Thesis for the Degree of Master of Engineering

Analysis of the microbial community changes in Jaran bay by the metagenomics platform

by

Sun Young Yoon

Interdisciplinary Program of Biomedical Mechanical & Electrical Engineering

The Graduate School

Pukyong National University

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(메타지노믹스 플랫폼을 이용한 자란만 내 미생물 군집 변화 분석)

Advisor: Prof. Hyun-Woo Kim

by

Sun Young Yoon

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Approved by :

(Chairman) Dr. Chul-Woong Oh

(Member) Dr. Kyeong-dong Park

(Member) Dr. Hyun-Woo Kim

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Abstract

A microbial community represents the environmental conditions, which is the result of complicate interactions of living organisms and the physical and chemical environmental factors. In order to investigate the environmental conditions in Jaran bay, a regional and seasonal variations of autotrophic phytoplankton and heterotrophic bacterial communities were studied using Miseq platform. Water temperature and salinity measured in five stations ranged between 5.1℃ and 29.4℃ and between 28.5 psu and 33.3 psu, respectively. Concentration of Chlorophyll *a* (Chl *a*) was between 0.1015 μgL^{-1} and 6.4974 μgL^{-1}. The ratio of phytoplankton to total microbial community were determined by qPCR and its value was lowest in February and highest in April. NGS data showed that the increased phytoplankton ratio in April was mainly due to the increased pico-sized chlorophyte, *Ostreococcus tauri*. NGS data from two stations, St.1 and St.5 in May, showed that community structures between two stations were highly similar to each other and difference in phytoplankton ratio may have come from the relatively lower amount of *O. tauri* in St.1 compared to St.5. This

result showed that changes in community structures in Jaran bay may have come from the seasonal factors, not from the regional factors. In conclusion, we were able to know that the microbial communities may provide an important information about the environmental conditions and a long-term surveys should be made to understand the various environmental events such as algal bloom, hypoxia, and thermal stress and following effects on the ecosystem in Jaran bay.

Introduction

The Jaran Bay is located in the eastern coast of the South Sea of Korean peninsula and five rivers supply nutrients to the bay to provide a favorable environment for the growth of primary producers. Therefore, there are high numbers of the aquaculture farms for the oyster, the sea squirt, and several fin fish. Among the cultured species, the oyster is major product in the bay and Jaran Bay is designated as a production area of shellfish for export (MIFAFF, 2008). Recently production of oyster in the bay is decreasing whose reason is still not clearly understood yet and the scientific strategy for the sustainable use of the Jaran bay.

In fact, several studies on the physical factors including temperature, salinity or light intensity (Han, 2017) or chemical factors such as dissolved inorganic nutrients (Jeong, 2017; Kwon et al., 2014) have been made in the bay. A study on the phytoplankton community in the bay was also conducted by the chromatography (HPLC) (Han, 2017). However, those traditional measurements may not provide the useful information enough to explain the ecological or environmental factors for the decreased oyster productivity in the Jaran Bay.

Recently, metagenomic methods using the next-generation sequencing (NGS) are used to analyze the microbial community (Howard et al., 2011; Li et al., 2011; Margulies et al., 2005; Wegley et al., 2007). This cost-effective strategy

enables researchers to analyze thousands of environmental water samples simultaneously producing a large amount of microbial community data. This data have profoundly changed ways of ecological studies in aquatic environment and metagenomic approaches are now not only limited to know the microbial biodiversity as in early introduction stage (Andersson et al., 2010). Since they are the result of complicate interactions between the numerous microorganisms and environmental factors, the microbial communities provide considerable information about the environmental and ecological conditions. In fact, several metagenomic studies are being introduced to understand the relationship between the microbial community and the environmental conditions including salinity, oxygen profile, or nitrogen cycle (Dupont et al., 2014; Thureborn et al., 2013).

The most widely used microbial metagenomics is the PCR-based massive sequencing using the universal primer set. Molecular techniques are currently being applied to improve results of phytoplankton community analysis. The most widely used DNA markers contain two protein-coding genes including protein D1 of photosystem-II reaction center (psbA) and a large subunit of the ribulose-1,5-diphophate carxoylase/oxygenase (rbcL) or plastid 16S rDNA region (Kirkham et al., 2013; Man-Aharonovich et al., 2010; McDonald et al., 2007; Paul et al., 2000; Zeidner et al., 2003). Particularly, the 16S rDNA sequence has been well recorded in the GenBank database (http://www.ncbi.nlm.nih.gov/) and more than 6,490

phytoplanktonic 16S sequences are currently accumulated in the PhytoREF database (http://phytoref.org). In spite of constant debate, universal primers targeting 16S rDNA are now most widely used (Decelle et al., 2015; Herlemann et al., 2011). However, it has been hard to design a universal primer set that will amplify all phytoplankton taxa from cyanobacteria to eukaryotic algae in the 16S rDNA region, and most studies have analyzed specific taxonomic groups, especially for the bacterial communities (Asudi et al., 2016; Cruaud et al., 2014; Kitamura et al., 2016; Le Bescot et al., 2016; Logares et al., 2014; Massana et al., 2015; Valenzuela-González et al., 2016; Vierheilig et al., 2015). Although the sequence information from the 23S rDNA region is only a subset of that for 16S rDNA in the database, this region is regarded as an important marker for phytoplankton community structure when designing better universal primer sets that will cover most phytoplankton taxa (Folmer et al., 1994; Sherwood and Presting, 2007).

In this study, changes in the autotrophic phytoplankton and heterotrophic bacterial communities were analyzed by the NGS to understand their correlations with either ecological or environmental conditions in Jaran bay. We here especially concentrated on the correlations between photosynthetic phytoplankton and heterotrophic bacterial communities.

Material and Method

1. Sample collection

Surface water samples were collected from five sample stations (from St.1 to St.5) monthly from December 2016 to August 2017 in the coast of Jaran bay, Gosung, Korea (Fig. 1). Sample collection was performed as the part of a project titled “environmental carrying capacity for mariculture in Jaran bay”, which was funded by the National Institute of Fisheries Science (NIFS) from 2016 to 2017.

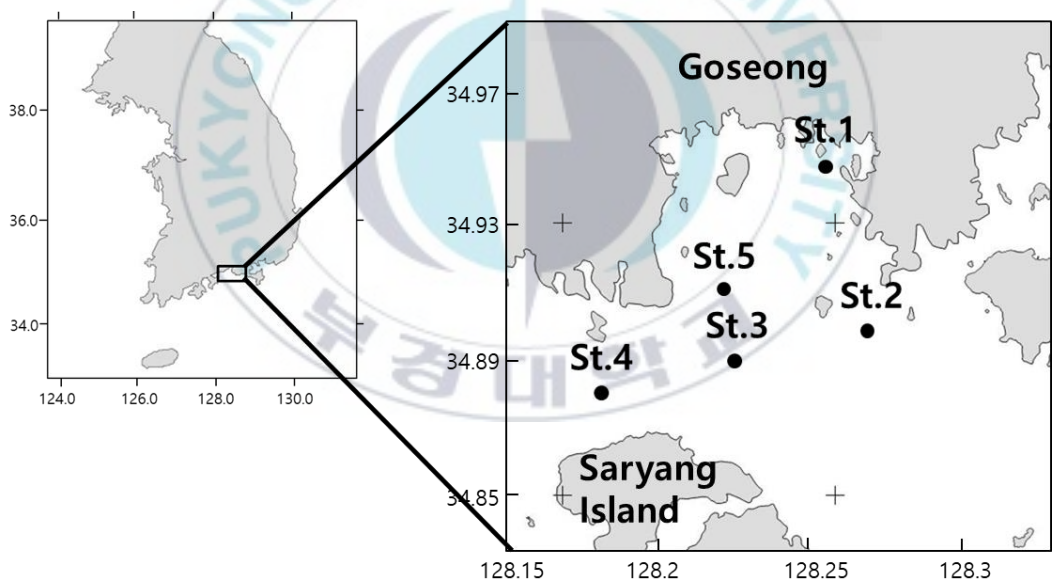

Fig. 1. Map of the sampling stations in Jaran Bay, Korea (St.1: 34°55′48.49″N 128°15′12.64″E; St.2: 34°53′16.8″N 128°15′54″E; St.3: 34°52′42.6″N 128°13′16.68″E; St.4: 34°52′ 8.4″N 128°10′51.51.6″E; St.5: 34°53′44.57″N 128°13′ 5.02″E)

2. Environmental factors (Temperature, Salinity, Concentration of Chl *a*, Amount of precipitation)

Water temperature and salinity were measured using an SBE-43 unit (Sea-Bird Electronics, USA). Water sample (3 L) was collected from each sample station and directly put into the ice until used in the lab. 500 ml of water samples was used for the quantification of Chlorophyll-a (Chl*a*). Chl*a* was measured by spectrophotometry according to the previous study (Jeong, 2017). Briefly, water sample was filtered using a 0.45-μm GN6 membrane filter (Pall Corporation, USA). After 10 ml of 90 % acetone (Sigma-Aldrich, German) was added, the membrane filter was vigorously stirred by vortex (Daeiltech, Korea). After 24 hours of incubation at 4°C, the supernatant was used for spectrophotometry (DU 730, USA) as a result of centrifugation at 2,000 rpm for 10 minutes by 5810R centrifuge (Eppendorf, German). Absorbance was measured at 630 nm, 645 nm, 665 nm and 750 nm. Chl*a* concentration was calculated by Scor-Unesco (Humphrey and Wootton, 1966). Monthly precipitation data were obtained from the Meteorological Administration (KMA, http://www.kma.go.kr).

3. Genomic DNA extraction and qPCR

Genomic DNA from was extracted using a DNeasy ® plant mini kit (Qiagen, Germany) following the manufacturer's instructions. One hundred milliliter of water was filtered using a 0.45-μm GN6 membrane filter with three replications. The filter was put into 650 μL lysis buffer, cut into smaller pieces using scissors, and homogenized using Tissuelyzer II (Qiagen Korea, Korea). The homogenized samples were then incubated at 65 °C for 2 hours. Extracted genomic DNA was quantified and qualified using a NanoDrop spectrophotometer ND-1000 (Thermo Scientific, USA) and was stored at −80°C until it was used for further analysis.

Copy numbers of total microorganisms and photosynthetic phytoplankton were measured by qPCR. Two universal primer sets (16S for total microorganisms and 23S for photosynthetic phytoplankton) were used (Table 1). The qPCR reaction mixture (20 μL) contained 4 μL of template, 1 μL of each primer (10 pmol), 4 μL of D.W., 10 μL SYBR. qPCR was performed by Chromo4 thermocycler (Bio-Rad, USA) following cycling conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. Standard curve for each primer set was drawn as shown in the previous study (Medler and Mykles, 2003; Yu and Mykles, 2003).

4. NGS analysis

4.1. Library preparation and sequencing

Equal amount of triplicated genomic DNA samples in each sample were pooled together for the library construction. A Nextera XT index kit (Illumina, USA) was used for constructing the libraries for sequencing (Illumina, USA). To conduct library preparation, the PCR amplifications by two times PCR were performed under the following cycling conditions: initial denaturation at 94°C for 3 min, followed by cycles resulted from such samples at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 3 min (Herlemann et al., 2011; Yoon et al., 2016). The amplicon was purified using the AccuPrep®Gel Purification Kit (Bioneer, Korea) and eluted with 21 μL TE buffer. Each sample ordered triplet arrangement was pooled. Firstly, PCR amplifications were performed with three primer sets with overhanging adapter sequences of 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3' and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3' on forward and reverse primers, respectively (Table 1). A library was constructed from the NGS data using the TruSeq®Sample Preparation kit V2 (Illumina, USA). The quality and quantity of the library were measured using a 2100 Bioanalyzer (Agilent

Technologies, Santa Clara, CA, USA) and sequencing was performed using Illumina MiSeq (2 X 300 bp pair-ends) (Illumina, USA).

