Terr. Atmos. Ocean. Sci., Vol. 30, No. 4, 575-587, August 2019 doi: 10.3319/TAO.2018.09.26.01

Attribution of the growth of a distinct population of *Synechococcus* to the coverage of lateral water on an upwelling

Chih-Ching Chung[1,2,*] and Gwo-Ching Gong[1,2]

[1] *Institute of Marine Environment and Ecology, National Taiwan Ocean University, Keelung, Taiwan*
[2] *Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan*

Article history:
Received 6 September 2017
Revised 18 July 2018
Accepted 26 September 2018

Keywords:
Upwelling, Kuroshio Current, 16S rDNA phylogeny, Prokaryotic picoplankton, *Synechococcus*

Citation:
Chung, C.-C. and G.-C. Gong, 2019: Attribution of the growth of a distinct population of *Synechococcus* to the coverage of lateral water on an upwelling. Terr. Atmos. Ocean. Sci., 30, 575-587, doi: 10.3319/TAO.2018.09.26.01

ABSTRACT

Upwelling water generally transports abundant nutrients to fertilize the euphotic zone and promotes larger phytoplankton to thrive. In contrast, knowledge about the composition of prokaryotic picoplankton associated with the scale of upwelling is limited. In this study, the population compositions of prokaryotic picoplankton, with particular focus on *Synechococcus*, under two diverse hydrographic conditions in the shelf-margin upwelling system were compared in detail. During the study period of 2009, an upwelling event was observed. In contrast to conventional upwelled water, the surface of this upwelling was covered with a thin layer of nutrient-depleted water. Although the water exchange created a low-nutrient stock environment, which fell below traditional chemical detection limits, its flux was likely sufficient to support the growth of the *Synechococcus* clade-II lineage. In comparison with the hydrography in 2009, no obvious upwelling occurred, and oligotrophic water primarily occupied the upper layer during the study period of 2010. The abundance of *Synechococcus* significantly declined to approximately half its numbers observed in 2009. While the *Synechococcus* clade-II were still the predominant population, their proportion in the 16S rRNA gene library decreased to approximately 50%. The remaining part was replaced with α-*Proteobacteria* and various heterotrophic bacteria. The results of the present study, combined with those obtained in previous studies, yield a more comprehensive understanding of the phytoplankton community dynamics in this varied ecosystem.

1. INTRODUCTION

Prokaryotic picophytoplankton (≤ 2 μm in size), which primarily comprise *Prochlorococcus* and *Synechococcus*, are important primary producers that are responsible for up to half of the CO_2 fixation in the open ocean (Waterbury et al. 1979; Chisholm et al. 1988; Goericke and Welschmeyer 1993; Liu et al. 1995, 1997; Richardson and Jackson 2007). Because of their tiny cell sizes, episodic environmental disturbances can easily influence their distribution and succession. For instance, an Asian dust storm event promoted the transient growth of *Synechococcus* in oligotrophic water in which *Prochlorococcus* was predominant (Chung et al. 2011). In addition, the injection of abundant freshwater rich in terrestrial materials from an extreme rainfall-induced flood in the Changjiang River basin resulted in a transient change in the picoplankton assemblage in the East China Sea (Chung et al. 2014, 2015). Therefore, global changes, including those resulting from human activities, may strongly affect picophytoplankton ecology and the marine biogeochemical cycle.

Upwelling, which is characterized by the uplift of cold and nutrient-rich deep water, is an important nutrient source in oligotrophic oceans. Larger phytoplankton (i.e., microphytoplankton and macrophytoplankton) have been suggested to be the most dominant primary producers during upwelling events (Malone 1971; Chen 1992; Mackey et al. 2014). In contrast, current knowledge about the picophytoplankton in this dynamic ecosystem is limited. Previous studies have indicated that the occurrence and strength of upwelling events and their interactions with surrounding waters are closely associated with the properties of the upper water column (i.e., stratification intensity and nutrient concentration), which in turn regulate the community structure of picophytoplankton (Partensky et al. 1996; Van

* *Corresponding author*
E-mail: chungcc@mail.ntou.edu.tw

Dongen-Vogels et al. 2012; Ahlgren et al. 2014). For instance, *Synechococcus* represented the largest populations of picophytoplankton during a period of weak upwelling in the subsurface of a coastal upwelling system in South Australia, which was typically dominated by *Prochlorococcus* during periods of non-upwelling (Van Dongen-Vogels et al. 2012). Moreover, Ahlgren et al. (2014) suggested that the distinct distribution of diverse *Synechococcus* populations surrounding the Costa Rica upwelling dome was derived from the surface concentration gradients of nutrients and trace metals, which were carried by the upwelling water.

The Kuroshio Current, which is one of the major western boundary currents in global oceans, originates from the western equatorial Pacific Ocean and flows northward along the outer margin of the East China Sea (Fig. 1). The Kuroshio surface water is characterized by high temperature, high salinity, and ultra-oligotrophicity, with chlorophyll *a* concentrations consistently below 0.2 mg m^{-3}. In contrast, the Kuroshio subsurface is colder and more nutrient-rich. When the Kuroshio Current flows by the shelf break of the southeastern East China Sea, the subsurface water is often elevated by topographically induced upwelling. Depending on the scale of this upwelling, the hydrographic condition is highly dynamic and governs the biogeochemistry and fishery activities at the border of the subtropical Northwest Pacific Ocean (Gong et al. 1997, 1999; Wong et al. 2000; Kwon et al. 2010). The community structure and primary productivity of microphytoplankton associated with the intensity of the Kuroshio-related upwelling have been well described (Chen 1992; Gong et al. 1997, 1999). To our knowledge, however, there are no studies of the changes in picophytoplankton community assemblage under diverse upwelling conditions in this region. For this reason, the information obtained from the flow cytometric enumeration and assessment of molecular phylogeny in the present study, combined with the results of previous studies, will provide a more comprehensive understanding of the phytoplankton community dynamics in this varied ecosystem. Furthermore, there is growing evidence that the dynamic changes in different *Synechococcus* populations are highly associated with ambient hydrographic conditions. Therefore, a particular emphasis was placed on the assemblage composition of *Synechococcus* in this study.

2. MATERIAL AND METHODS

2.1 Sample Collection

Four stations were established from the mid-shelf to the Kuroshio Current and were visited onboard the research vessel Ocean Researcher I during the periods of 11 to 13 July 2009 and from 16 to 17 July 2010 (Fig. 1). Moreover, Station Ref (also known as Station E) (Chung et al. 2015) was also visited during the same cruise in 2009 (Fig. 1). Temperature and salinity were measured using a conductivity/temperature/depth recorder (CTD) (SBE 9/11 plus, Sea-Bird Electronics), and these values were further applied to calculate seawater density. Water samples used for the determination of nutrient concentrations and picoplankton abundances were collected using Teflon-coated 20 L Go-Flo bottles (General Oceanics) mounted on a CTD rosette sampler. From the surface water of Station 2 (Fig. 1), where upwelling usually occurred, environmental DNA was isolated to

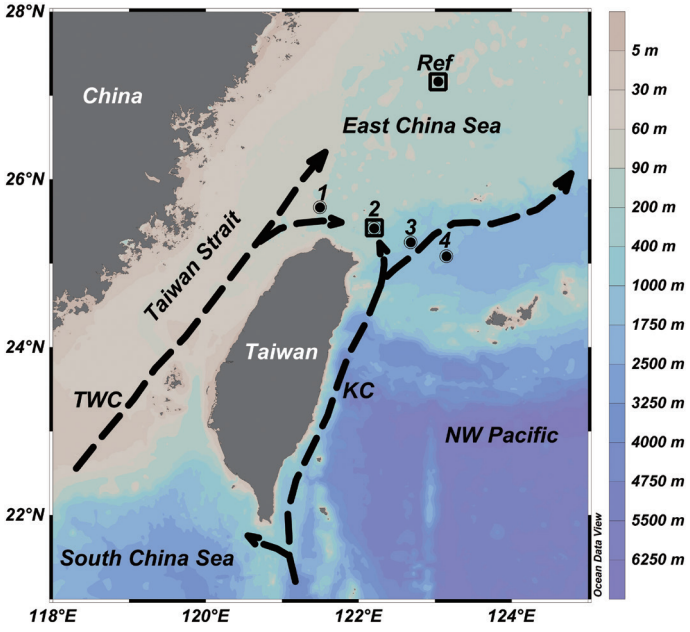

Fig. 1. Locations of the observation stations on the southern East China Sea during the summers of 2009 and 2010. Station 2, which is located on the shelf-break, is the station with year-round upwelling. KC and TWC indicate the Kuroshio Current and the Taiwan warm current, respectively.

analyze the phylogenetic diversity of 16S rRNA genes.

