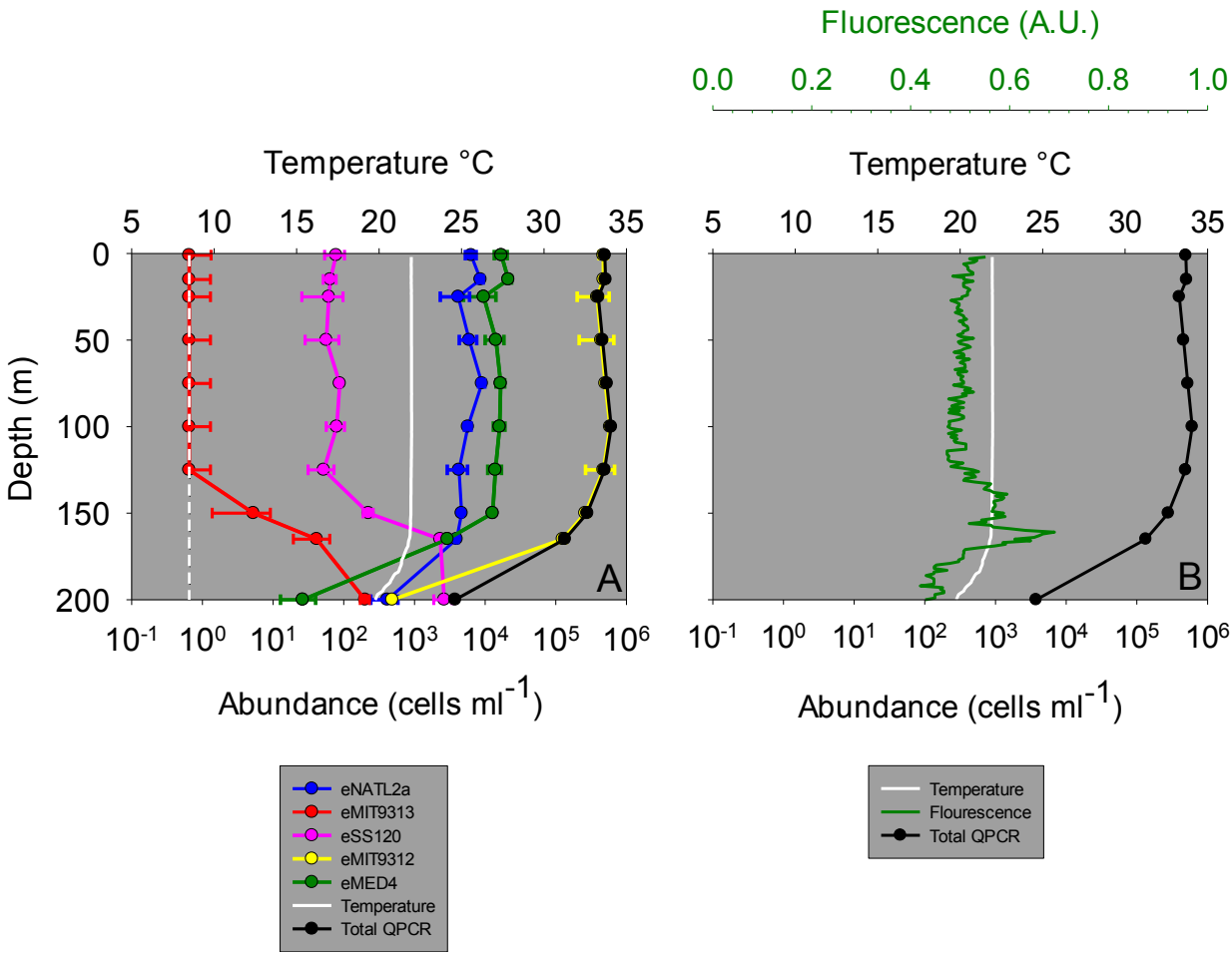
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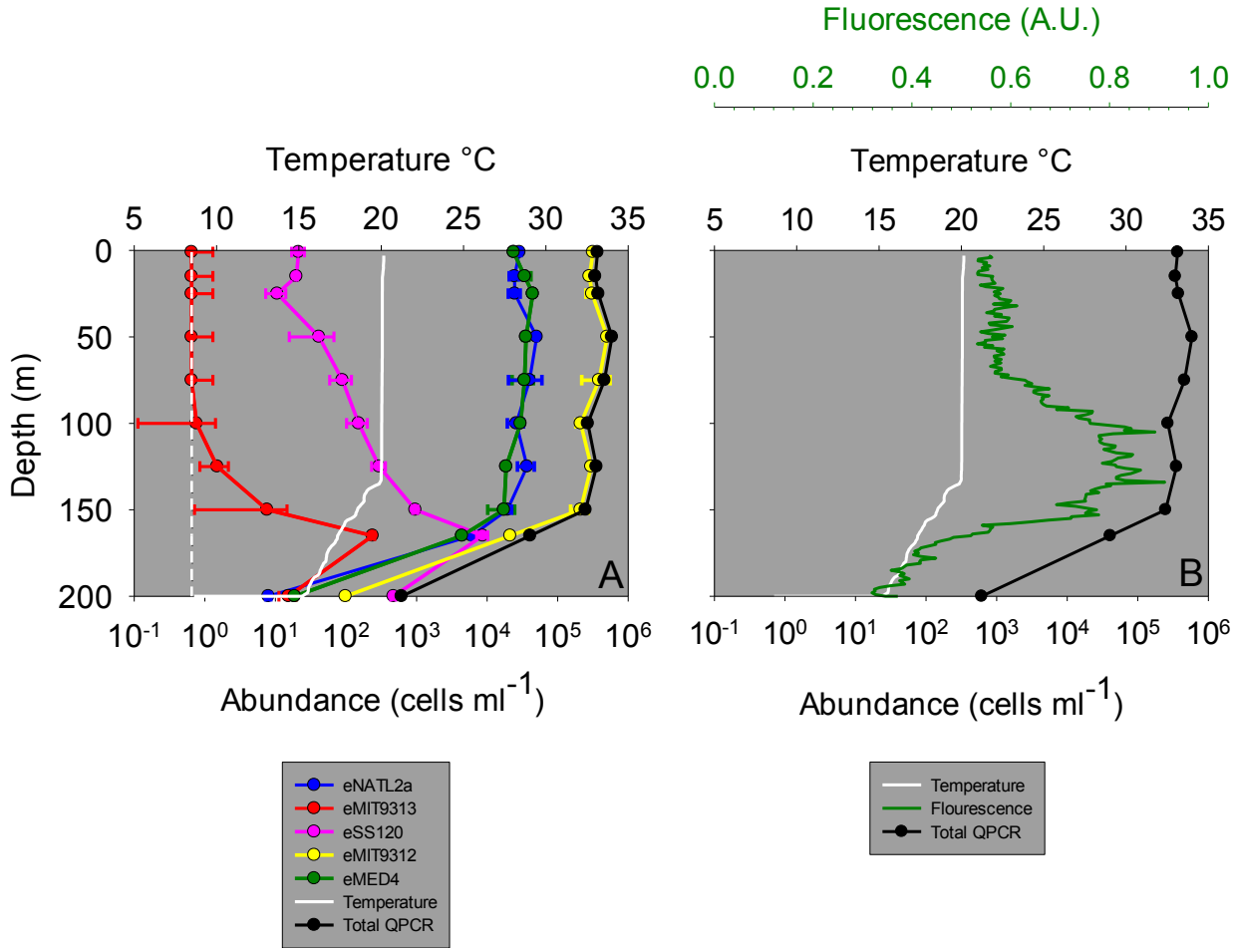
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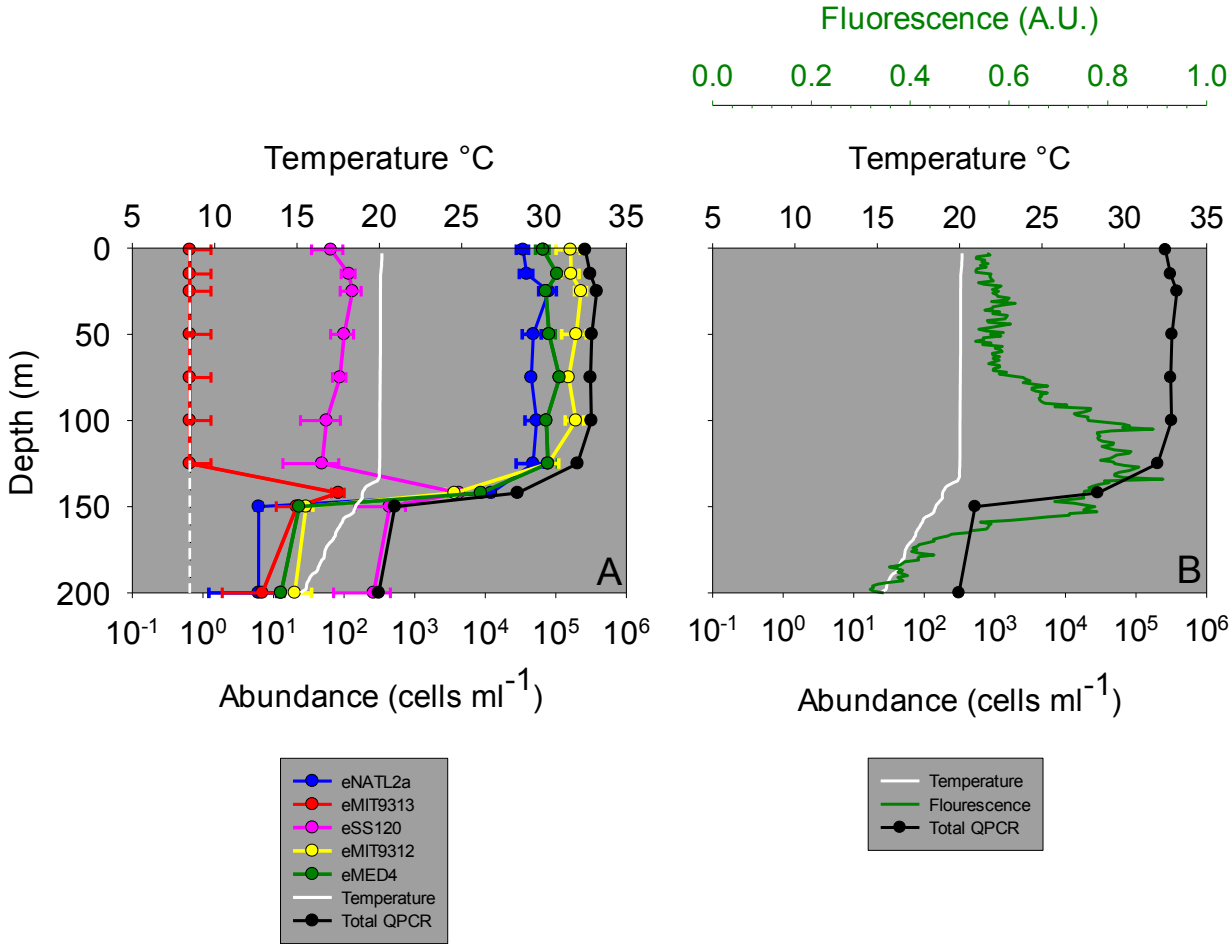
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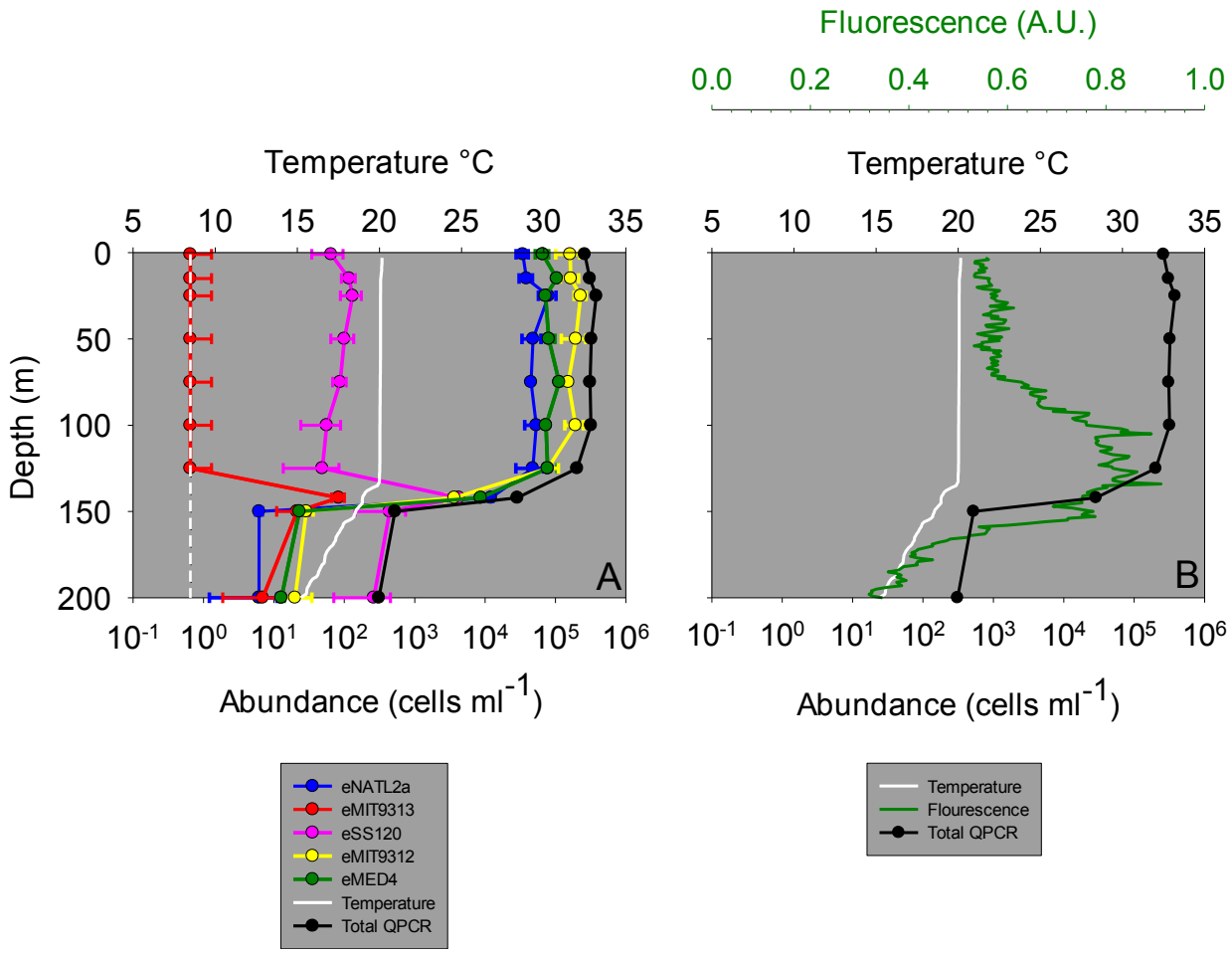
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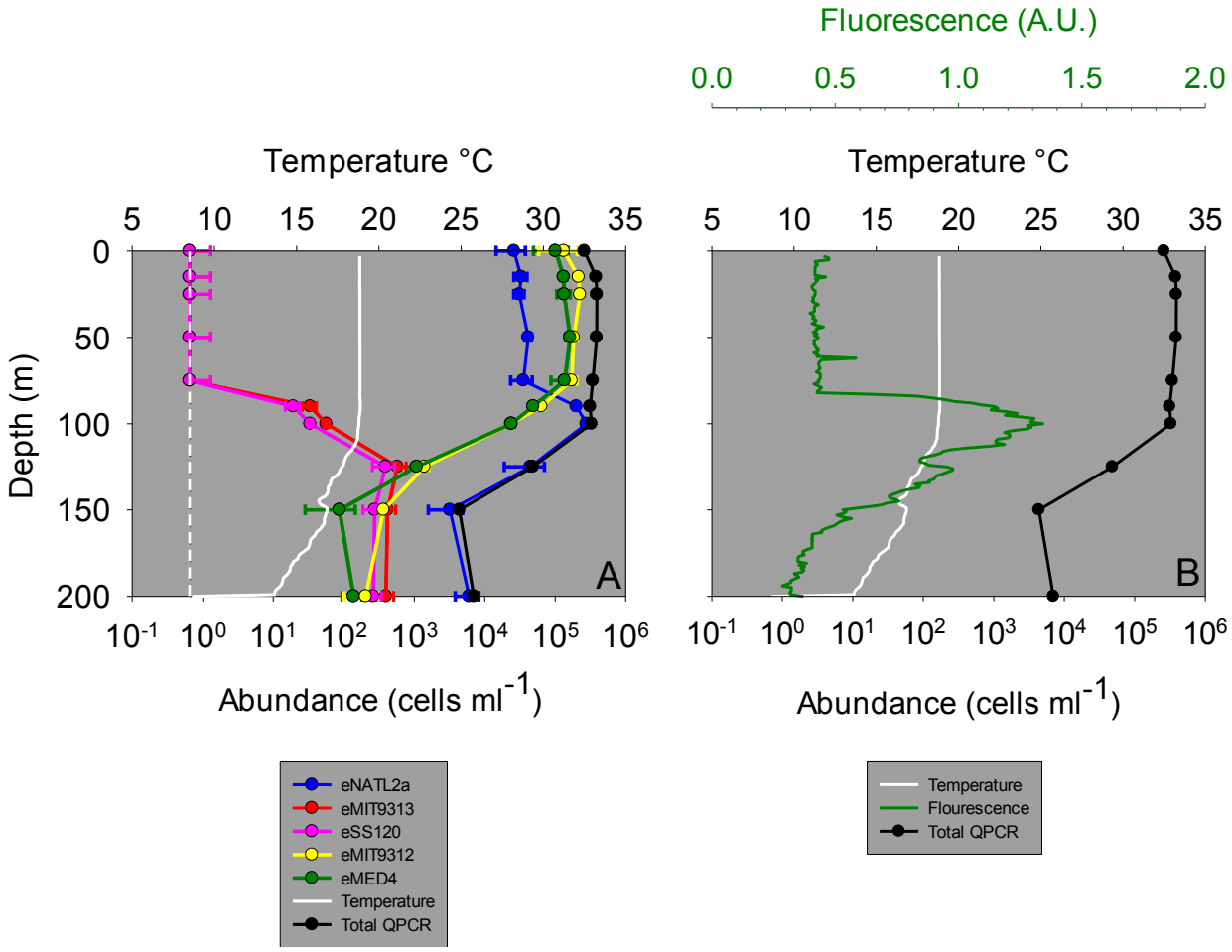
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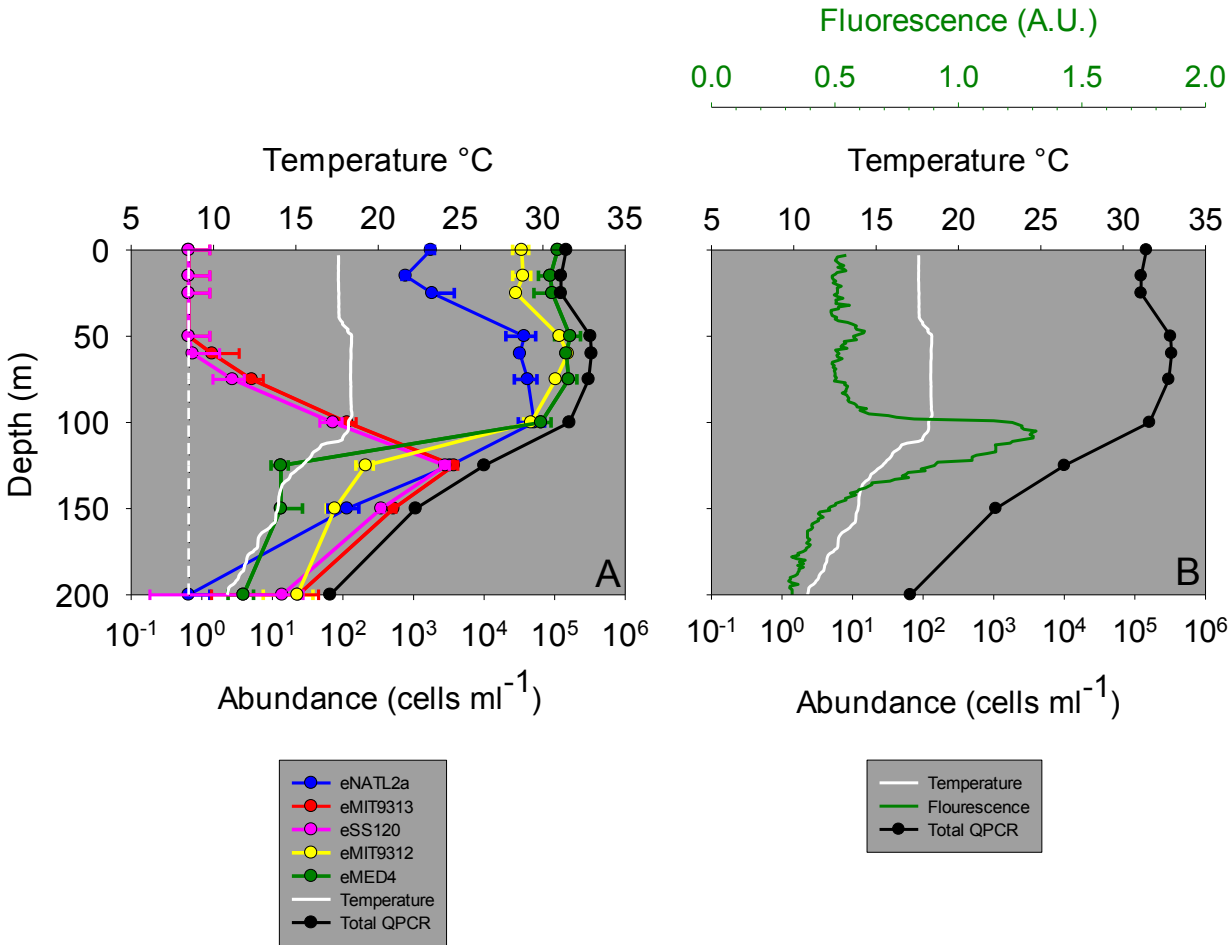
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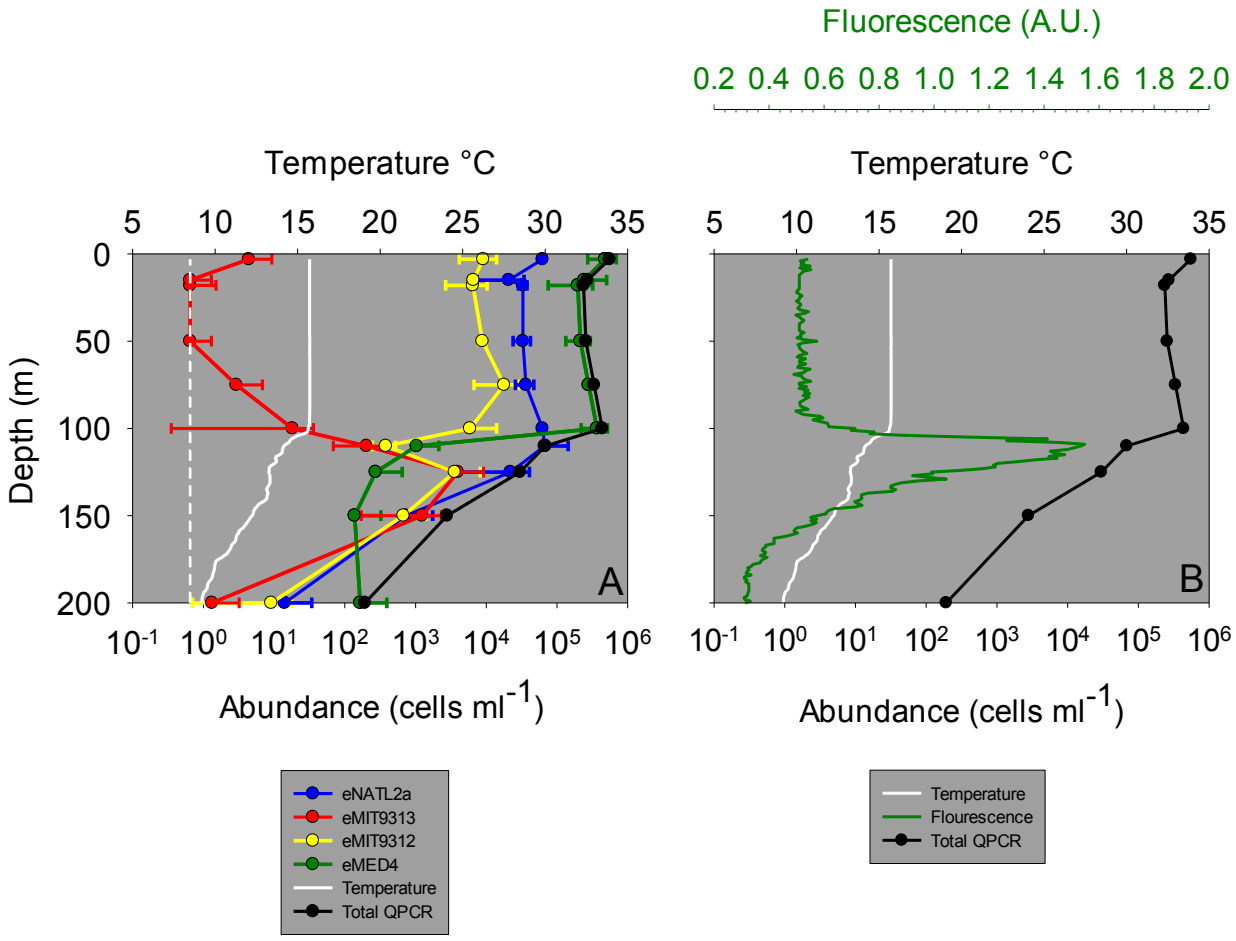
POWOW Station 10