4.2. Bioinformatics analysis NGS data

Raw MiSeq reads were imported into CLC Genomic Workbench v.8.0 (CLC Bio, USA). The adapters and index sequences with low quality values (QV < 20) were trimmed. Paired-end assembly, size selection (400 ~ 500 for 16S and 350 ~ 450 for 23S rRNA-based approaches), and primer trimming were performed by Mothur software v.1.35.0 (Schloss et al., 2009) with 6 bp and higher overlapping sequences and without any mismatch option. Sequences were clustered into operational taxonomic units (OTUs) with 99.6 % similarity and chimeras were removed using UCHIME software v8.1 (http://drive5.com/uchime). The sequences of OTUs were compared against known species from the NCBI-NT database using BLAST (BLASTN, version 2.230+). OTU sequences with less than 90 % identity were described as “Unknown”. And, OTUs with similarity between 90 % and 98 % were assigned to the genus level. At last, the species level was above 98 % in samples by 16S universal primer set (Herlemann et al., 2011). In samples by 23S universal primer set (Kang, 2017; Yoon et al., 2016), OTUs with similarity between 90 % and 97 % were assigned to the genus level and the species level was above 97 % (Table 1).

Table 1. Primer for NGS analysis

Primer	5'–3'	Target region	Reference
Bakt_341F	CCTACGGGNGGCWGCAG	16S	(Herlemann et al., 2011)
Bakt_805R	GACTACHVGGGTATCTAATCC	16S	
P23MISQF1	GGACARWAAGACCCTATGMAG	23S	(Kang, 2017; Yoon et al., 2016)
P23MISQR1	AGATYAGCCTGTTATCCCT	23S	
Forward adapter sequences	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG		
Reverse adapter sequences	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG		

5. Statistical analysis

Each measurement was analyzed by T-test and variation of microbial community was analyzed by Chi-square test (PRIMER v6 program, PRIMER-E, UK).

Result

1. Environmental factors (Temperature, Salinity, Chlorophyll *a*, Amount of Precipitation)

Annual water temperature ranged from 5.1 °C to 29.4 °C (Fig. 2). The highest average temperature was identified in St. 4 (17. 3 °C) followed by St. 5 (16. 7 °C) and St. 1 (16. 5 °C) and St. 3 (16. 5 °C). The lowest temperature was identified in St. 2 (16. 1 °C). The highest difference in water temperature showed in July (0.9 °C) and the lowest one was in Apr (0.1 °C). The average salinity were between 30.5 psu and 31.2 psu, which was not significant different in each station. The salinity ranges in five sample stations were between 28.5 psu (St.4 in December) and 33.3 psu (St.5 in February) (Fig. 3). Concentrations of Chl*a* were between 0.1015 μgL^{-1} and 6.4974 μgL^{-1} (Fig. 4). The lowest Chl*a* was identified at St.5 in March and the highest was at St.1 in December. It was the lowest by 0.2103 μgL^{-1} in March and the highest by 2.8568 μgL^{-1} in December. The values were higher in December and January than the others. The difference between survey stations was low by 0.0464 μgL^{-1} in April. In December, it was high by 2.0411 μgL^{-1} (Fig. 4). The factors were significantly different monthly ($P < 0.05$). Total precipitation during the survey was 863. 2 mm and it was lowest in January (0.52 mm) and highest in July (7.20 mm) (Fig. 5).

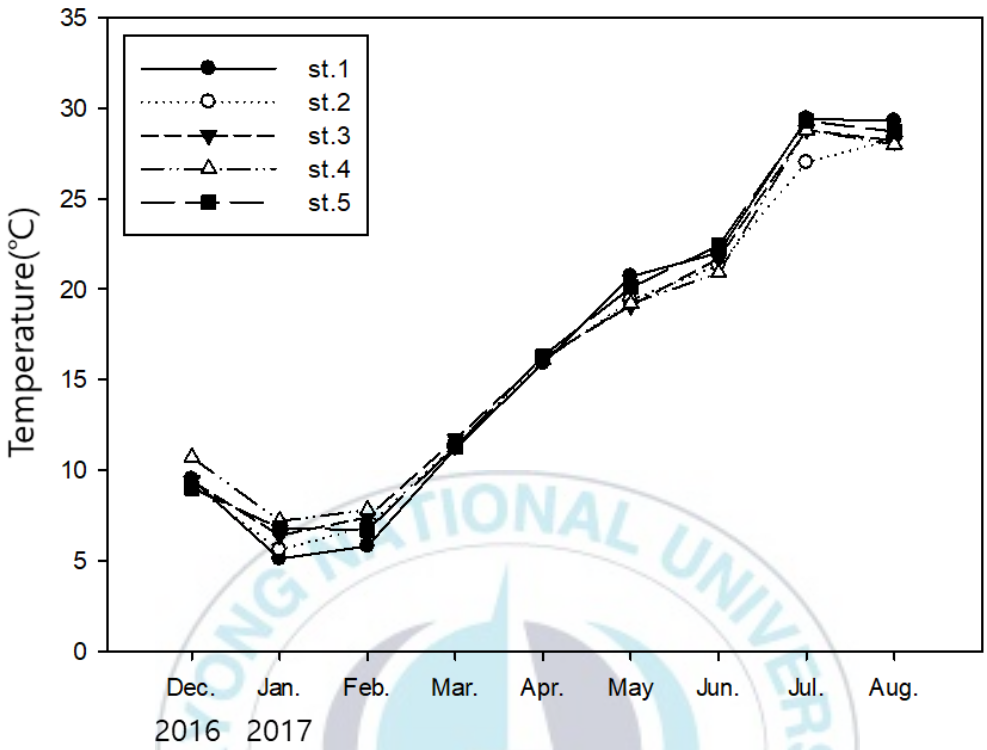

Fig. 2. Temperature from December 2016 to August 2017 in Jaran bay by each station

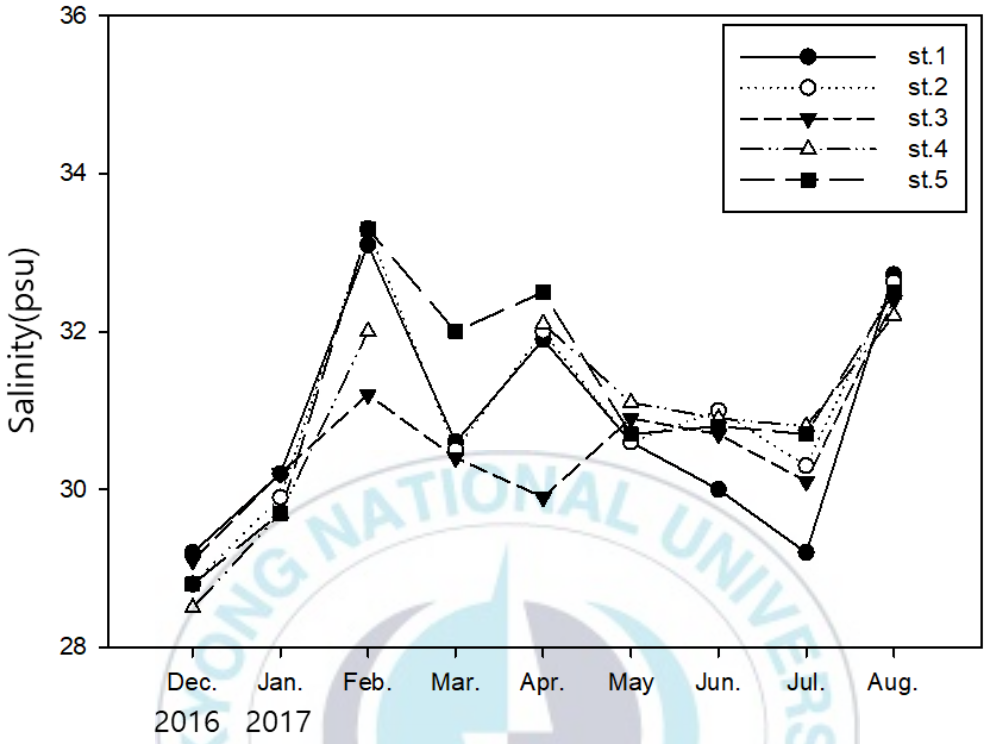

Fig. 3. Salinity from December 2016 to August 2017 in Jaran bay by each station

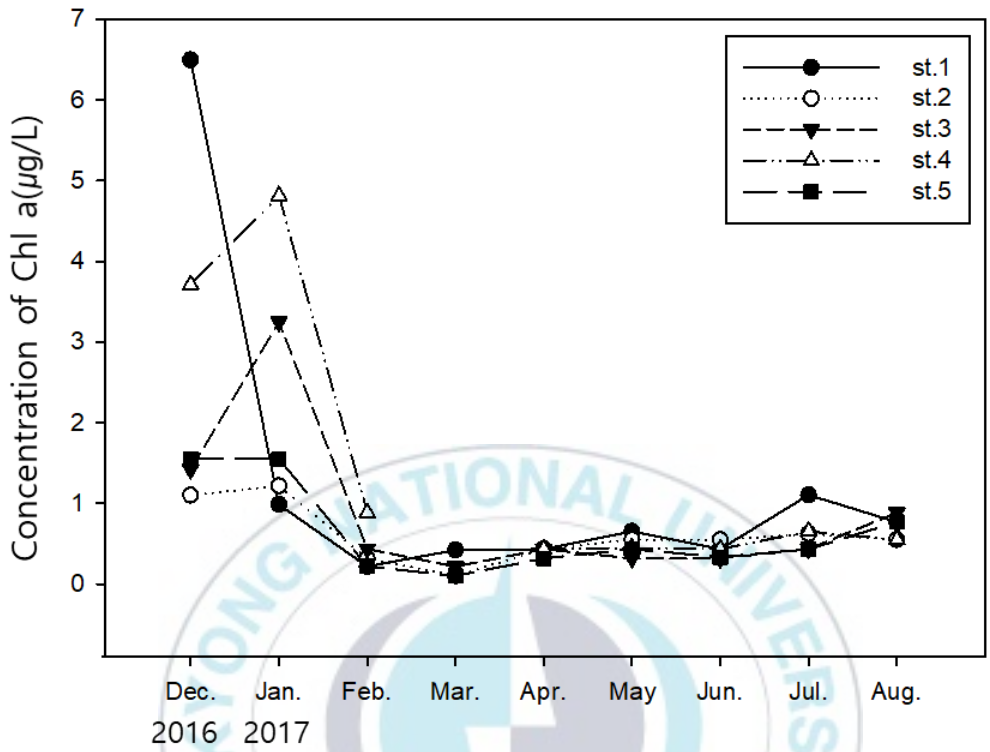

Fig. 4. Concentration of Chl*a* from December 2016 to August 2017 in Jaran bay by each station

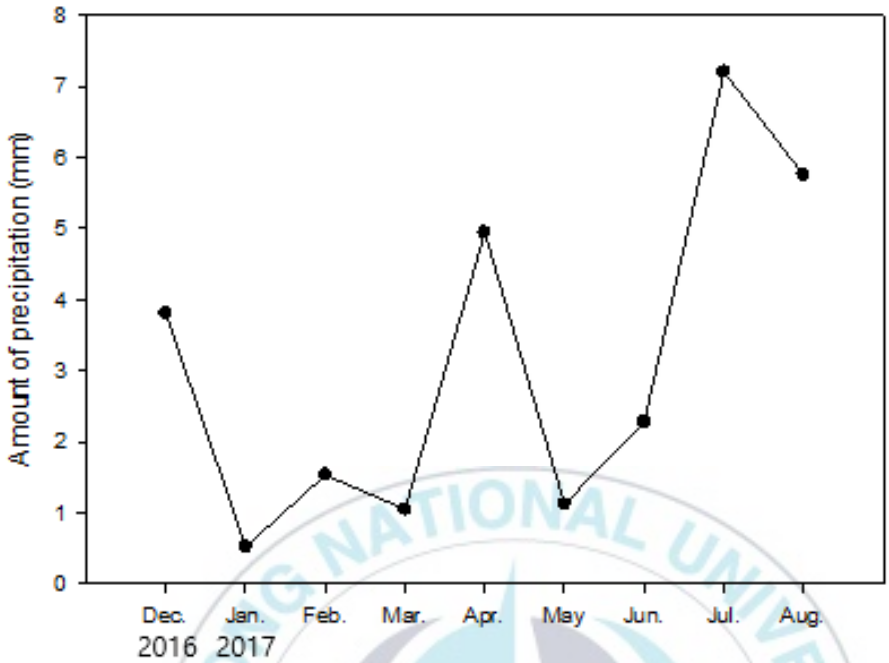

Fig. 5. Amount of precipitation from December 2016 to August 2017 in Jaran bay by each station

2. qPCR analysis

The qPCR has been employed to quantify phytoplankton and total microorganisms of collected water samples. There was no statistically significant difference among five sample stations in average copy numbers of both heterotrophic bacteria and phytoplankton. The range of average heterotrophic bacterial copy numbers were between 231.39±125.48 (St. 1) and 141.43±91.07 (St. 5). The lowest was St.4, 141.43±84.30 (Fig. 5). The range of average phytoplankton copy numbers were between 32.86±36.90 (St. 2) and 24.44±26.36 (St. 4).