2.2 Determination of Dissolved Inorganic Nutrient Concentrations

The water samples were rapidly frozen in liquid nitrogen and subsequently stored at -20°C until further analysis. The methods employed to determine the concentrations of dissolved inorganic nutrients (i.e., NH_4, NO_3, and PO_4) have been well described in previous studies (Gong et al. 2000, 2003, 2006).

2.3 Determination of Picoplankton Abundance

Samples used for picoplankton enumeration were fixed in seawater-buffered paraformaldehyde at a final concentration of 0.2% (w/v) in the dark; they were subsequently frozen and stored in liquid nitrogen until further analysis. Different populations of picoplankton were identified and enumerated using a flow cytometer (FACSAria, Becton-Dickinson). The abundances of *Synechococcus* and *Prochlorococcus* was determined based on cell size (forward- and side-scattering) and autofluorescence in the ranges of orange (from phycoerythrin, 575 ± 15 nm) and red (from divinyl chlorophyll, > 670 nm) under excitation at 488 nm. Heterotrophic bacteria stained with SYBR-Green I dye (Molecular Probes) were enumerated in a separate subsample. A known number of fluorescent beads (TruCOUNT Tubes, Becton-Dickinson) was simultaneously counted to calculate the original cell abundance in each sample (Chung et al. 2015).

2.4 Environmental DNA Isolation

Approximately 10 L of seawater was filtered through a 10-μm mesh nylon net to remove larger plankton. To understand the population composition of *Synechococcus*, the cells in the filtrate were simultaneously harvested on three 0.8-μm pore size polycarbonate membranes (diameter = 45 mm) (Nucleopore, Whatman) under gentle vacuum (< 100 mmHg). The cell sizes, that were collected on the membranes ranged from 0.8 - 10 μm. The membranes were pooled together in one cryovial and immediately frozen in liquid nitrogen until DNA isolation. The cell walls of the prokaryotic picoplankton retained on the membranes were disrupted via a single treatment with lysozyme (10 mg ml^{-1}) (Sigma-Aldrich) at 37°C for 30 min, followed by incubation in lysis buffer containing 0.1 M EDTA (pH 8.0), 1 mM Tris-HCl (pH 8.0), 0.25% sodium dodecyl sulfate, and 0.1 mg ml^{-1} proteinase K (Roche) at 55°C overnight. A 1% hexadecyltrimethylammonium bromide solution (Sigma-Aldrich) was added to remove cellular debris and residual polysaccharides, followed by chloroform extraction and an additional round of phenol/chloroform/isoamyl alcohol extraction (25/24/1, v/v/v). The DNA pellet was precipitated using isopropanol and resuspended in Tris-EDTA buffer (pH 8.0) (Chung et al. 2015). The concentration and purity of DNA were determined by spectrophotometry (NanoDrop, Thermo Scientific) at wavelengths of 260 and 280 nm.

2.5 Pyrosequencing and Sequence Analysis

Approximately 50 ng of environmental DNA was used as the template for the polymerase chain reaction (PCR) to specifically amplify the hypervariable V6 - V9 region of 16S rRNA genes using the high-fidelity Phusion DNA Polymerase (New England Biolabs) and the primer set 16S-909F (5'-ACTCAAAKGAATWGACGG-3') and 16S-1492R (5'-NTACCTTGTTACGACT-3') (Ngugi et al. 2012). Equimolar amounts of amplicons from two libraries were pooled for sequencing on a Roche 454 Genome Sequencer FLX+ Instrument (Roche) (Chung et al. 2015).

To reduce the sequence error rate, raw reads were first processed using the PyroNoise program with quality cutoff scores between 360 and 720 flows (Quince et al. 2009). Any ambiguous sequence having more than one mismatch with the barcode or more than two mismatches with the PCR primers was subsequently eliminated. Chimeras were detected and removed using the UCHIME program (Edgar et al. 2011). Sequences that were identified as chloroplast, mitochondrial, archaeal, and eukaryotic rRNAs were removed after alignment to the SILVA-based small subunit rRNA reference dataset (version 123). Eventually, the resulting high-quality sequences (≥ 350 bp) were aligned using the Clustal Omega program (Sievers et al. 2011). Pairwise distances between all aligned reads were calculated to generate operational taxonomic units (OTU), with a cutoff value of 3% sequence divergence. The OTUs were employed for the assessment of rarefaction curves, abundance-based species richness estimators (Chao1), abundance-based coverage estimators (ACE), and the Shannon diversity index. The taxonomy of each OTU was determined using the Ribosomal Database Project Classifier (Wang et al. 2007). All of the above analyses were implemented using the MOTHUR software package (version 1.36.0) (Schloss et al. 2009, 2011). Moreover, subgroups of *Synechococcus* were inferred from the 16S rRNA gene sequences of 10 known clades deposited in the GenBank database using neighbor-joining and maximum likelihood algorithms with bootstrap values of ≥ 50 under both methods (PHYLIP ver. 3.695, distributed by Felsenstein; Department of Genome Sciences, University of Washington, Seattle, USA).

2.6 Nucleotide Sequence Deposition

All sequences collected from the Stations 2 and Ref (also known as Station E, Chung et al. 2015) were deposited in the Sequence Read Archive Database (SRA) of the National Center for Biotechnology Information (https://

www.ncbi.nlm.nih.gov) under the accession numbers SRX1890707 and KM469544 to KM471290, respectively.

3. RESULTS

3.1 Hydrographic Conditions

The hydrographic characteristics of Stations 1, 3, and 4 in the study periods of 2009 and 2010 were similar. Station 1 is located on the mid-shelf of the East China Sea, and its hydrography was extensively affected by the Taiwan Warm Current (TWC), resulting in surface water with salinity levels below 34 (Figs. 1, 2) (Gong et al. 1996). In contrast, warmer waters with salinity levels higher than 34 in Stations 3 and 4 were due to the throughflow of the Kuroshio Current (Figs. 1, 2) (Gong et al. 1996). In comparison with other stations, the hydrography in Station 2 is more dynamic because upwelling events frequently occur due to the topographic elevation of subsurface Kuroshio water. During the study period in 2009, the water column exhibited approximately consistent distributions of high salinity and density, which suggested that an upwelling event occurred. However, the intrusive Kuroshio subsurface water was just lifted to a depth of 20 - 30 m. It appeared that the coverage of a thin layer of lower salinity water on the surface constrained the extension of the upwelled water (Figs. 2a - c, 3a - c). In 2010, the upper layer (at a depth of < 40 m) was occupied by less-saline water compared with that observed in 2009. The stratification of the water column, with a significant halocline (pycnocline) between depths of 40 and 45 m, was also observed (Figs. 2d - e, 3b - c). This stratification suggested that no upwelling event occurred (Figs. 3b - c). Moreover, with respect to the nutrient composition at Station 2, the surface waters were depleted in NO_3 and PO_4 during both years (Table 1, Fig. 3d). Nevertheless, in 2009, the NH_4 concentration in the surface was 2.5 μM, which was obviously higher than that of 0.6 μM in 2010 (Table 1). Additionally, to reveal the effects of upwelling events on the community composition of picophytoplankton, Station Ref, which was located far from the upwelling site, was chosen as a reference (Fig. 1). In general, the surface of Station Ref in 2009 was primarily covered by TWC water, which caused its hydrographic features to be analogous to those of Station