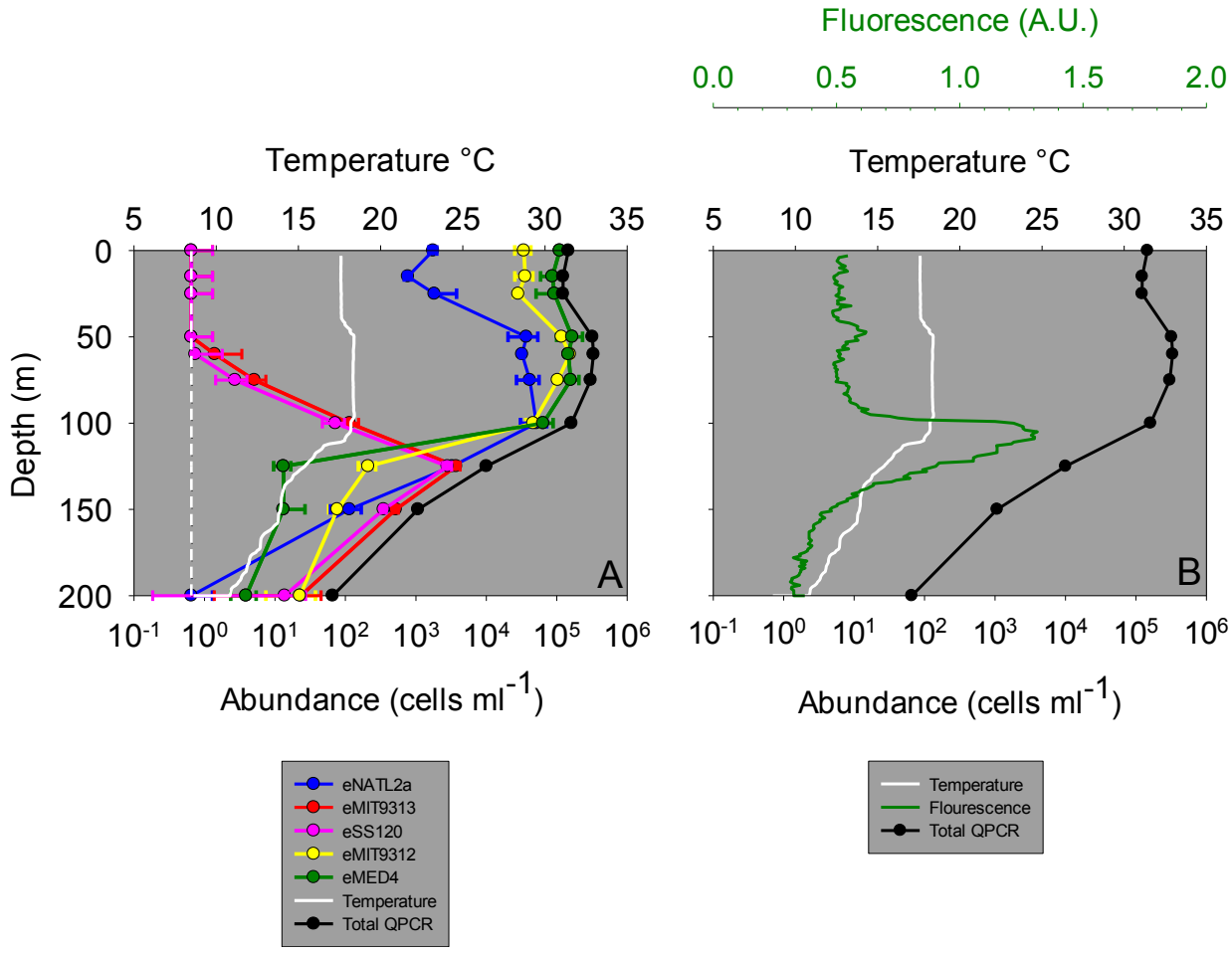
POWOW Station 11



POWOW Station 12



POWOW Station 13



Vita

Jeremy Werner Chandler was born in 1982 during a Memphis, TN ice storm to John and Susi Chandler. He grew up in a loving family who surrounded him with the wonders of the outdoor environment from an early age. At the age of 11 he got his first taste of Mississippi mud in his toes, while duck hunting in naturally flooded pin oak and cypress swamps of the Malmaison State Wildlife Management area. Here nature's spectacle provided educations in beaver run navigation, subsequent chest wader swimming techniques, alligator