In heterotrophic bacteria, the values (copy number/10^4 by C(t) value) were between 24.01±4.81 and 454.67±59.47. It was the lowest at St.3 in August and the highest at St.1 in May. At St.1, the values estimated monthly was the highest by 454.67±59.47 in May. The lowest was 89.65±13.08 in July. At St.2, the highest was 315.66±26.76 in May and the lowest was 29.49±14.85 in August. At St.3, the highest was 453.51±86.14 in March and the lowest was 24.01±4.81 in August. At St.4, the highest was 262.79±17.75 in February and the lowest was 25.43±1.51 in August. At St.5, the highest was 328.81±205.52 in March and the lowest was 28.36±16.95 in August (Fig. 9, Fig. 15).

In autotrophic phytoplankton, the values (copy number/10^4 by C(t) value)

were between 1.13±0.13 and 107.87±13.51. It was the lowest in August at St.4 and the highest in April at St.3. At St.1, the values estimated monthly was the highest by 88.28±2.57 in May. The lowest was 3.66±0.50 in February. At St.2, the highest was 91.54±10.69 in May and the lowest was 1.61±0.25 in July. At St.3, the highest was 107.87±13.51 in April and the lowest was 2.21±0.33 in July. At St.4, the highest was 69.91±6.95 in April and the lowest was 1.13±0.13 in August. At St.5, the highest was 70.21±5.06 in May and the lowest was 1.61±0.24 in July (Fig. 10, Fig. 16).

In order to know the difference in growth rate between phytoplankton and bacteria, we calculated the ratios between them. The monthly ratios of phytoplankton to heterotrophic bacteria (P/B ratio) were between 0.01 and 0.52 (Fig. 10). Regardless of sample stations, there was a common patterns in P/B ratio which was the lowest in February and highest was in April. Among five sample stations, St.1 showed the lowest changes in P/B ratio. The monthly ratios of heterotrophic bacteria to phytoplankton (B/P ratio) were between 1.92 and 105.13 (Fig. 11). Regardless of sample stations, there was a common patterns in P/B ratio which was the lowest in April and highest was in February. Among five sample stations, St.3 showed the lowest changes in B/P ratio. Collectively, bacterial bloom occurred in February and March and phytoplankton bloom was identified in April and May (Fig. 10 and 11).

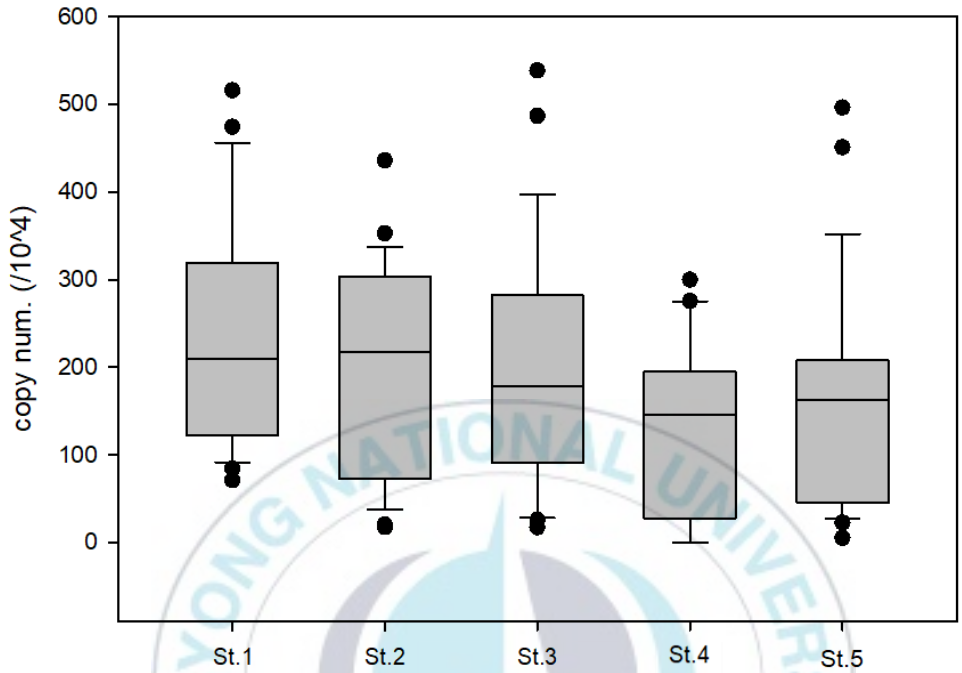

Fig. 6. Average of copy numbers of heterotrophic bacteria in five sample stations of Jaran bay from December 2016 to August 2017 using qPCR

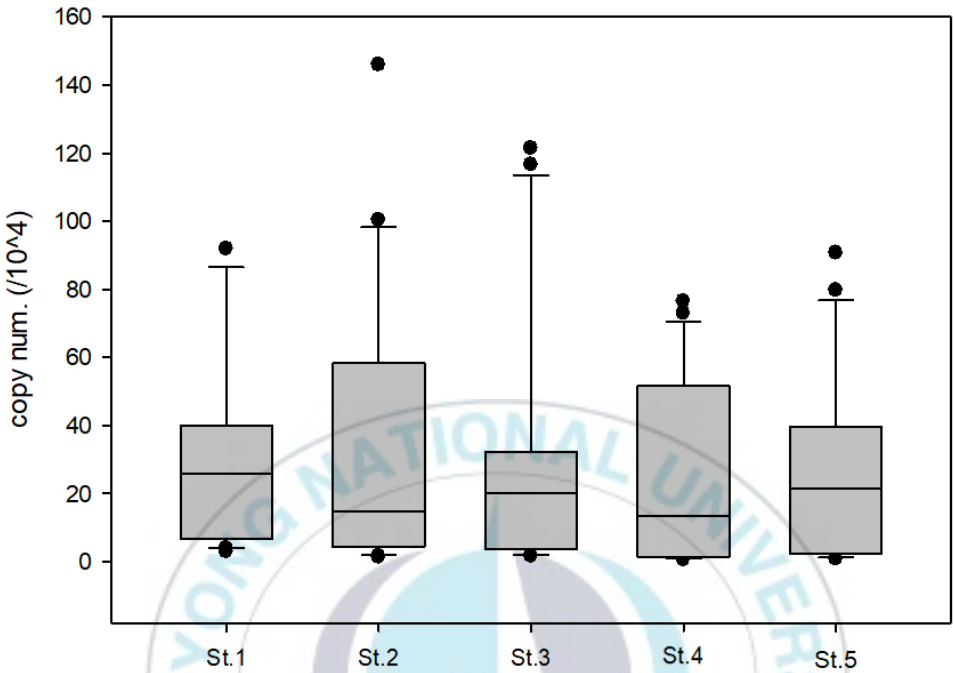

Fig. 7. Average of copy numbers of phytoplankton in five sample stations of Jaran bay from December 2016 to August 2017 using qPCR

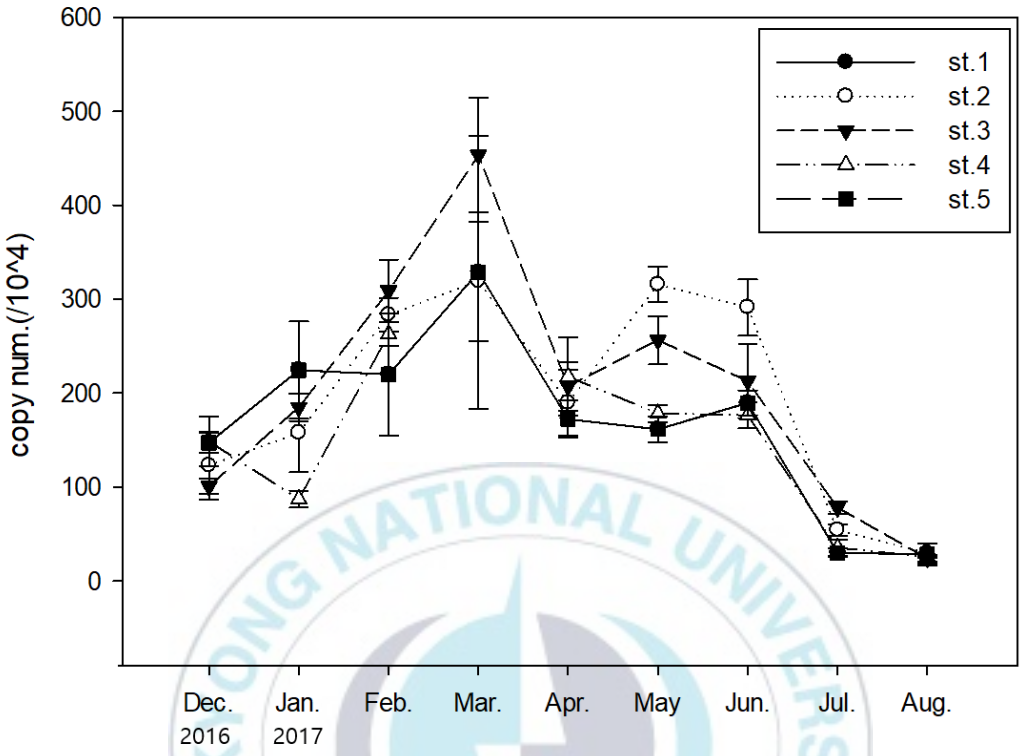

Fig. 8. Changes in bacterial copy numbers in five sample stations in Jaran bay from December 2016 to August 2017 using qPCR

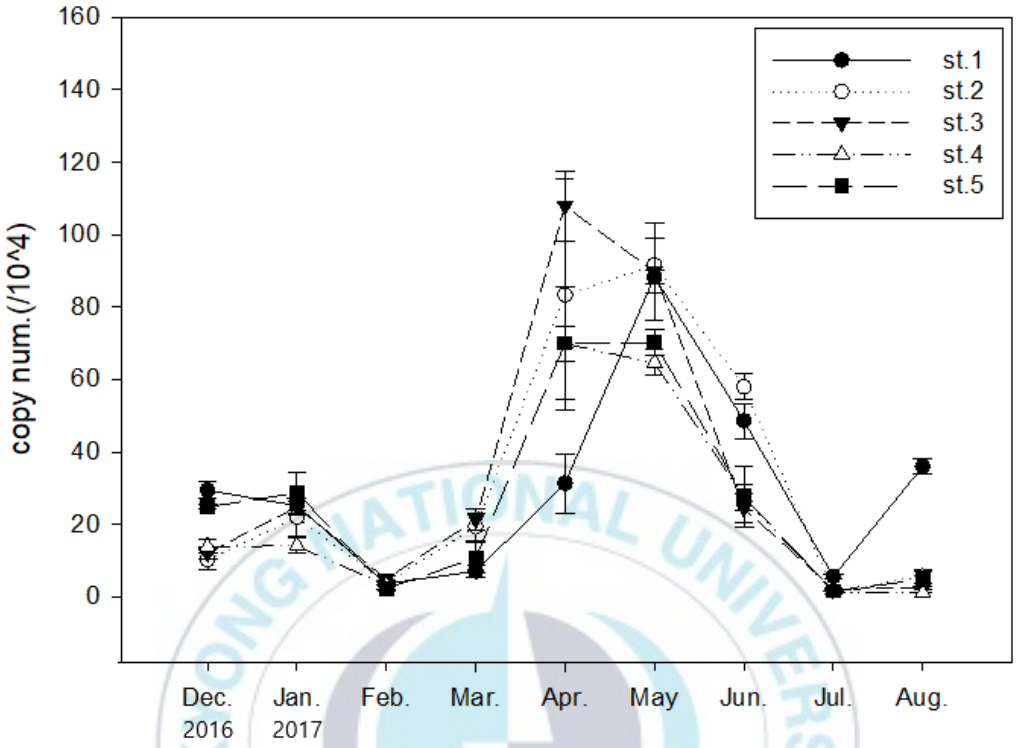

Fig. 9. Changes in phytoplankto copy numbers in five sample stations in Jaran bay from December 2016 to August 2017 using qPCR