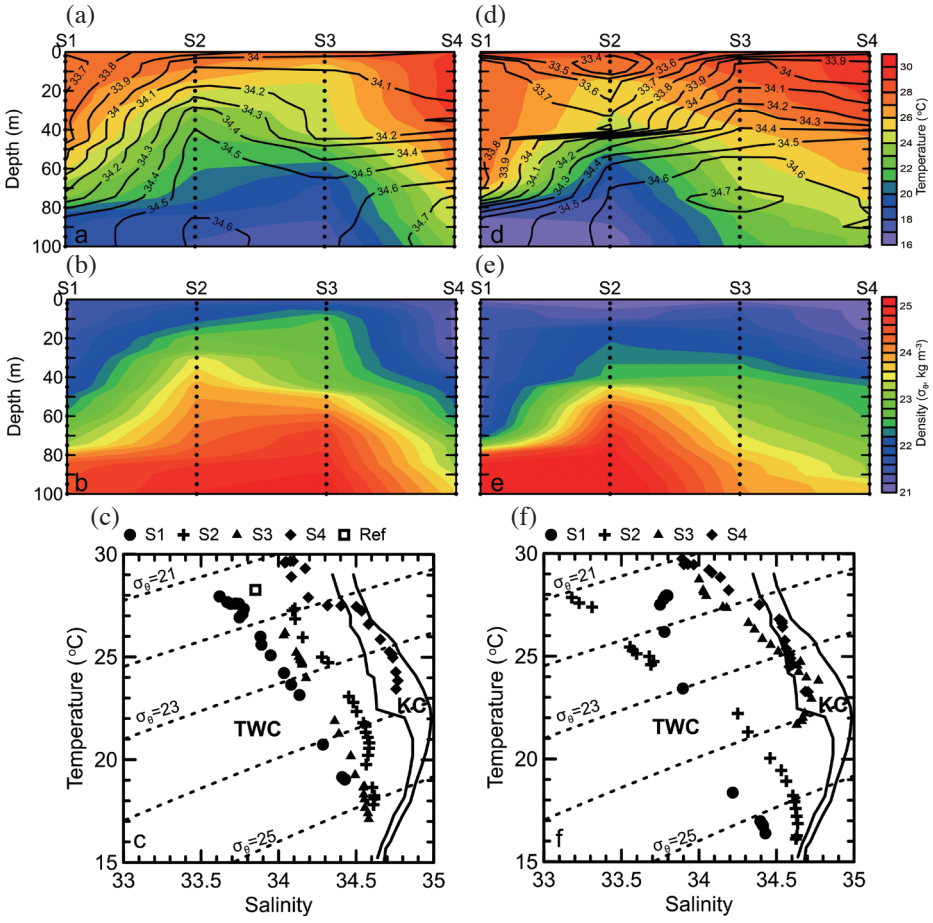

Fig. 2. Profiles of (a) (d) temperature (°C), salinity, (b) (e) water density, and (c) (f) diagrams of temperature versus salinity. The data obtained from observation stations during the summers of 2009 and 2010 are presented in panels (a) - (c) and (d) - (f), respectively. In panels (c) and (f), the characteristics of the Kuroshio Current water (KC) are denoted using solid lines. Except for the data points within the demarcation of the KC, the other points represent the hydrographic features consistent with the characteristics of the Taiwan Warm Current (TWC) (Gong et al. 1996).

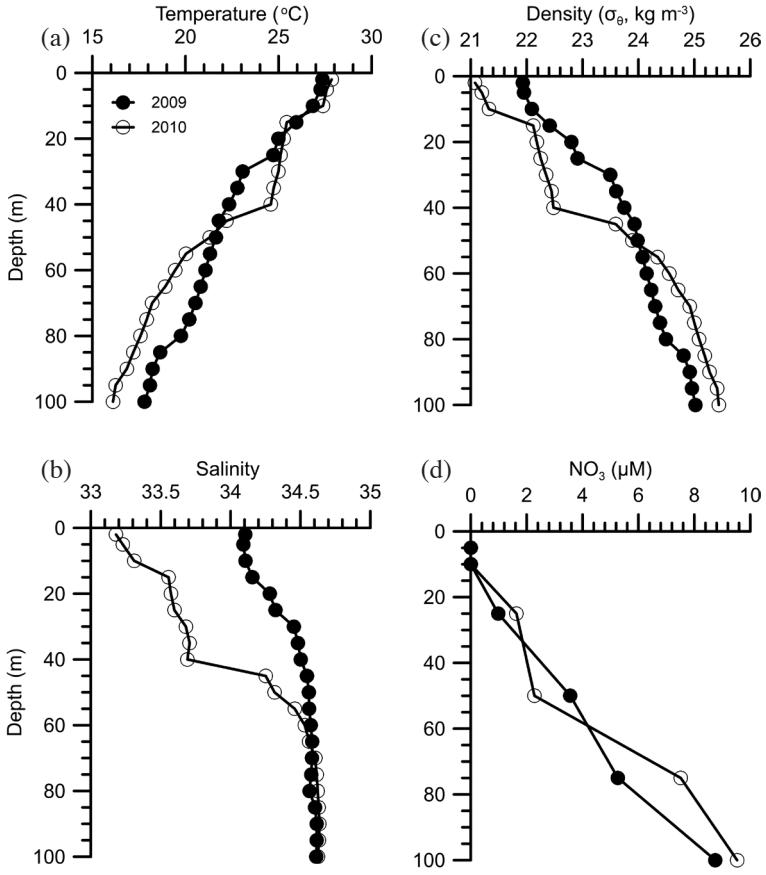

Fig. 3. Vertical distributions of (a) temperature, (b) salinity, (c) density, and (d) nitrate (NO_3) in Station 2 in the summers of 2009 (solid circles) and 2010 (opened circles).

Table 1. Hydrographic features and *Synechococcus* cell numbers in the surface waters of Stations 2 and Ref.

	Station 2		**Station Ref***
	2009	**2010**	**2009**
Temperature (°C)	27.4	27.7	28.2
Salinity	34.1	33.1	33.8
NO_3 (μM)	n.d.	n.d.	n.d.
NH_4 (μM)	2.5	0.6	0.3
PO_4 (μM)	0.06	0.04	0.04
Chlorophyll *a* (mg m^{-3})	0.16	0.34	0.24
Synechococcus (10^3 cells ml^{-1})	59	23	44

Note: n.d.: non-detectable.

**: data obtained from Chung et al. (2015).*

2 in 2009 and 2010, except it had the lowest concentration of NH_4 (Table 1, Fig. 2c).

3.2 Distribution of Prokaryotic Picoplankton

Synechococcus and *Prochlorococcus* showed diverse distribution patterns between these stations during both summers. *Synechococcus* were the predominant prokaryotic picophytoplankton species in the surface waters of all stations, except Station 4 (Figs. 4a, d). However, the abundance of these species in the surface waters of Station 2 in 2009 was 5.9×10^4 cells ml^{-1}, which was approximately twice as high as that in 2010 (Figs. 4a, d). *Prochlorococcous* primarily occupied the subsurface waters in the stations through which the Kuroshio Current passed (i.e., Stations 3 and 4) (Figs. 4b, e). Moreover, because of the uplift of subsurface Kuroshio water, abundant *Prochlorococcus* were also detected in the upper layer of Station 2 in 2009 (Fig. 4b). In addition to picocyanobacteria, heterotrophic bacteria also showed different distributions between both years. In 2009, high abundances of more than 10^6 bacteria cell ml^{-1} were observed at all stations, particularly Stations 2 and 4 (Fig. 4c). Compared to those in 2009, the number of bacteria in all stations in 2010 was less than 10^6 cells ml^{-1}, and it exhibited a constantly decreasing trend from the shelf to the open ocean (Fig. 4f).