avoidance in warmer years, and on some days with no hunting, an appreciation for flocks of waterfowl so large that the sun was obscured by their very flight and amazement at thick water vapor wisps that hung over unfrozen waters for minutes subsequent to their departure. Further bolstering of these appreciations was supported by the discovery of southern author and humorist Havilah Babcock at an early age.

Boy Scouts of America provided more outdoors educational experiences from southeastern coastal marsh camping, to rock climbing the 14,000 ft. high mountain peaks of Colorado in the Chicago basin, and the blistering heat of southwestern deserts at Philmont Scout Ranch. As a merit badge instructor Jeremy enjoyed teaching environmental science and teaching younger scouts the simple joys of the natural world around us on nature hikes. Order of the Arrow provided a lasting impression of the importance of cheerful and unselfish service to others. Jeremy's Vigil honor name Allummochwalan (to guide someone) reinforced his love of teaching others his passions he still carries to this day. Physical and mental fortitude along with a strong code of morals and ethics were developed at a young age through scouting, family ties and Shito-ryu Karate under the tutelage of Kyoshi Howard Smith, where Jeremy achieved the rank of Shodan before departing for college.

Jeremy joined the Knoxville Volunteer Rescue squad in 2004 during his undergraduate career, with encouragement of his friend Colin Ickes, to fulfill his desire to devote volunteer service to the local community. He can still be found driving an emergency crash truck, rappelling down a cliff or cave, or plunging into swift water environments to help those in need to this day.

As an undergraduate at the University of Tennessee Jeremy volunteered in several