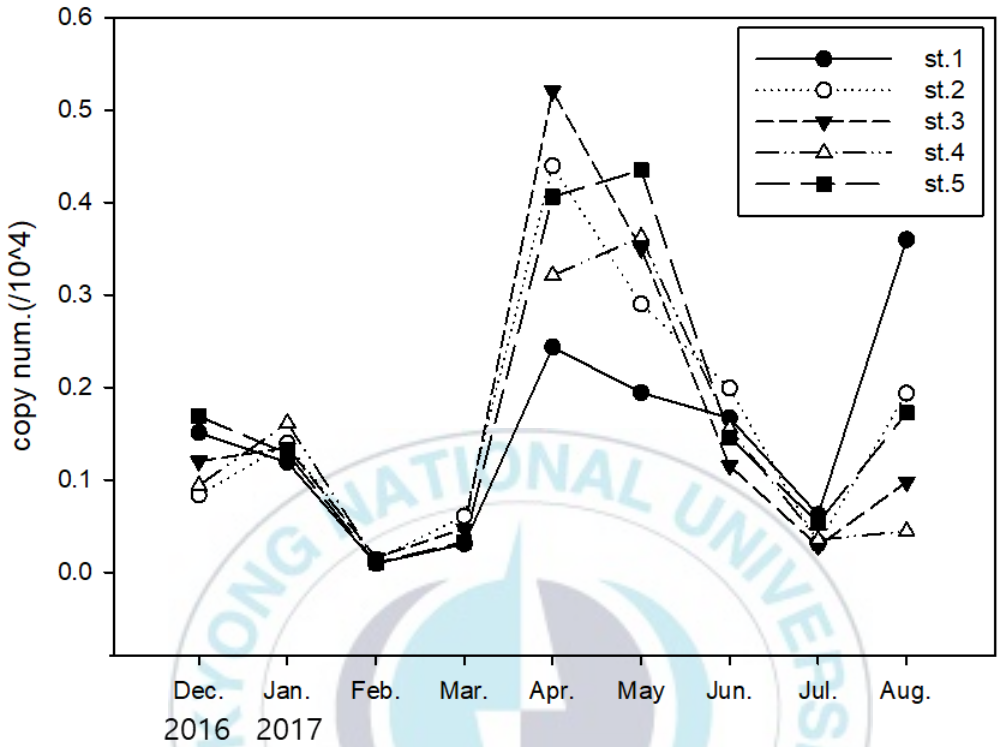

Fig. 10. Changes in ratio of phytoplankton copy numbers to bacterial numbers (P/B ratio) in five sample stations in Jaran bay from December 2016 to August 2017 using qPCR

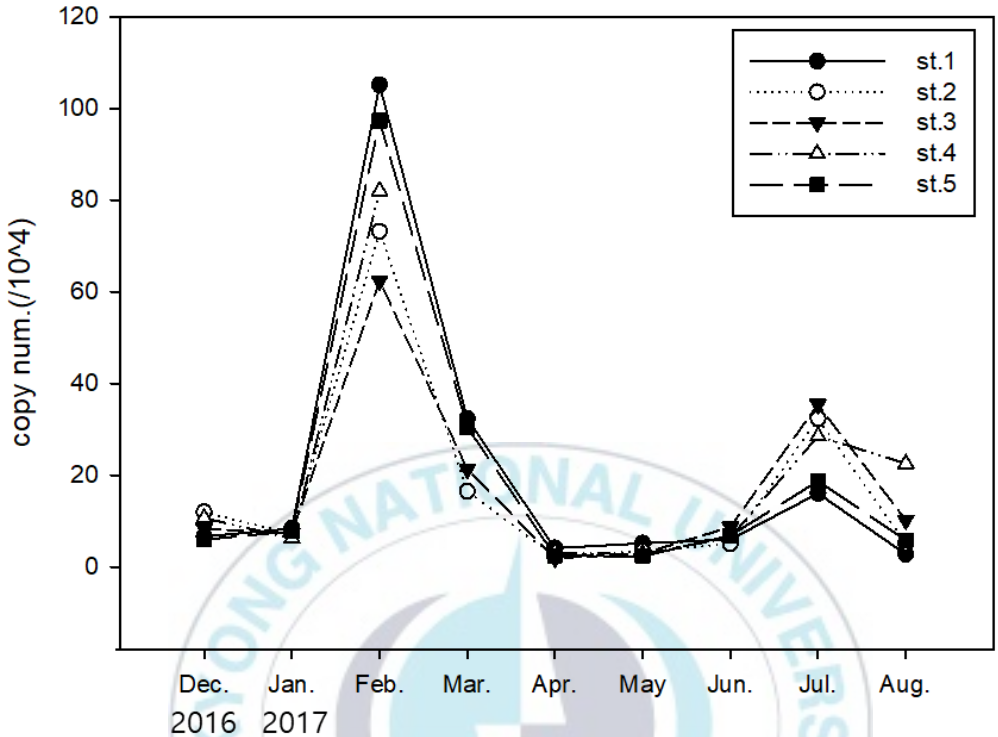

Fig. 11. Changes in ratio of bacterial copy numbers to phytoplankton numbers (B/P ratio) in five sample stations in Jaran bay from December 2016 to August 2017 using qPCR

3. Microbial community structures by NGS analysis

Based on the difference in the ratios between phytoplankton and bacteria, we analyzed the microbial community structures of St.4 in February and April and St. 1and St. 5 in May by MiSeq platform (Fig. 12). We used 16S universal primer sets to analyze the samples. After trimming and clustering the raw reads, 276,719 and 96,656 contigs were obtained from St. 4 in February and April, respectively. Total 3,857 OTUs (February) and 1,550 OTUs (April) were generated at 99 % sequence identity as cutoff. OTUs in St. 4 were classified into 32 phyla including 22 prokaryotic heterotrophs (Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria, and Verrucomicrobia etc.), one prokaryotic autotroph (Cyanobacteria) and nine eukaryotic autotrophs (Bacillariophyta, Chlorophyta, Cryptophyta, Haptophyta, and Ochrophyta etc.) (Table 2, 6, 8 and 10; Fig. 12 and 14). Among 32 phyla, Proteobacteria occupied highest proportion (59.79 %) in February and 97.39 % in April. Proportions of eukaryotic algae in February (2.57 %) was higher than in April (0.01 %). The numbers of 'Unknown' OTUs, which showed less than 90% sequence identity to database, were 171 (0.37 %) in February and 65 (1.12 %) in April. Major difference in the microbial community structures at St. 4 between February and April were proportions of Proteobacteria (59.79 %

in February and 97.39 % in April). The patterns between February and April were significantly different (x^2 = 79.914, df = 8, $P < 0.05$).

In order to know the difference between February and April in microbial community structure, commonly identified OTUs both samples and monthly specific ones were analyzed (Table 8 and 10, Fig. 18). Among 95 OTUs generated by 16S universal primer set, 12 OTUs were commonly identified and their proportions were 59.39 % (in February) and 95.14 % (in April). The common species included Proteobacteria, Verrucomicrobia and Bacteroidetes. *Candidatus pelagibacter* was dominant in both February (17.35 %) and April (36.84 %).

In order to know the difference in microbial community between two sample stations, St. 1 and St. 5, we analyzed the samples using 16S universal primer sets. After trimming and clustering the raw data, total 51,157 contigs at St.1 and 82,672 contigs at St. 5 was obtained, respectively. Total 831 OTUs from St. 1 and 1,253 OTUs from St. 5 were generated with 99 % as cutoff value. Obtained OTUs could be divided into 23 phyla including 13 prokaryotic heterotrophs (Actinobacteria, Bacteroidetes, Marinimicrobia, Planctomycetes, Proteobacteria, and Verrucomicrobia etc.), one eukaryotic protist (Foraminifera), one prokaryotic autotroph (Cyanobacteria) and nine eukaryotic autotrophs

(Bacillariophyta, Cercozoa, Chlorophyta, Cryptophyta, and Orchrophyta etc.) (Table 3, 6, 8 and 11; Fig. 12 and 16). Among 23 phyla, Proteobacteria occupied highest proportion (64.22 %) from St.1 and 61.59 % from St.5. The numbers of 'Unknown' OTUs, which showed less than 90 % sequence identity to database, were 55 (1.89 %) at St.1 and 82 (2.22 %) at St.5. The microbial community between two sample stations showed similar patterns (x^2 = 3.253, df = 8, P > 0.05).

In order to know the difference between at St.1 and St.5 in microbial community structure, commonly identified OTUs both samples and site- specific ones were analyzed (Table 8 and 11, Fig. 19). Among the samples at St.1 and St.5 generated by 16S universal primer set, 94 OTUs were commonly identified and their proportions were 94.76 % (at St.1) and 95.89 % (at St.5). Among them, Proteobacteria comprise high proportion over 60 % in both sites. In the dominant phylum, the proportion of *Rhodobacteraceae bacterium* and *C. pelagibacter* were high about 10 %, respectively.

In order to know the difference in microbial community between two months, February and April, we analyzed the samples using 23S universal primer sets. After trimming and clustering the raw reads, 239,200 and 230,203 contigs were obtained from St. 4 in February and April, respectively. Total 4,771 OTUs

(February) and 2,135 OTUs (April) were generated at 99 % sequence identity as cutoff. OTUs in St. 4 were classified into 15 phyla including two prokaryotic heterotrophs (Bacteroidetes, Verrucomicrobia), one prokaryotic autotroph (Cyanobacteria) and 12 eukaryotic autotrophs (Bacillariophyta, Biliphyta, Chlorophyta, Cryptophyta, Haptophyta, and Ochrophyta etc.) (Table 4, 7, 9 and 12; Fig. 13 and 15). The numbers of 'Unknown' OTUs, which showed less than 90 % sequence identity to database, were 228 (1.87 %) in February and 31 (0.25 %). Major difference in the microbial community structures at St. 4 between February and April were proportions of Chlorophyta (23.37 %) in February and 83.80 % in April. Among the phyla, *Ostreococcus tauri* was dominant by 63.89 % in April. The patterns between February and April were significantly different ($x^2 = 48.720$, df = 8, $P < 0.05$).

In order to know the difference between in February and April in microbial community structure, commonly identified OTUs both samples and monthly specific ones were analyzed (Table 9 and 12, Fig. 20). Among the samples in February and April generated by 23S universal primer set, 12 OTUs were commonly identified and their proportions were 34.09 % (in February) and 89.77 % (in April). Among them, Chlorophyta was dominant by 21.93 % and 83.20 %, respectively. In the dominant phylum, the proportion of *Micromonas pusilla* dominated by 15.44 % in February. In particular, *O. tauri* was dominant

species by 67.92 % in April. In April, monthly specific species was 1 Chlorophyta, *Ostreococcus* sp.. But the species in February were significantly diverse. They were composed of 2 Bacillariophyta (*Cerataulina daemon*, *Cerataulina* sp., *Nitzschia* sp., 1 Cryptophyta (*Teleaulax* sp.) and 3 Haptophyta (*Chrysochromulina* sp., *Phaeocystis globosa*, Unknown).

In order to know the difference in microbial community between two sample stations, St. 1 and St. 5, we analyzed the samples using 23S universal primer sets. After trimming and clustering the raw data, total 75,302 contigs at St.1 and 60,022 contigs at St. 5 was obtained, respectively. Total 484 OTUs from St. 1 and 612 OTUs from St. 5 were generated with 99 % as cutoff value. Obtained OTUs could be divided into 14 phyla including one prokaryotic heterotroph (Verrucomicrobia), one prokaryotic autotroph (Cyanobacteria) and 12 eukaryotic autotrophs (Chlorophyta, Haptophyta, Miozoa, and Ochrophyta etc.) (Table 5, 7, 9 and 13; Fig. 13 and 17). The numbers of 'Unknown' OTUs, which showed less than 90 % sequence identity to database, were 33 (0.57 %) at St.1 and 37 (0.97 %) at St.5. The microbial community between two sample stations showed similar patterns ($x^2 = 2.754$, $df = 9$, $P > 0.05$).