3.3 Assemblage Composition of Prokaryotic Picoplankton

The 16S rRNA gene phylogenetic diversity was assessed in the surface waters of Station 2 to examine the effects of upwelling on prokaryotic picoplankton community dynamics. A total of 21327 and 44648 high-quality reads for the V6 to V9 region of the 16S rRNA genes were obtained in 2009 and 2010, respectively. With a cutoff of 3% sequence divergence, the reads that were defined as singletons (a total of 1258 reads, which were approximately 1.9% of the total reads) were excluded from further analyses to avoid expanding the richness and diversity estimations (Table 2). As a result, a total of 421 OTUs were obtained in 2009 and 1572 OTUs were obtained in 2010. The rarefaction curves reached the plateau phase, and the coverage of reads to OTUs was more than 98%, suggesting that the sampling efforts utilized here were sufficient for analyzing 16S rRNA diversity (Table 2, Fig. 5).

The greater observed values of the indices of richness (Chao1 and ACE) and diversity (Shannon) suggested that the surface of Station 2 in 2010 possessed a more complex assemblage composition of prokaryotic picoplankton than those of Station 2 in 2009 (Table 2). Most of the reads consisted of five bacterial orders: the photosynthetic bacterium *Synechococcus*, and the heterotrophic bacteria α-*Proteobacteria*, γ-*Proteobacteria*, *Actinobacteria*, and *Flavobacteria*. In 2009, 96% of the reads were affiliated with *Synechococcus*. Although *Synechococcus* remained the dominant species in 2010, their proportion dramatically decreased to 52% (Fig. 6). With respect to the population composition of *Synechococcus*, the reads acquired from both years were principally composed of three OTUs, namely OTU01, OTU09, and OTU13, which could be further categorized into clades II, X, and XI, respectively (Fig. 7). While the abundance of *Synechococcus* differed between 2009 and 2010, there was no change in the population composition of these bacteria. In both years, clade II (OTU01) was the most dominant subgroup, accounting for up to 96% of the total reads belonging to *Synechococcus*. However, the overall proportion of heterotrophic bacteria increased from an unnoticeable level in 2009 to 48% of the total picoplankton population in 2010. Among these bacteria, α-*Proteobacteria*, which primarily comprised *Pelagibacterales* (clade SAR11), was the predominant order, accounting for 70% of the heterotrophic bacteria (Fig. 6). Furthermore, the community composition and diversity of Station Ref in 2009 were similar to those of Station 2 in 2010 (Table 2, Fig. 6) (Chung et al. 2015).

4. DISCUSSION

Under the northeast wind that usually occurs in winter, the uplift of subsurface Kuroshio water frequently emerges in the shelf-margin of the southeastern East China Sea. In contrast, due to Ekman transport, which is driven by a strong southwest monsoon in summer, the intrusion of the oligotrophic Taiwan Warm Current (TWC) is often observed (Jan et al. 2002; Chang et al. 2010; Wu et al. 2014). In association with the development of upwelling, the intrusion of the TWC and lateral water exchange create highly dynamic hydrographic conditions and a complex ecosystem in this area (Takahashi et al. 1986; Chen 1992; Gong et al. 1997, 1999; Liu et al. 2010; Van Dongen-Vogels et al. 2012). Upwelled water is typically characterized by abundant nutrients and benefits that allow microphytoplankton to thrive. For instance, larger chain-forming diatoms (e.g., *Chaetoceros* spp. and *Skeletonema* spp.) were observed as the dominant phytoplankton species in the upwelling water examined in the study area. However, the water in the upwelled region is not always fertile; when this is the case, smaller dinoflagellates and diazotrophic cyanobacteria (i.e., *Trichodesmium* spp.) prevailed (Chen 1992; Liu et al. 2010; Chung et al. 2012). In contrast, to date, changes in the assemblage composition of picophytoplankton with the development of upwelling have rarely been investigated. *Synechococcus* are the most widely distributed picophytoplankton in global oceans. Based on the diversity of genetic markers, such as the 16S rRNA genes, the internal transcribed spacer of 16S-23S rRNA genes and *rpoC1*, as least 10 clades of phycoerythrobilin (PE)-rich *Synechococcus* have been defined. Although *Synechococcus* are usually believed to thrive in

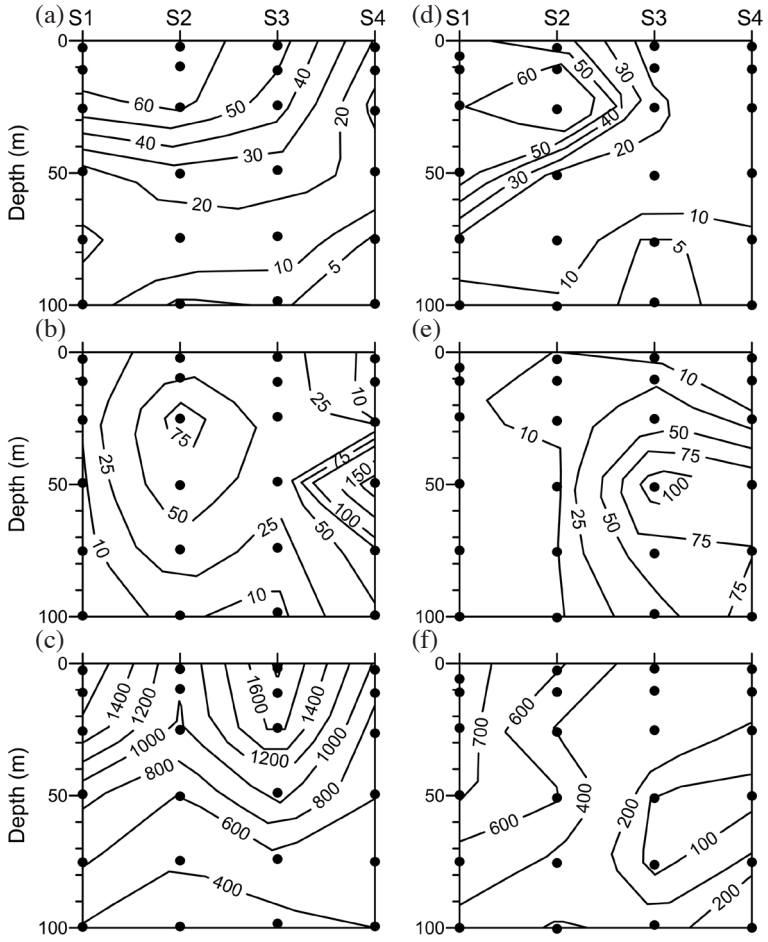

Fig. 4. Vertical distributions of (a) (d) *Synechococcus*, (b) (e) *Prochlorococcus*, and (c) (f) heterotrophic bacteria in Station 2. The cell number is presented as $\times 10^3$ cells ml^{-1}. The data obtained during the summers of 2009 and 2010 are shown in panels (a) - (c) and (d) - (f), respectively.

Table 2. Summary of pyrosequencing results, richness estimates (Chao1 and ACE) and diversity index (Shannon) for the overall 16S ribosomal RNA gene sequences present in Stations 2 and Ref (Fig. 1) in 2009 and 2010. The OTUs defined at the threshold of 3% sequence divergence. The OTUs defined as singletons were excluded in the estimation of richness and diversity.