ecological studies on the agricultural campus as well as worked in a laboratory for Dr. Bruce McKee working with *drosophila melanogaster* and parental failure genes. At the closure of his undergraduate experience Jeremy was captivated with all things microbial under Dr. Wilhelm, and continued on in graduate school with Dr. Erik Zinser, whom facilitated the majority of Jeremy's scientific research development and excitement in addition to helping him become an experienced world traveler.

Jeremy met Abby Tucker in 2000 when he and his roommate defeated the entire women's rowing team in a sand volleyball match, and as a result of a bet they placed, all had to go swing dancing because of their loss to two exuberant college freshmen. Abby subsequently got Jeremy into rowing where he met Derek Jamison and other lifelong friends, while enjoying the absolute bliss of a perfectly set 8 man shell on glass-like water during 5AM practice sessions. Abby and Jeremy were married in 2007. In 2008 for their honeymoon they ventured into the wilds of Yellowstone National park, where they were chased by forest fires and grizzly bears in the Pelican wilderness. They finished their honeymoon with a light mountaineering ascent of Grand Teton via the Owen Spalding route in Grand Teton National Park. Jeremy and Abby acquired their first dog, Boogy, on October 31st 2006 and had their first child, Tucker Werner Chandler, on May 5th 2013.

Jeremy enjoys riding and racing his quiver of bicycles, hunting, archery, martial arts, gardening, beer, tending to his flock of 20 chickens, and building an assortment of contraptions and home additions around the small patch of land he calls home in west Knox county. Jeremy can still be found in a community near you volunteering and inoculating into those willing to listen, his enthusiasm of the wonders of the natural world from the microbial and beyond.

Scientia ipsa potential est