In order to know the difference between St.1 and St.5 in microbial community structure, commonly identified OTUs both samples and site- specific

ones were analyzed (Table 9 and 13, Fig. 21). Among 68 OTUs generated by 23S universal primer set, 48 OTUs were commonly identified and their proportions were 98.69 % (at St.1) and 96.97 % (at St.5). The proportion was significantly high. And their composition was similar. Among them, Chlorophyta was dominant about 50 % at both sites. And then Cyanobacteria was followed by 28.40 % (at St.1) and 30.43 % (at St.5). In the dominant phylum, the proportion of *O. tauri* and *Synechococcus* sp. occupied over 50 % at both St.1 and St.5.

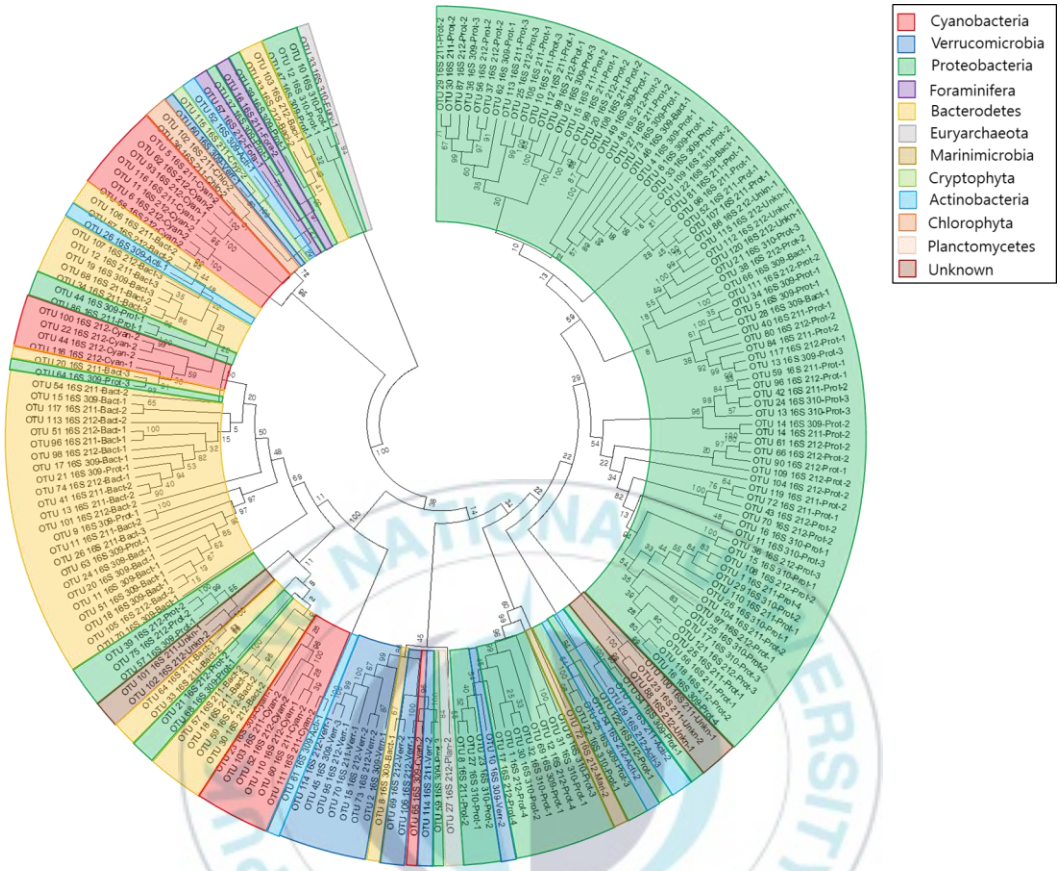

Fig. 12. Phylogenetic tree for 16S sequences in Jaran bay sample (>0.1 %)

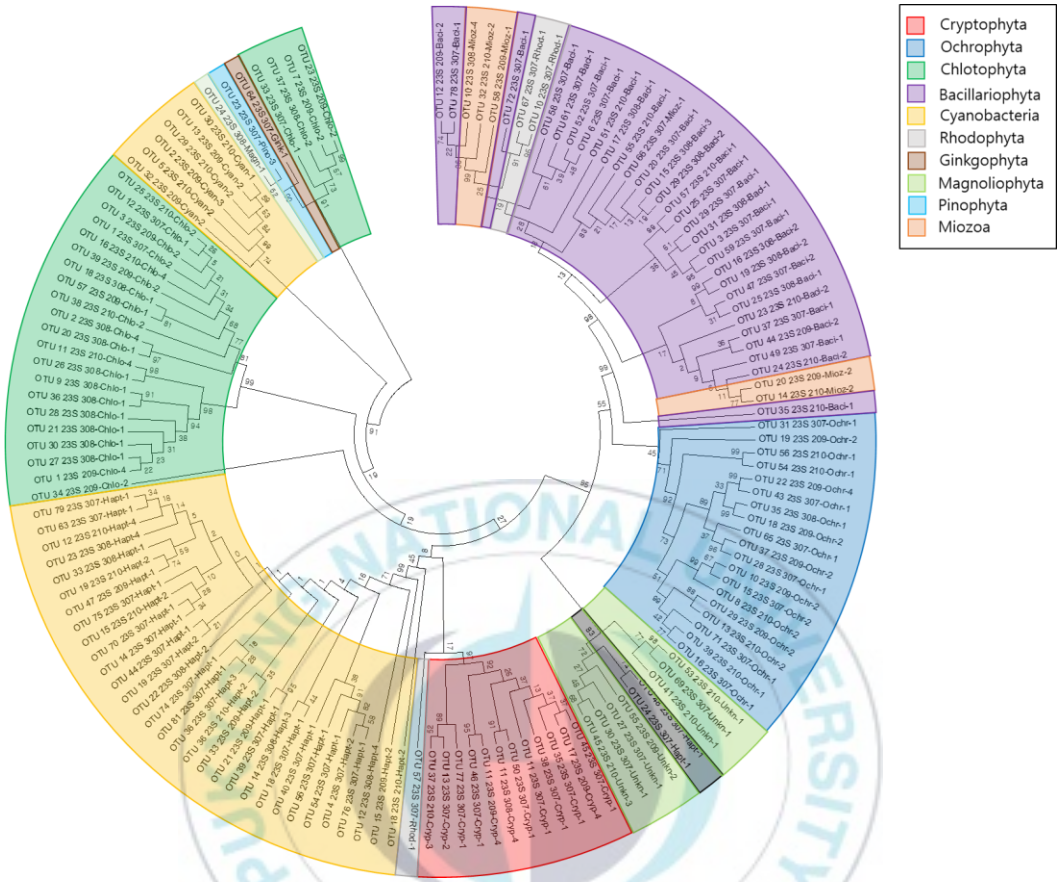

Fig. 13. Phylogenetic tree for 23S sequences in Jaran bay sample (>0.1 %)

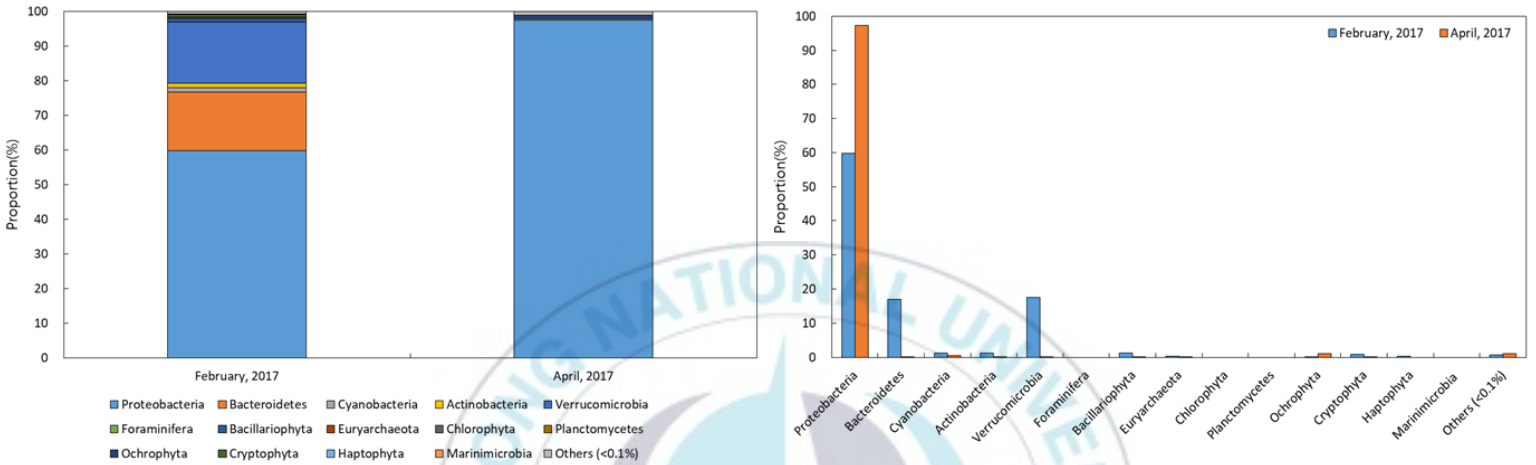

Fig. 14. Microbial community structure at phylum level for 16S sequences in Jaran bay sample collected at St.4 in February and April. Each bar shows the proportion of microbial phyla according to 98 % sequence similarity.

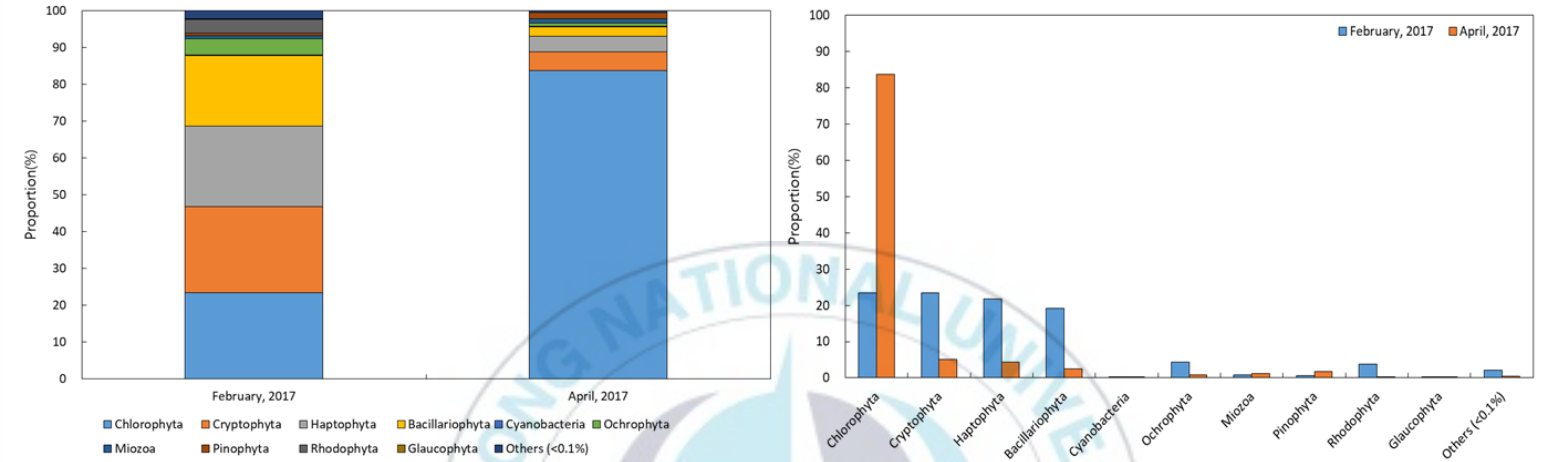

Fig. 15. Microbial community structure at phylum level for 23S sequences in Jaran bay sample collected at St.4 in February and April. Each bar shows the proportion of microbial phyla according to 97 % sequence similarity.