	Station 2		**Station Ref***
	2009	**2010**	**2009**
Total Reads	21327	44648	50122
Singleton	255	1003	602
Total OTUs (without singleton)	421	1572	554
Coverage	99%	98%	99%
Chao1	180	570	607
ACE	197	578	650
Shannon	0.9	3.0	2.2

*Note: *: data obtained from Chung et al. (2015).*

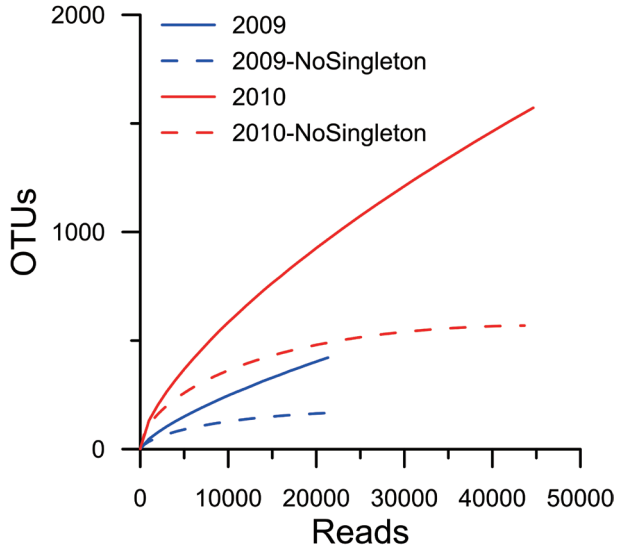

Fig. 5. Rarefaction curves inferred from the reads versus the OTUs in Station 2 during the summers of 2009 (blue lines) and 2010 (red lines). The solid and dashed lines denote all reads and the reads excluding singletons for rarefaction analysis, respectively.

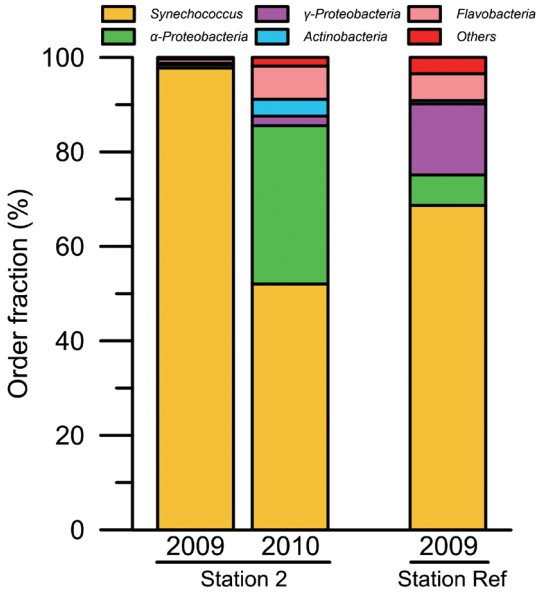

Fig. 6. Assemblages of prokaryotic picoplankton in Station 2, as indicated based on their order level, during the summers of 2009 and 2010 and in Station Ref during the summer of 2009 (Chung et al. 2015).

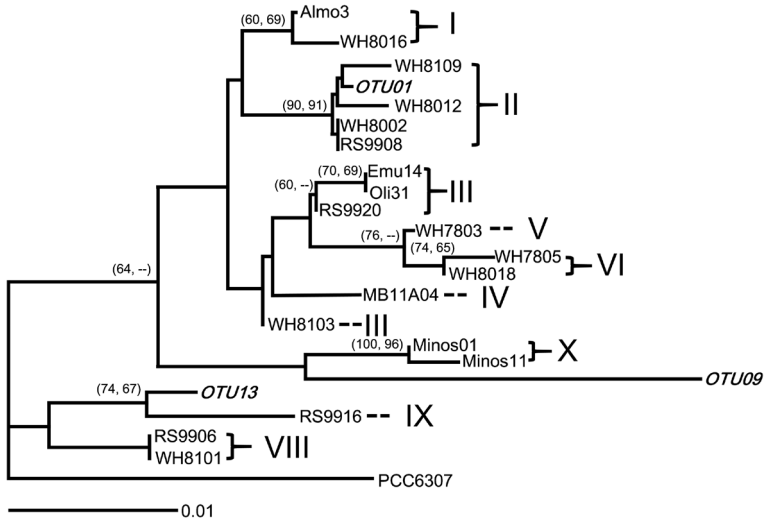

Fig. 7. Neighbor-joining phylogenetic tree inferred from the 16S rRNA gene sequences of *Synechococcus* in Station 2 during the summers of 2009 and 2010. All sequences affiliated with *Synechococcus* were further grouped into OTU01, OTU09, and OTU13 based on 3% divergence. One representative sequence of these 3 OTUs was used for the tree construction. Furthermore, all sequences of identified strains were obtained from the GenBank database. The effects of sampling error on tree inference and the stability of branch nodes were examined using neighbor-joining (NJ) and maximum likelihood (ML) bootstrap analyses, with 1000 replications. Bootstrap values of > 60% are shown at the branches (NJ, ML). The freshwater strain *Synechococcus* PCC6307 served as the root. The length of the scale bar on the lower left indicates a 0.01 nucleotide substitution unit.

oligotrophic waters, the distinct distribution of the 10 clades in different ocean systems has been revealed by their genetically diverse nature (Rocap et al. 2002; Fuller et al. 2003; Mühling et al. 2006; Zwirglmaier et al. 2007, 2008). Among all clades, the clade II lineage prevails in subtropical and tropical coasts and continental shelf waters (Zwirglmaier et al. 2007, 2008). In addition, compared with other lineages of PE-rich *Synechococcus*, the clade II lineage favors waters with higher nutrients, but excess amounts of nutrients could constrain their growth (Agawin et al. 2000a, b; Lu et al. 2001; Chung et al. 2011, 2015; Ahlgren et al. 2014). For instance, trace nutrients introduced by dust deposition could promote clade II to transiently bloom in the oligotrophic ocean, where *Prochlorococcus* dominates (Chung et al. 2011). Conversely, the injection of overabundant nutrients into the East China Sea shelf as a result of flooding limited the growth of PE-rich *Synechococcus*, the majority of which were clade II lineage (Chung et al. 2015). The results of chemical analyses indicated that all of the surface waters of Station 2 in both years and Station Ref in 2009 were nutrient-deprived due to the intrusion of TWC water; however, the hydrographic conditions of Station 2 in 2009 were more appropriate for the growth of *Synechococcus*, predominantly the clade-II lineage. In 2009, the coverage of TWC water upon the upwelling was thin, as it was constrained in the upper 20 m. Deeper nutrients were smuggled with the uplifted water and in turn mixed with the oligotrophic TWC water in the surface. Unlike other nutrient-deficient waters, this exchange of water created a low-nutrient environment that was below the limit of traditional chemical detection, but its flux was sufficient to sustain the growth of *Synechococcus*. The similar scenario was observed in the boundary region of Costa Rica upwelling dome (CRD). The exchange of lateral oligotrophic water and upwelled water in the surface CRD resulted in an environment lacking trace metals and nutrients and specifically promoted the *Synechococcus* clade-II to thrive (Ahlgren et al. 2014).

In addition to the influence of lateral water intrusion, the development of an upwelling associated with picoplankton succession should also be considered. Unfortunately, without continuous onboard observation, it is difficult to comprehensively describe hydrographic changes and phytoplankton succession throughout an upwelling process. To compensate this insufficiency, several indicators, such as "the Aging Index of Upwelling" (AIU) (Takahashi et al. 1986), have been established to define the upwelling stage, instead of continuous observation. Nevertheless, most of them only examine changes in temperature, salinity, inorganic nutrients, and dissolved oxygen, etc. The lack of dissolved organic nutrient data makes it difficult to precisely determine the stage of upwelling (Chen et al. 2004). Another evaluator, the "Degree of Nutrient Consumption" (DNC), based on the ratio of dissolved organic nutrients, particulate organic nutrients, and dissolved inorganic nutrients to assess the relative age of upwelling water was established (Chen et al. 2004). Although the DNC compensates for the insufficiency of conventional upwelling indicators, how to precisely measure all parameters for the DNC is a serious technical challenge. In contrast to other upwelling events reported in previous studies, the surface water of the upwelling occurred during the study period in 2009 was ultraoligotrophic, which lacked NO_3 and contained extreme low chlorophyll. While the dissolve inorganic nutrients were scarce, abundant NH_4 was detected in the euphotic layer. In the open oceans, NH_4 is typically derived from organic nitrogen compounds (e.g., amino acids and excretion of zooplankton). Chung et al. (2012) found large amounts of fecal pellets excreted from copepods after the decline of diatom blooms in the same study area. Correspondingly, subsequent appearance of high NH_4 concentration in the upper water column might reflect active zooplankton grazing on microphytoplankton. It also implied that the upwelling in 2009 should be in the aging stage. Furthermore, we suggest that the NH_4 concentration can serve as a complimentary factor, combined with the information obtained from traditional upwelling indicators, for evaluating the development stage of upwelling.