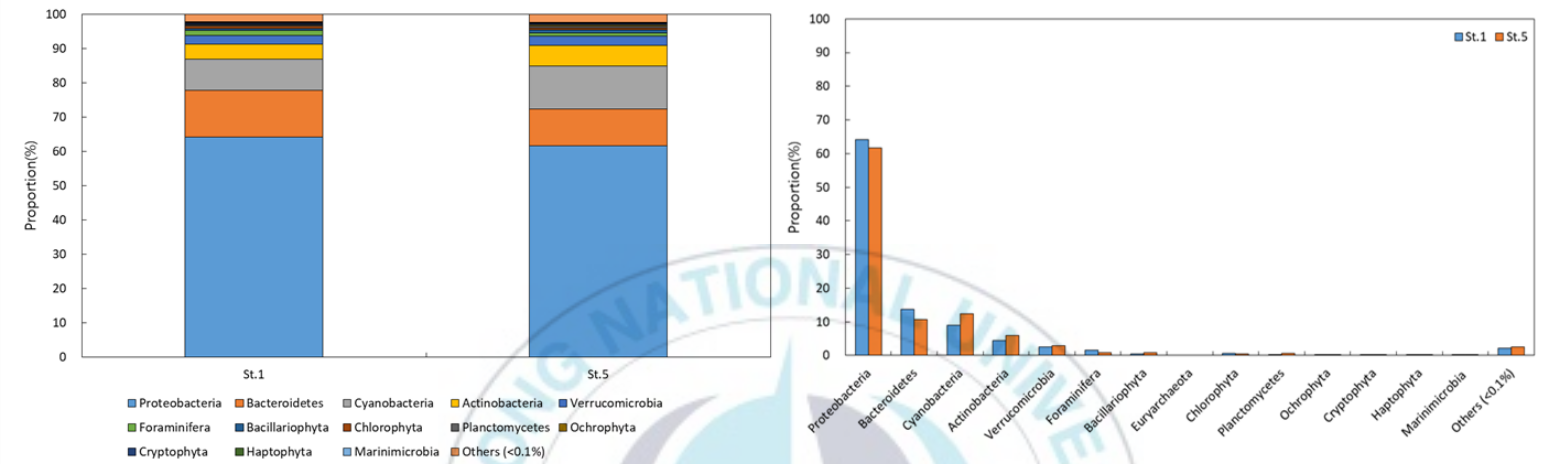

Fig. 16. Microbial community structure at phylum level for 16S sequences in Jaran bay sample collected at St.1 and St.5 in May. Each bar shows the proportion of microbial phyla according to 98 % sequence similarity.

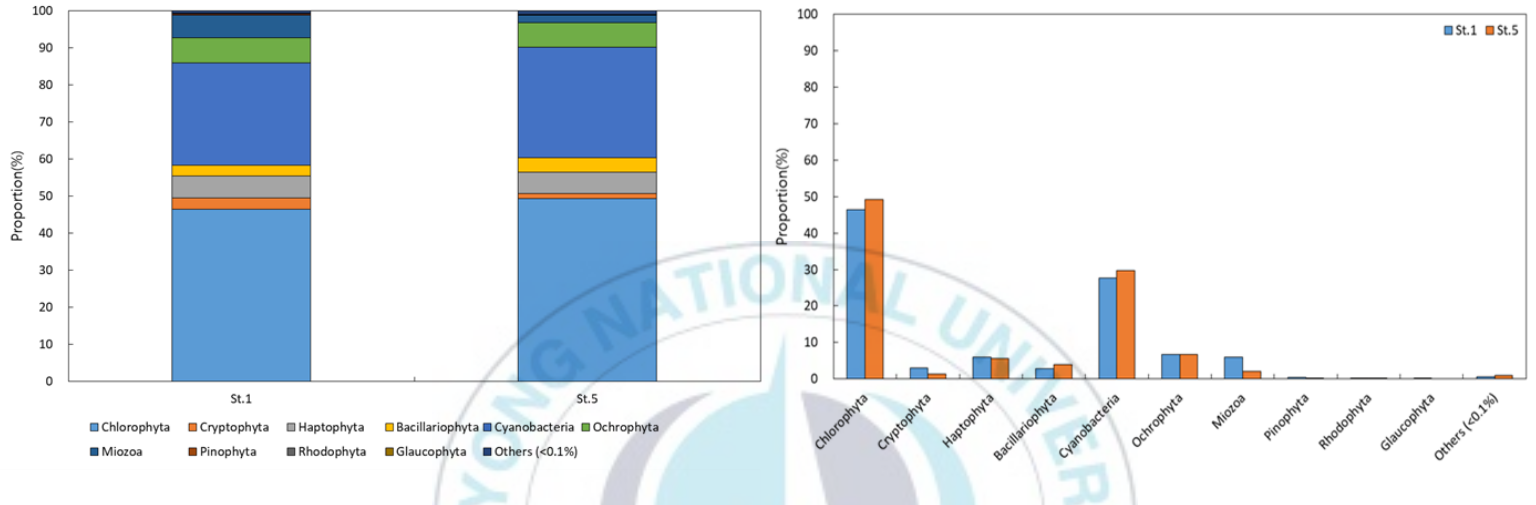

Fig. 17. Microbial community structure at phylum level for 23S sequences in Jaran bay sample collected at St.1 and St.5 in May. Each bar shows the proportion of microbial phyla according to 97 % sequence similarity.

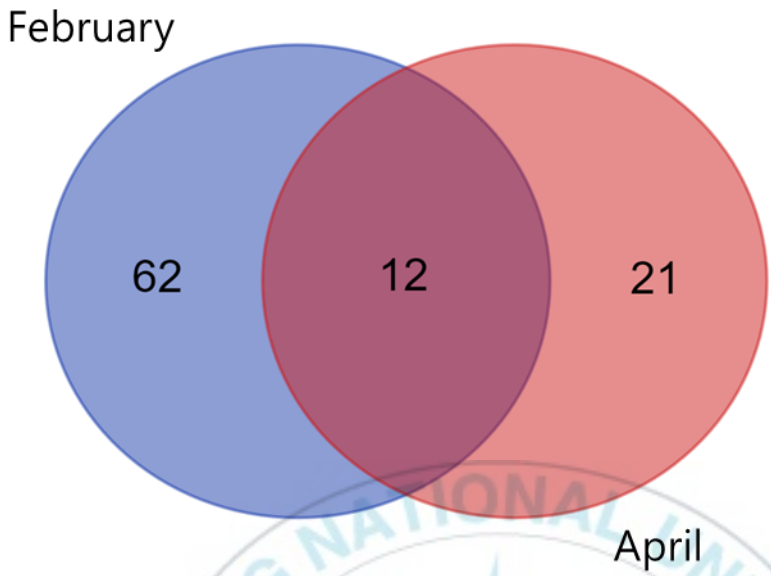

Fig. 18. Two-way Venn diagram illustrating the number of unique and shared OTUs in 16S sequences among the sampling period (>0.1 %). The venn diagram was carried out by Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/)

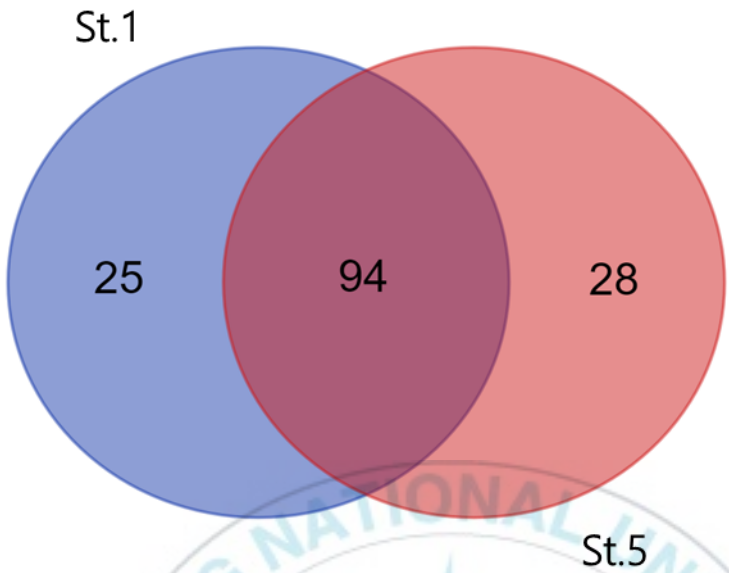

Fig. 19. Two-way Venn diagram illustrating the number of unique and shared OTUs in 16S sequences among the sampling station (>0.1 %). The venn diagram was carried out by Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/)

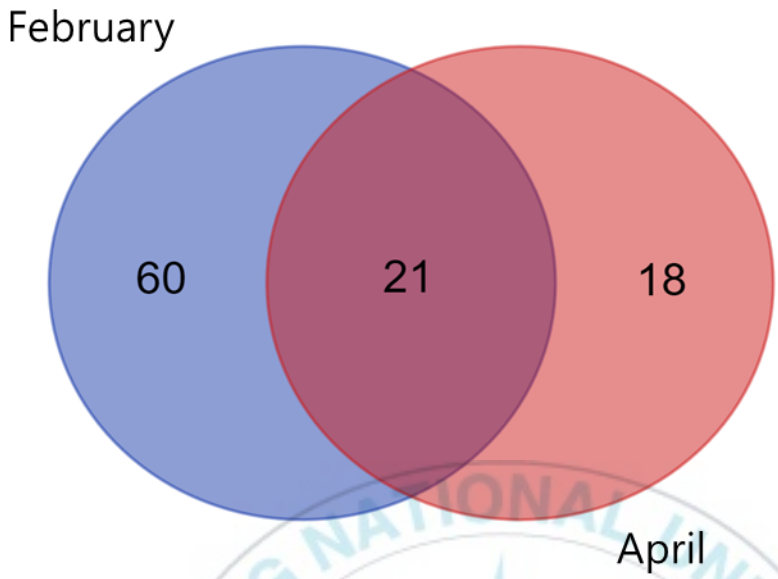

Fig. 20. Two-way Venn diagram illustrating the number of unique and shared OTUs in 23S sequences among the sampling period (>0.1 %). The venn diagram was carried out by Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/)

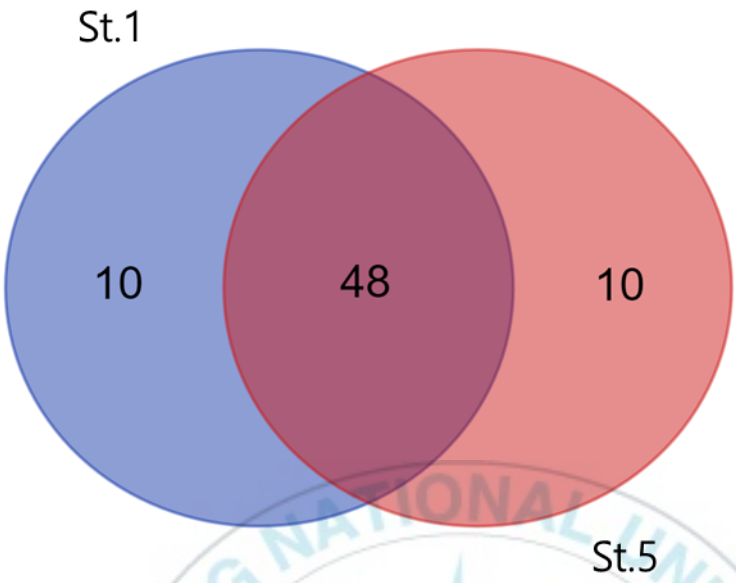

Fig. 21. Two-way Venn diagram illustrating the number of unique and shared OTUs in 23S sequences among the sampling station (>0.1 %). The venn diagram was carried out by Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/

Table 2. Summary of OTUs at St.4 by 16S universal primer set

Phylum	Description	Feb.				Apr.			
		OTUs	Subtotal	Proportion(%)	Subtotal(%)	OTUs	Subtotal	Proportion(%)	Subtotal(%)
Acidobacteria	Prokaryotic heterotroph	12		0.02		0		0.00	
Actinobacteria	Prokaryotic heterotroph	42		1.23		14		0.42	
Ascomycota	Prokaryotic heterotroph	1		0.00		0		0.00	
Bacteroidetes	Prokaryotic heterotroph	516		16.99		11		0.03	
Chlamydiae	Prokaryotic heterotroph	1		0.00		0		0.00	
Chloroflexi	Prokaryotic heterotroph	11		0.01		0		0.00	
Deferribacteres	Prokaryotic heterotroph	2		0.00		0		0.00	
Euryarchaeota	Prokaryotic heterotroph	2		0.00		11		1.01	
Firmicutes	Prokaryotic heterotroph	6		0.01		1		0.00	
Fusobacteria	Prokaryotic heterotroph	5	3477	0.01	95.72	0	1480	0.00	98.87
Gemmatimonadetes	Prokaryotic heterotroph	2		0.00		0		0.00	
Ignavibacteriae	Prokaryotic heterotroph	2		0.00		0		0.00	
Lentisphaerae	Prokaryotic heterotroph	2		0.01		0		0.00	
Marinimicrobia	Prokaryotic heterotroph	3		0.02		0		0.00	
Nitrospirae	Prokaryotic heterotroph	1		0.00		0		0.00	
Planctomycetes	Prokaryotic heterotroph	12		0.02		0		0.00	
Proteobacteria	Prokaryotic heterotroph	2430		59.79		1438		97.39	
Thaumarchaeota	Prokaryotic heterotroph	2		0.00		0		0.00	
Tm6(Dependentiae)	Prokaryotic heterotroph	1		0.00		0		0.00	
Verrucomcrobia	Prokaryotic heterotroph	424		17.59		5		0.02	
Cyanobacteria	Prokaryotic autotroph	39	39	1.23	1.23	1	1	0.00	0.00
Foraminifera	Eukaryotic protest	21	21	0.11	0.11	0	0	0.00	0.00
Bacillariophyta	Eukaryotic autotroph	40		1.17		1		0.00	
Chlorophyta	Eukaryotic autotroph	16		0.10		0		0.00	
Cryptophyta	Eukaryotic autotroph	22		0.78		1		0.00	
Euglenophyta	Eukaryotic autotroph	1		0.00		0		0.00	
Haptophyta	Eukaryotic autotroph	32	149	0.27	2.57	1	4	0.00	0.01

Table 2. Continued

Phylum	Description	Feb.				Apr.			
		OTUs	Subtotal	Proportion(%)	Subtotal(%)	OTUs	Subtotal	Proportion(%)	Subtotal(%)
Miozoa	Eukaryotic autotroph	2		0.00		0		0.00	
Ochrophyta	Eukaryotic autotroph	30		0.23		0		0.00	
Pinophyta	Eukaryotic autotroph	3		0.01		1		0.00	
Rhodophyta	Eukaryotic autotroph	3		0.01		0		0.00	
Unknown		171	171	0.37	0.37	65	65	1.12	1.12
Total	32	3857	3857	100.00	100.00	1550	1550	100.00	100.00

Table 3. Summary of OTUs in May by 16S universal primer set

Phylum	Description	St.1				St.5			
		OTUs	Subtotal	Proportion(%)	Subtotal(%)	OTUs	Subtotal	Proportion(%)	Subtotal(%)
Actinobacteria	Prokaryotic heterotroph	20		4.41		35		5.98	
Bacteroidetes	Prokaryotic heterotroph	122		13.64		157		10.74	
Balneolaeota	Prokaryotic heterotroph	1		0.01		1		0.02	
Deferribacteres	Prokaryotic heterotroph	3		0.04		6		0.04	
Euryarchaeota	Prokaryotic heterotroph	6		0.13		7		0.15	
Firmicutes	Prokaryotic heterotroph	0	632	0.00	85.48	1	924	0.00	82.28
Fusobacteria	Prokaryotic heterotroph	1		0.00		0		0.00	
Marinimicrobia	Prokaryotic heterotroph	2		0.16		2		0.18	
Planctomycetes	Prokaryotic heterotroph	4		0.36		9		0.68	
Proteobacteria	Prokaryotic heterotroph	435		64.22		668		61.59	
Tenericutes	Prokaryotic heterotroph	0		0.00		1		0.00	
Verrucomicrobia	Prokaryotic heterotroph	38		2.49		37		2.89	
Cyanobacteria	Prokaryotic autotroph	62	62	8.99	8.99	133	133	12.47	12.47
Foraminifera	Eukaryotic protest	15	15	1.52	1.52	18	18	0.80	0.80
Bacillariophyta	Eukaryotic autotroph	16		0.54		20		0.88	
Cercozoa	Eukaryotic autotroph	1		0.01		1	1	0.00	
Chlorophyta	Eukaryotic autotroph	12		0.71		18		0.55	
Cryptophyta	Eukaryotic autotroph	11		0.33		12		0.25	
Euglenophyta	Eukaryotic autotroph	1	67	0.03	2.12	1	95	0.00	2.23
Haptophyta	Eukaryotic autotroph	11		0.20		18		0.18	
Miozoa	Eukaryotic autotroph	2		0.05		4		0.04	
Ochrophyta	Eukaryotic autotroph	12		0.24		22		0.32	
Pinophyta	Eukaryotic autotroph	1		0.03		0		0.00	
Unknown		55	55	1.89	1.89	82	82	2.22	2.22
Total	23	831	831	100.00	100.00	1253	1253	100.00	100.00

Table 4. Summary of OTUs at St.4 by 23S universal primer set

Phylum	Description	Feb.				Apr.			
		OTUs	Subtotal	Proportion(%)	Subtotal(%)	OTUs	Subtotal	Proportion(%)	Subtotal(%)
Bacteroidetes	Prokaryotic heterotroph	1		0.00	0.00	1	2	0.00	0.00
Verrucomicrobia	Prokaryotic heterotroph	1		0.00		1		0.00	
Cyanobacteria	Prokaryotic autotroph	28	28	0.22	0.22	16	16	0.20	0.20
Bacillariophyta	Eukaryotic autotroph	974		19.13		69		2.40	
Chlorophyta	Eukaryotic autotroph	957		23.37		1691		83.80	
Cryptophyta	Eukaryotic autotroph	721		23.50		56		5.06	
Euglenophyta	Eukaryotic autotroph	4		0.09		1		0.00	
Ginkgophyta	Eukaryotic autotroph	3		0.11		1		0.02	
Glaucophyta	Eukaryotic autotroph	19	4513	0.22	97.91	3	2086	0.00	99.55
Haptophyta	Eukaryotic autotroph	1220		21.81		161		4.33	
Magnoliophyta	Eukaryotic autotroph	0		0.00		5		0.20	
Miozoa	Eukaryotic autotroph	254		0.89		32		1.19	
Ochrophyta	Eukaryotic autotroph	187		4.36		37		0.86	
Pinophyta	Eukaryotic autotroph	17		0.70		26		1.66	
Rhodophyta	Eukaryotic autotroph	157		3.72		4		0.02	
Unknown		228	228	1.87	1.87	31	31	0.25	0.25
Total	15	4771	4771	100	100	2135	2135	100.00	100.00

Table 5. Summary of OTUs in May by 23S universal primer set

Phylum	Description	St.1				St.5			
		OTUs	Subtotal	Proportion(%) of contigs number	Subtotal(%)	OTUs	Subtotal	Proportion(%) of contigs number	Subtotal(%)
Verrucomicrobia	Prokaryotic heterotroph	1	1	0.01	0.01	1	1	0.01	0.01
Cyanobacteria	Prokaryotic autotroph	116	116	27.74	27.74	122	122	29.78	29.78
Bacillariophyta	Eukaryotic autotroph	51		2.85		49		3.99	
Cercozoa	Eukaryotic autotroph	2		0.10		2		0.04	
Chlorophyta	Eukaryotic autotroph	199		46.50		133		49.31	
Cryptophyta	Eukaryotic autotroph	20		2.96		11		1.41	
Ginkgophyta	Eukaryotic autotroph	1		0.00		0	324	0.00	69.23
Glaucophyta	Eukaryotic autotroph	2		0.01		0		0.00	
Haptophyta	Eukaryotic autotroph	83	462	5.93	71.68	64		5.67	
Magnoliophyta	Eukaryotic autotroph	1		0.01		1		0.00	
Miozoa	Eukaryotic autotroph	35		6.06		21		2.03	
Ochrophyta	Eukaryotic autotroph	60		6.76		40		6.71	
Pinophyta	Eukaryotic autotroph	3		0.44		2		0.02	
Rhodophyta	Eukaryotic autotroph	5		0.06		1		0.04	
Unknown		33	33	0.57	0.57	37	37	0.97	0.97
Total	12	612	612	100.00	100.00	484	484	100.00	100.00

Table 6. Comparison of assigned to the taxa level OTUs generated by 16S universal primer set

Identity	St.4						May					
	Feb.			Apr.			St.1			St.5		
	OTUs	Contigs	Proportion (%)	OTUs	Contigs	Proportion (%)	OTUs	Contigs	Proportion (%)	OTUs	Contigs	Proportion (%)
Above 98%	2399	211598	76.47	1316	91930	95.11	472	39728	77.66	755	65578	79.32
98% to 90%	1332	64403	23.27	198	3780	3.91	306	10465	20.46	430	15294	18.50
Below 90%	126	718	0.26	36	946	0.98	53	964	1.88	68	1800	2.18
Total	3857	276719	100	1550	96656	100	831	51157	100	1253	82672	100

Table 7. Comparison of assigned to the taxa level OTUs generated by 23S universal primer set

Identity	St.4						May					
	Feb.			Apr.			St.1			St.5		
	OTUs	Contigs	Proportion (%)	OTUs	Contigs	Proportion (%)	OTUs	Contigs	Proportion (%)	OTUs	Contigs	Proportion (%)
Above 97%	2823	182725	76.39	1017	214645	93.24	364	66307	88.05	298	53334	88.86
97% to 90%	1721	51991	21.74	1087	14983	6.51	215	8563	11.37	149	6103	10.17
Below 90%	227	4484	1.87	31	575	0.25	33	432	0.57	37	585	0.97
Total	4771	239200	100	2135	230203	100	612	75302	100	484	60022	100

Table 8. Comparative analysis of shared OTUs generated by 16S universal primer set in samples collected at St.4 in February and April and at St.1 and St.5 in May, respectively

Data	OTUs	Feb.		Apr.		Data	OTUs	St.1		St.5	
		OTU(%)	Proportion(%)	OTU(%)	Proportion(%)			OTU(%)	Proportion(%)	OTU(%)	Proportion(%)
Feb. and Apr.	12	16.22	59.39	36.36	95.14	St.1 and St.5	94	78.99	94.76	77.05	95.89
Feb.	62	83.78	40.61			St.1	25	21.01	5.24		
Apr.	21			63.64	4.86	St.5	28			22.95	4.11
Total	95	100.00	100.00	100.00	100.00		147	100.00	100.00	100.00	100.00

Table 9. Comparative analysis of shared OTUs generated by 23S universal primer set in samples collected at St.4 in February and April and at St.1 and St.5 in May, respectively

Data	OTUs	Feb.		Apr.		Data	OTUs	St.1		St.5	
		OTU(%)	Proportion(%)	OTU(%)	Proportion(%)			OTU(%)	Proportion(%)	OTU(%)	Proportion(%)
Feb. and Apr.	21	25.93	34.09	76.92	89.77	St.1 and St.5	48	82.76	98.69	82.76	96.97
Feb.	60	74.07	65.91			St.1	10	17.24	1.31		
Apr.	18			23.08	10.23	St.5	10			17.24	3.03
Total	99	100.00	100.00	100.00	100.00		68	100.00	100.00	100.00	100.00

Table 10. Top 20 OTUs obtained by 16S universal primer set in samples collected at St.4 in February and April