The recent result obtained from a global survey of genes used for the assimilation of the organic compounds in picocyanobacteria directly indicated that the *Synechococcus* clade-II prefer to take up amino acids and NH_4 for use in biogenesis (Yelton et al. 2016). During the study period in 2009, besides excretion of zooplankton, the diazotrophic cyanobacteria, *Trichodesmium*, usually thriving in the Kuroshio Current in summer, might provide considerable amount of NH_4, (Chen et al. 2008; Chung et al. 2012; Wu et al. 2018). Additionally, the study area is an excellent fishing ground, and the contribution of fish excrement to the organic nitrogen inventory cannot be ignored. All of above were possible sources to provide enough NH_4 to sustain the growth of the *Synechococcus* clade II in the surface of Station 2 in 2009.

During the study period in 2010, a severe flood occurred in the basin of the Changjiang River. Whereas abundant freshwater injected nutrient-rich terrestrial material into the East China Sea and thus enhancing the primary production and causing the changes in the picoplankton community composition, it did not affect the hydrography of the southern East China Sea (Gong et al. 2011; Chung et al. 2014, 2015). Based on the scarce nutrients in the surface water and other hydrographic data, the surface in this area was covered by TWC water. Although *Synechococcus* (i.e., clade II) still dominated the surface water, the proportion of these bacteria obviously decreased to approximately 50%. The other half of the picoplankton community was principally replaced by *α-Proteobacteria* (i.e., *Pelagibacterales* clade SAR11). Analogous hydrographic features and community structures of picoplankton in the same area has

also been described by Yeh et al. (2015). Compared with other heterotrophic bacteria, the SAR11 clade is more versatile (e.g., smaller cell size, slower growth rate and having proteorhodopsin for ATP production) at acclimatizing to an oligotrophic environment (Karl 2007; Fuhrman 2009; Chung et al. 2015). It is challenging to accurately determine nutrient concentrations using chemical analysis methods in oligotrophic oceans. Therefore, the alternating appearance patterns of *Synechococcus* and SAR 11 in water may be able to serve as a substitute index to reflect the real-time hydrographic conditions.

Because picophytoplankton are too small to sink independently or to be grazed by fecal pellet-producing zooplankton, most of their carbon export is limited in the microbial cycle via the processes of protist grazing and virus lysis (Michaels and Silver 1988; Karl 2007; Jiao et al. 2010). While there were some studies indicated that picophytoplankton cells could directly incorporate into sinking aggregates to the deep ocean, their contribution were less than 10% of total particulate carbon flux (Richardson and Jackson 2007; Stukel et al. 2013; De Martini et al. 2018). Most organic carbon produced by picophytoplankton might be utilized and recycled in the microbial loop in the euphotic zone. In the surface of the study area, *Synechococcus* contributed up to 40% of total chlorophyll *a*, which was calculated by the conversion factor 1.1 fg chlorophyll *a* cell^{-1} (Morel et al. 1993). In the past, relative research works conducted in the upwelling area have focused on the contribution of larger phytoplankton to the carbon cycle. While picophytoplankton have a significant contribution to the chlorophyll concentration in the upwelling province, their importance was seldom elucidated (Stukel et al. 2013; Ahlgren et al. 2014). Therefore, in order to completely describe the carbon cycle in this dynamic ecosystem, the importance of picophytoplankton have to be revealed in the future.

With upwelling attributable to the intrusion of the Kuroshio Current usually occurring in this area, the hydrography is highly dynamic and associated with the development of upwelling events. Overall, the surface warming rate of subtropical western boundary currents (WBC), including the Kuroshio Current, in the past century has been two to three times faster than the global mean surface ocean warming rate. In the context of global climate change, this accelerated warming phenomenon can affect the flow direction and strength of WBC, thereby changing the ecosystems around them (Wu et al. 2012, 2016; Yang et al. 2016). Prokaryotic picophytoplankton are highly susceptible to, and their community assemblages are responsible for, environmental changes, and they are thus better than other biogeochemical parameters (Zwirglmaier et al. 2007, 2008). Our observations are also consistent with this argument. Therefore, the community assemblages of picophytoplankton around the Kuroshio are important indicators of changes in this environment. However, in order to comprehensively describe these conditions, it is necessary to obtain more information (e.g., hydrography changes and succession of phytoplankton community) from continuous on board observation. This information will also facilitate a complete evaluation of the impact of global environment changes on the upwelling ecosystem and its fishery resources.

Acknowledgements We deeply appreciate the assistance received from the captain and crew of the research vessel 'Ocean Researcher I'. We also thank the National Center for High-performance Computing (NCHC) of the National Applied Research Laboratories (NARLabs) of Taiwan and the National Research Program for Biopharmaceuticals (NRPB, MOST 104-2325-B-492-001). This study was financially supported by the Ministry of Science and Technology of Taiwan (MOST 104-2611-M-019-011).

REFERENCES

Agawin, N. S. R., C. M. Duarte, and S. Agustí, 2000a: Response of Mediterranean *Synechococcus* growth and loss rates to experimental nutrient inputs. *Mar. Ecol. Prog. Ser.*, **206**, 97-106, doi: 10.3354/meps206097. [Link]

Agawin, N. S. R., C. M. Duarte, and S. Agustí, 2000b: Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. *Limnol. Oceanogr.*, **45**, 591-600, doi: 10.4319/lo.2000.45.3.0591. [Link]

Ahlgren, N. A., A. Noble, A. P. Patton, K. Roache-Johnson, L. Jackson, D. Robinson, C. McKay, L. R. Moore, M. A. Saito, and G. Rocap, 2014: The unique trace metal and mixed layer conditions of the Costa Rica upwelling dome support a distinct and dense community of *Synechococcus*. *Limnol. Oceanogr.*, **59**, 2166-2184, doi: 10.4319/lo.2014.59.6.2166. [Link]

Chang, Y.-L., L.-Y. Oey, C.-R. Wu, and H.-F. Lu, 2010: Why are there upwellings on the northern shelf of Taiwan under northeasterly winds? *J. Phys. Oceanogr.*, **40**, 1405-1417, doi: 10.1175/2010JPO4348.1. [Link]

Chen, C.-T. A., L.-Y. Hsing, C.-L. Liu, and S.-L. Wang, 2004: Degree of nutrient consumption of upwelled water in the Taiwan Strait based on dissolved organic phosphorus or nitrogen. *Mar. Chem.*, **87**, 73-86, doi: 10.1016/j.marchem.2004.01.006. [Link]

Chen, Y.-L. L., 1992: Summer phytoplankton community structure in the Kuroshio current-related upwelling northeast of Taiwan. *Terr. Atmos. Ocean. Sci.*, **3**, 305-319, doi: 10.3319/TAO.1992.3.3.305(KEEP). [Link]

Chen, Y.-L. L., H.-Y. Chen, S. Tuo, and K. Ohki, 2008: Seasonal dynamics of new production from *Trichodesmium* N_2 fixation and nitrate uptake in the upstream Kuroshio and South China Sea basin. *Limnol. Oceanogr.*, **53**, 1705-1721, doi: 10.4319/lo.2008.53.5.1705. [Link]

Chisholm, S. W., R. J. Olson, E. R. Zettler, R. Goericke,

J. B. Waterbury, and N. A. Welschmeyer, 1988: A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature*, **334**, 340-343, doi: 10.1038/334340a0. [Link]

Chung, C.-C., J. Chang, G.-C. Gong, S.-C. Hsu, K.-P. Chiang, and C.-W. Liao, 2011: Effects of Asian dust storms on *Synechococcus* populations in the subtropical Kuroshio Current. *Mar. Biotechnol.*, **13**, 751-763, doi: 10.1007/s10126-010-9336-5. [Link]

Chung, C.-C., G.-C. Gong, and C.-C. Hung, 2012: Effect of Typhoon Morakot on microphytoplankton population dynamics in the subtropical Northwest Pacific. *Mar. Ecol. Prog. Ser.*, **448**, 39-49, doi: 10.3354/meps09490. [Link]

Chung, C.-C., C.-Y. Huang, G.-C. Gong, and Y.-C. Lin, 2014: Influence of the Changjiang River flood on *Synechococcus* ecology in the surface waters of the East China Sea. *Microb. Ecol.*, **67**, 273-285, doi: 10.1007/s00248-013-0299-8. [Link]

Chung, C.-C., G.-C. Gong, C.-Y. Huang, J.-Y. Lin, and Y.-C. Lin, 2015: Changes in the *Synechococcus* assemblage composition at the surface of the East China Sea due to flooding of the Changjiang River. *Microb. Ecol.*, **70**, 677-688, doi: 10.1007/s00248-015-0608-5. [Link]

De Martini, F., S. Neuer, D. Hamill, J. Robidart, and M. W. Lomas, 2018: Clade and strain specific contributions of *Synechococcus* and *Prochlorococcus* to carbon export in the Sargasso Sea. *Limnol. Oceanogr.*, **63**, S448-S457, doi: 10.1002/lno.10765. [Link]

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight, 2011: UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, **27**, 2194-2200, doi: 10.1093/bioinformatics/btr381. [Link]

Fuhrman, J. A., 2009: Microbial community structure and its functional implications. *Nature*, **459**, 193-199, doi: 10.1038/nature08058. [Link]

Fuller, N. J., D. Marie, F. Partensky, D. Vaulot, A. F. Post, and D. J. Scanlan, 2003: Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea. *Appl. Environ. Microbiol.*, **69**, 2430-2443, doi: 10.1128/AEM.69.5.2430-2443.2003. [Link]

Goericke, R. and N. A. Welschmeyer, 1993: The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. *Deep-Sea Res. Part I-Oceanogr. Res. Pap.*, **40**, 2283-2294, doi: 10.1016/0967-0637(93)90104-B. [Link]

Gong, G.-C., Y.-L. L. Chen, and K.-K. Liu, 1996: Chemical hydrography and chlorophyll *a* distribution in the East China Sea in summer: Implications in nutrient dynamics. *Cont. Shelf Res.*, **16**, 1561-1590, doi: 10.1016/0278-4343(96)00005-2. [Link]

Gong, G.-C., F.-K. Shiah, K.-K. Liu, W.-S. Chuang, and J. Chang, 1997: Effect of the Kuroshio intrusion on the chlorophyll distribution in the southern East China Sea during spring 1993. *Cont. Shelf Res.*, **17**, 79-94, doi: 10.1016/0278-4343(96)00022-2. [Link]

Gong, G.-C., J. Chang, and Y.-H. Wen, 1999: Estimation of annual primary production in the Kuroshio waters northeast of Taiwan using a photosynthesis-irradiance model. *Deep-Sea Res. Part I-Oceanogr. Res. Pap.*, **46**, 93-108, doi: 10.1016/S0967-0637(98)00057-0. [Link]

Gong, G.-C., F.-K. Shiah, K.-K. Liu, Y.-H. Wen, and M.-H. Liang, 2000: Spatial and temporal variation of chlorophyll a, primary productivity and chemical hydrography in the southern East China Sea. *Cont. Shelf Res.*, **20**, 411-436, doi: 10.1016/S0278-4343(99)00079-5. [Link]

Gong, G.-C., Y.-H. Wen, B.-W. Wang, and G.-J. Liu, 2003: Seasonal variation of chlorophyll a concentration, primary production and environmental conditions in the subtropical East China Sea. *Deep-Sea Res. Part II-Top. Stud. Oceanogr.*, **50**, 1219-1236, doi: 10.1016/S0967-0645(03)00019-5. [Link]

Gong, G.-C., J. Chang, K.-P. Chiang, T.-M. Hsiung, C.-C. Hung, S.-W. Duan, and L. A. Codispoti, 2006: Reduction of primary production and changing of nutrient ratio in the East China Sea: Effect of the Three Gorges Dam? *Geophys. Res. Lett.*, **33**, L07610, doi: 10.1029/2006GL025800. [Link]

Gong, G.-C., K.-K. Liu, K.-P. Chiang, T.-M. Hsiung, J. Chang, C.-C. Chen, C.-C. Hung, W.-C. Chou, C.-C. Chung, H.-Y. Chen, F.-K. Shiah, A.-Y. Tsai, C. Hsieh, J.-C. Shiao, C.-M. Tseng, S.-C. Hsu, H.-J. Lee, M.-A. Lee, I.-I. Lin, and F. Tsai, 2011: Yangtze River floods enhance coastal ocean phytoplankton biomass and potential fish production. *Geophys. Res. Lett.*, **38**, L13603, doi: 10.1029/2011GL047519. [Link]

Jan, S., J. Wang, C.-S. Chern, and S.-Y. Chao, 2002: Seasonal variation of the circulation in the Taiwan Strait. *J. Mar. Syst.*, **35**, 249-268, doi: 10.1016/S0924-7963(02)00130-6. [Link]

Jiao, N., G. J. Herndl, D. A. Hansell, R. Benner, G. Kattner, S. W. Wilhelm, D. L. Kirchman, M. G. Weinbauer, T. Luo, F. Chen, and F. Azam, 2010: Microbial production of recalcitrant dissolved organic matter: Long-term carbon storage in the global ocean. *Nat. Rev. Microbiol.*, **8**, 593-599, doi: 10.1038/nrmicro2386. [Link]

Karl, D. M., 2007: Microbial oceanography: Paradigms, processes and promise. *Nat. Rev. Microbiol.*, **5**, 759-769, doi: 10.1038/nrmicro1749. [Link]

Kwon, Y.-O., M. A. Alexander, N. A. Bond, C. Frankignoul, H. Nakamura, B. Qiu, and L. A. Thompson, 2010: Role of the gulf stream and Kuroshio–Oyashio systems in large-scale atmosphere–ocean interaction: A review. *J. Climate*, **23**, 3249-3281, doi: 10.1175/2010JCLI3343.1. [Link]

Liu, H., L. Campbell, and M. R. Landry, 1995: Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. *Mar. Ecol. Prog. Ser.*, **116**, 277-287, doi: 10.3354/meps116277. [Link]

Liu, H., H. A. Nolla, and L. Campbell, 1997: *Prochlorococcus* growth rate and contribution to primary production in the equatorial and subtropical North Pacific Ocean. *Aquat. Microb. Ecol.*, **12**, 39-47, doi: 10.3354/ame012039. [Link]

Liu, H.-C., G.-C. Gong, and J. Chang, 2010: Lateral water exchange between shelf-margin upwelling and Kuroshio waters influences phosphorus stress in microphytoplankton. *Mar. Ecol. Prog. Ser.*, **409**, 121-130, doi: 10.3354/meps08603. [Link]

Lu, J., F. Chen, and R. E. Hodson, 2001: Distribution, isolation, host specificity, and diversity of cyanophages infecting marine *Synechococcus* spp. in river estuaries. *Appl. Environ. Microbiol.*, **67**, 3285-3290, doi: 10.1128/AEM.67.7.3285-3290.2001. [Link]

Mackey, K. R. M., C.-T. Chien, and A. Paytan, 2014: Microbial and biogeochemical responses to projected future nitrate enrichment in the California upwelling system. *Front. Microbiol.*, **5**, doi: 10.3389/fmicb.2014.00632. [Link]

Malone, T. C., 1971: The relative importance of nannoplankton and netplankton as primary producers in the California Current system. *Fish. Bull.*, **69**, 799-820.

Michaels, A. F. and M. W. Silver, 1988: Primary production, sinking fluxes and the microbial food web. *Deep-Sea Res. Part I-Oceanogr. Res. Pap.*, **35**, 473-490, doi: 10.1016/0198-0149(88)90126-4. [Link]

Morel, A., Y.-H. Ahn, F. Partensky, D. Vaulot, and H. Claustre, 1993: *Prochlorococcus* and *Synechococccus*: A comparative study of their optical properties in relation to their size and pigmentation. *J. Mar. Res.*, **51**, 617-649, doi: 10.1357/0022240933223963. [Link]

Mühling, M., N. J. Fuller, P. J. Somerfield, A. F. Post, W.-H. Wilson, D. J. Scanlan, I. Joint, and N. H. Mann, 2006: High resolution genetic diversity studies of marine *Synechococcus* isolates using *rpoC1*-based restriction fragment length polymorphism. *Aquat. Microb. Ecol.*, **45**, 263-275, doi: 10.3354/ame045263. [Link]

Ngugi, D. K., A. Antunes, A. Brune, and U. Stingl, 2012: Biogeography of pelagic bacterioplankton across an antagonistic temperature-salinity gradient in the Red Sea. *Mol. Ecol.*, **21**, 388-405, doi: 10.1111/j.1365-294X.2011.05378.x. [Link]

Partensky, F., J. Blanchot, F. Lantoine, J. Neveux, and D. Marie, 1996: Vertical structure of picophytoplankton at different trophic sites of the tropical northeastern Atlantic Ocean. *Deep-Sea Res. Part I-Oceanogr. Res. Pap.*, **43**, 1191-1213, doi: 10.1016/0967-0637(96)00056-8. [Link]

Quince, C., A. Lanzén, T. P. Curtis, R. J. Davenport, N. Hall, I. M. Head, L. F. Read, and W. T. Sloan, 2009: Accurate determination of microbial diversity from 454 pyrosequencing data. *Nat. Methods*, **6**, 639-641, doi: 10.1038/nmeth.1361. [Link]

Richardson, T. L. and G. A. Jackson, 2007: Small phytoplankton and carbon export from the surface ocean. *Science*, **315**, 838-840, doi: 10.1126/science.1133471. [Link]

Rocap, G., D. L. Distel, J. B. Waterbury, and S. W. Chisholm, 2002: Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. *Appl. Environ. Microbiol.*, **68**, 1180-1191, doi: 10.1128/AEM.68.3.1180-1191.2002. [Link]

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. van Horn, and C. F. Weber, 2009: Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.*, **75**, 7537-7541, doi: 10.1128/AEM.01541-09. [Link]

Schloss, P. D., D. Gevers, and S. L. Westcott, 2011: Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS ONE*, **6**, e27310, doi: 10.1371/journal.pone.0027310. [Link]

Sievers, F., A. Wilm, D. Dineen, T. J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert, J. Söding, J. D. Thompson, and D. G. Higgins, 2011: Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.*, **7**, doi: 10.1038/msb.2011.75. [Link]

Stukel, M. R., M. Décima, K. E. Selph, D. A. A. Taniguchi, and M. R. Landry, 2013: The role of *Synechococcus* in vertical flux in the Costa Rica upwelling dome. *Prog. Oceanogr.*, **112-113**, 49-59, doi: 10.1016/j.pocean.2013.04.003. [Link]

Takahashi, M., J. Ishizaka, T. Ishimaru, L. P. Atkinson, T. N. Lee, Y. Yamaguchi, Y. Fujita, and S. Ichimura, 1986: Temporal change in nutrient concentrations and phytoplankton biomass in short time scale local upwelling around the Izu Peninsula, Japan. *J. Plankton Res.*, **8**, 1039-1049, doi: 10.1093/plankt/8.6.1039. [Link]

Van Dongen-Vogels, V., J. R. Seymour, J. F. Middleton, J. G. Mitchell, and L. Seuront, 2012: Shifts in picophytoplankton community structure influenced by changing upwelling conditions. Estuar. *Coast. Shelf Sci.*, **109**, 81-90, doi: 10.1016/j.ecss.2012.05.026. [Link]

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole, 2007: Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.*, **73**, 5261-5267, doi:

10.1128/AEM.00062-07. [Link]

Waterbury, J. B., S. W. Watson, R. R. L. Guillard, and L. E. Brand, 1979: Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. *Nature*, **277**, 293-294, doi: 10.1038/277293a0. [Link]

Wong, G. T. F., S.-Y. Chao, Y.-H. Li, and F.-K. Shiah, 2000: The Kuroshio edge exchange processes (KEEP) study — an introduction to hypotheses and highlights. *Cont. Shelf Res.*, **20**, 335-347, doi: 10.1016/S0278-4343(99)00075-8. [Link]

Wu, C., F.-X. Fu, J. Sun, S. Thangaraj, and L. Pujari, 2018: Nitrogen Fixation by *Trichodesmium* and unicellular diazotrophs in the northern South China Sea and the Kuroshio in summer. *Sci. Rep.*, **8**, doi: 10.1038/s41598-018-20743-0. [Link]

Wu, C.-R., Y.-C. Hsin, T.-L. Chiang, Y.-F. Lin, and I.-F. Tsui, 2014: Seasonal and interannual changes of the Kuroshio intrusion onto the East China Sea shelf. *J. Geophys. Res.*, **119**, 5039-5051, doi: 10.1002/2013JC009748. [Link]

Wu, C.-R., Y.-L. Wang, Y.-F. Lin, T.-L. Chiang, and C.-C. Wu, 2016: Weakening of the Kuroshio intrusion into the South China Sea under the global warming hiatus. *IEEE J. Selected Topics Appl. Earth Observations Rem. Sens.*, **9**, 5064-5070, doi: 10.1109/JSTARS.2016.2574941. [Link]

Wu, L., W. Cai, L. Zhang, H. Nakamura, A. Timmermann, T. Joyce, M. J. McPhaden, M. Alexander, B. Qiu, M. Visbeck, P. Chang, and B. Giese, 2012: Enhanced warming over the global subtropical western boundary currents. *Nat. Clim. Change*, **2**, 161-166, doi: 10.1038/NCLIMATE1353. [Link]

Yang, H., G. Lohmann, W. Wei, M. Dima, M. Ionita, and J. Liu, 2016: Intensification and poleward shift of subtropical western boundary currents in a warming climate. *J. Geophys. Res.*, **121**, 4928-4945, doi: 10.1002/2015JC011513. [Link]

Yeh, Y.-C., P. R. Peres-Neto, S.-W. Huang, Y.-C. Lai, C.-Y. Tu, F.-K. Shiah, G.-C. Gong, and C. Hsieh, 2015: Determinism of bacterial metacommunity dynamics in the southern East China Sea varies depending on hydrography. *Ecography*, **38**, 198-212, doi: 10.1111/ecog.00986. [Link]

Yelton, A. P., S. G. Acinas, S. Sunagawa, P. Bork, C. Pedrós-Alió, and S. W. Chisholm, 2016: Global genetic capacity for mixotrophy in marine picocyanobacteria. *The ISME Journal*, **10**, 2946-2957, doi: 10.1038/ismej.2016.64. [Link]

Zwirglmaier, K., J. L. Heywood, K. Chamberlain, E. M. S. Woodward, M. V. Zubkov, and D. J. Scanlan, 2007: Basin-scale distribution patterns of picocyanobacterial lineages in the Atlantic Ocean. *Environ. Microbiol.*, **9**, 1278-1290, doi: 10.1111/j.1462-2920.2007.01246.x. [Link]

Zwirglmaier, K., L. Jardillier, M. Ostrowski, S. Mazard, L. Garczarek, D. Vaulot, F. Not, R. Massana, O. Ulloa, and D. J. Scanlan, 2008: Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. *Environ. Microbiol.*, **10**, 147-161, doi: 10.1111/j.1462-2920.2007.01440.x. [Link]