No.	Feb.				Apr.			
	Species	GeBank No.	Proportion(%)	Phylum	Species	GeBank No.	Proportion(%)	Phylum
1	*Candidatus pelagibacter*	LT840186	15.48	Proteobacteria	*Candidatus pelagibacter*	LN850161	34.22	Proteobacteria
2	*Verrucomicrobium* sp.	GQ262724	13.98	Verrucomicrobia	*Rhodobacteraceae bacterium*	KU173771	22.17	Proteobacteria
3	*Rhodobacteraceae bacterium*	KU173711	7.68	Proteobacteria	*Candidatus pelagibacter*	LN850159	9.26	Proteobacteria
4	*Gamma proteobacterium*	EF195480	6.09	Proteobacteria	*Roseobacter* sp.	KX465757	6.97	Proteobacteria
5	*Candidatus pelagibacter*	LN850159	2.85	Proteobacteria	*Alpha proteobacterium*	HQ675217	4.86	Proteobacteria
6	*Pseudoalteromonas strain*	CP011026	2.83	Proteobacteria	*Alpha proteobacterium*	HQ675195	3.74	Proteobacteria
7	*Alteromonadaceae bacterium*	KP770091	2.65	Proteobacteria	*Alpha proteobacterium*	HQ675244	0.98	Proteobacteria
8	*Flavobacteriaceae bacterium*	KF023505	2.53	Bacteroidetes	*Rhodobacteraceae bacterium*	KU173771	0.90	Proteobacteria
9	*Bacterium* sp.	JN699216	2.46	Proteobacteria	*Euryarchaeote* sp.	U11042	0.89	Euryarchaeota
10	*Verrucomicrobia bacterium*	KT933189	2.18	Verrucomicrobia	*Amylibacter ulvae*	NR146351	0.88	Proteobacteria
11	*Flavicella* sp.	NR134724	1.82	Bacteroidetes	*Candidatus pelagibacter*	CP002511	0.82	Proteobacteria
12	*Methylophilaceae bacterium*	KP770084	1.44	Proteobacteria	*Alpha proteobacterium*	JF488476	0.68	Proteobacteria
13	*Gamma proteobacterium*	LC018892	1.38	Proteobacteria	*Alpha* sp.	AB458529	0.67	Proteobacteria
14	*Alpha proteobacterium*	JF488580	1.30	Proteobacteria	*Unknown*		0.63	Unknown
15	*Bacteroidetes bacterium*	JF488553	1.24	Bacteroidetes	*Alpha proteobacterium*	JF488329	0.58	Proteobacteria
16	*Roseobacter* sp.	KX465757	1.21	Proteobacteria	*Alpha* sp.	JF488530	0.42	Proteobacteria
17	*Flavobacteriaceae bacterium*	LC075351	1.19	Bacteroidetes	*Hyphomonadaceae bacterium*	EU642858	0.40	Proteobacteria
18	*Polaribacter* sp.	LT629794	1.14	Bacteroidetes	*Actinobacterium scgc*	JF488172	0.38	Actinobacteria
19	*Bacteroidetes bacterium*	JF488604	1.10	Bacteroidetes	*Sedimentitalea* sp.	KP172215	0.37	Proteobacteria
20	*Polaribacter* sp.	AF493675	0.94	Bacteroidetes	*Sulfur-Oxidizing* sp.	AF181991	0.36	Proteobacteria

Table 11. Top 20 OTUs obtained by 16S universal primer set in samples collected at St.1 and St.5 in May

No.	St.1				St.5			
	Species	GeBank No.	Proportion(%)	Phylum	Species	GeBank No.	Proportion(%)	Phylum
1	*Rhodobacteraceae bacterium*	KU173771	13.43	Proteobacteria	*Candidatus pelagibacter*	LT840186	13.33	Proteobacteria
2	*Candidatus pelagibacter*	LT840186	10.72	Proteobacteria	*Rhodobacteraceae bacterium*	KU173771	7.89	Proteobacteria
3	*Roseobacter* sp.	KX467571	7.04	Proteobacteria	*Roseobacter* sp.	KX467571	7.64	Proteobacteria
4	*Gamma proteobacterium*	JF488603	5.97	Proteobacteria	*Gamma proteobacterium*	JF488603	6.04	Proteobacteria
5	*Unicellular* sp.	KY789460	2.77	Cyanobacteria	*Actinobacterium scgc*	JF488172	4.19	Actinobacteria
6	*Actinobacterium scgc*	KF488172	2.59	Actinobacteria	*Synechococcus* sp.	KU867931	3.87	Cyanobacteria
7	*Synechococcus* sp.	KU867931	2.48	Cyanobacteria	*Unicellular* sp.	KY789460	3.04	Cyanobacteria
8	*Alpha proteobacterium*	HQ675159	2.35	Proteobacteria	*Alpha proteobacterium*	HQ675159	2.20	Proteobacteria
9	*Alpha proteobacterium*	HQ675217	1.94	Proteobacteria	*Alpha proteobacterium*	JF488534	2.10	Proteobacteria
10	*Gamma proteobacterium*	NR134724	1.85	Proteobacteria	*Gamma proteobacterium*	HQ675210	1.93	Proteobacteria
11	*Flavicella* sp.	NR134724	1.84	Bacteroidetes	*Synechococcus* sp.	KU867940	1.69	Cyanobacteria
12	*Bacteroidetes* sp.	JF488593	1.55	Bacteroidetes	*Alpha proteobacterium*	HQ675217	1.56	Proteobacteria
13	*Formosa* sp.	CP017259	1.34	Bacteroidetes	*Formosa complete*	CP017259	1.45	Bacteroidetes
14	*Alpha proteobacterium*	HQ488534	1.33	Proteobacteria	*Bacteroidetes* sp.	JF488593	1.33	Bacteroidetes
15	*Gamma proteobacterium*	JF488180	1.28	Proteobacteria	*Verrucomicrobia bacterium*	JF488400	1.19	Verrucomicrobia
16	*Virgulinella fragilis*	JN207225	1.12	Foraminifera	*Methylophilaceae bacterium*	KP770084	1.03	Proteobacteria
17	*Rhodoluna* sp.	KU173555	1.09	Actinobacteria	*Alpha proteobacterium*	HQ675244	0.97	Proteobacteria
18	*Bacteroidetes bacterium*	JF488529	1.08	Bacteroidetes	*Bacteroidetes bacterium*	JF488529	0.96	Bacteroidetes
19	*Alpha proteobacterium*	HQ675244	0.93	Proteobacteria	*Rhodoluna* sp.	KU173555	0.82	Actinobacteria
20	*Flavobacterium* sp.	HQ175321	0.76	Bacteroidetes	*Obligately oligotrophic bacteria*	AB022713	0.81	Proteobacteria

Table 12. Top 20 OTUs obtained by 23S universal primer set in samples collected at St.4 in February and April

No.	Feb.				Apr.			
	Species	GeBank No.	Proportion(%)	Phylum	Species	GeBank No.	Proportion(%)	Phylum
1	*Micromonas pusilla*	FN563097	13.48	Chlorophyta	*Ostreococcus tauri*	KF285533	63.89	Chlorophyta
2	*Teleaulax amphioxeia*	KP899713	8.88	Cryptophyta	*Micromonas* sp.	FJ858267	7.84	Chlorophyta
3	*Nitzschia* sp.	EF426584	7.42	Bacillariophyta	*Micromonas pusilla*	FN563097	4.96	Chlorophyta
4	*Phaeocystis globosa*	KC900889	6.14	Haptophyta	*Teleaulax acuta*	FN563097	2.91	Cryptophyta
5	*Chrysochromulina sp.*	KJ201907	5.62	Haptophyta	*Pinus mugo*	KX833097	1.61	Pinophyta
6	*Cerataulina daemon*	KJ958484	4.74	Bacillariophyta	*Bathycoccus prasinos*	FO082259	1.36	Chlorophyta
7	*Teleaulax gracilis*	KP142643	4.33	Cryptophyta	*Phaeocystis globosa*	KC900889	1.25	Haptophyta
8	*Teleaulax acuta*	KP142645	3.93	Cryptophyta	*Teleaulax amphioxeia*	KP899713	1.13	Cryptophyta
9	*Micromonas pusilla*	L42847	3.20	Chlorophyta	*Bathycoccus* sp.	FO082259	1.06	Chlorophyta
10	*Neosiphonia japonica*	KC782888	3.04	Rhodophyta	*Kryptoperidinium foliaceum*	GU591328	0.89	Miozoa
11	*Cryptochloris* sp.	KP142652	2.03	Cryptophyta	*Teleaulax gracilis*	KP142643	0.70	Cryptophyta
12	*Micromonas pusilla*	FN563097	1.64	Chlorophyta	*Chrysochromulina* sp.	KJ201907	0.64	Haptophyta
13	*Rhodomonas lens*	KP142647	1.52	Cryptophyta	*Chrysochromulina* sp.	KJ201907	0.64	Haptophyta
14	*Chrysochromulina* sp.	KJ201907	1.47	Haptophyta	*Chrysochromulina* sp.	KJ201907	0.40	Haptophyta
15	*Mallomonas* sp.	KM817983	1.21	Ochrophyta	*Thalassiosira pseudonana*	EF067921	0.39	Bacillariophyta
16	*Mallomonas* sp.	KM817982	1.16	Ochrophyta	*Lithodesmium* sp.	KC509525	0.38	Bacillariophyta
17	*Bathycoccus prasinos*	FO082259	1.07	Chlorophyta	*Thalassiosira weissflogii*	KJ958485	0.34	Bacillariophyta
18	*Chrysochromulina* sp.	KJ201907	0.97	Haptophyta	*Micromonas pusilla*	FN563097	0.29	Chlorophyta
19	*Chrysochromulina* sp.	KJ201907	0.95	Haptophyta	*Cerataulina* sp.	KJ958484	0.29	Bacillariophyta
20	*Thalassiosira oceanica*	GU323224	0.73	Bacillariophyta	*Micromonas* sp.	FJ858267	0.26	Chlorophyta

Table 13. Top 20 OTUs obtained by 23S universal primer set in samples collected at St.1 and St.5 in May

No.	St.1				St.5			
	Species	GeBank No.	Proportion(%)	Phylum	Species	GeBank No.	Proportion(%)	Phylum
1	*Ostreococcus tauri*	KF285533	27.89	Chlorophyta	*Ostreococcus tauri*	KF285533	28.01	Chlorophyta
2	*Synechococcus* sp.	CP000435	20.83	Cyanobacteria	*Synechococcus* sp.	CP011941	19.68	Cyanobacteria
3	*Micromonas pusilla*	FN563097	5.42	Chlorophyta	*Micromonas pusilla*	FN563097	7.47	Chlorophyta
4	*Micromonas* sp.	FJ858267	5.03	Chlorophyta	*Micromonas* sp.	FJ858267	6.41	Chlorophyta
5	*Dinophysis acuta*	KP826904	4.84	Miozoa	*Synechococcus* sp.	CP011941	5.81	Cyanobacteria
6	*Synechococcus* sp.	CP000097	4.59	Cyanobacteria	*Chlorella* sp.	KF021304	3.30	Chlorophyta
7	*Chlorella cleb*	KF021304	3.17	Chlorophyta	*Synechococcus* sp.	CP011941	3.04	Cyanobacteria
8	*Bathycoccus prasinos*	FO082259	1.78	Chlorophyta	*Synura* sp.	KM590725	2.08	Ochrophyta
9	*Synura* sp.	KM590725	1.62	Ochrophyta	*Pseudo-Nitzschia multiseries*	KR709240	1.89	Bacillariophyta
10	*Chromulina* sp.	KM590766	1.40	Ochrophyta	*Chromulina* sp.	KM590766	1.80	Ochrophyta
11	*Teleaulax acuta*	KP142645	1.32	Cryptophyta	*Bathycoccus prasinos*	FO082259	1.75	Chlorophyta
12	*Pseudo-Nitzschia multiseries*	KR709240	1.18	Bacillariophyta	*Chrysochromulina* sp.	KJ201907	1.39	Haptophyta
13	*Synechococcus* sp.	CP011941	1.05	Cyanobacteria	*Mallomonas* sp.	KM817948	1.11	Ochrophyta
14	*Chrysochromulina* sp.	KJ201907	0.99	Haptophyta	*Dinophysis acuta*	KP826904	0.96	Miozoa
15	*Emiliania* sp.	JN022705	0.84	Haptophyta	*Chrysochromulina* sp.	KJ201907	0.67	Haptophyta
16	*Mallomonas* sp.	KM817948	0.71	Ochrophyta	*Micromonas pusilla*	L42847	0.47	Chlorophyta
17	*Teleaulax amphioxeia*	KP899713	0.71	Cryptophyta	*Teleaulax amphioxeia*	K899713	0.44	Cryptophyta
18	*Ochromonas* sp.	KJ877675	0.71	Ochrophyta	*Chrysochromulina* sp.	HM595078	0.44	Haptophyta
19	*Ectocarpus* sp.	FP102296	0.55	Ochrophyta	*Chrysochromulina* sp.	KJ201907	0.43	Haptophyta
20	*Dinophysis acuta*	KP826904	0.54	Miozoa	*Emiliania* sp.	JN022705	0.36	Haptophyta

Discussion

In present study, we analyze the microbial communities using NGS strategy to explain the ecological and environmental condition of Jaran bay. NGS technique has been known as the reliable strategy to analyze microbial community, which present unculturable microbes as well as culturable ones. Until now, only microbial 16S universal primers was used to analyze the microbial community. However, we adopted two primer sets 16S and 23S universal primer set. Generally, phytoplankton level is lower than bacterial level and phytoplankton communities could not be analyzed well with 16S universal primer set. Here, we used two primer sets and analyze both the microorganism and phytoplankton qualitatively and quantitatively. Metagenomic analysis with two primer sets would provide useful information about the microbial and phytoplankton communities, which would be further used to understand environmental and ecological conditions of coastal waters.

In April 2017, the ratio of phytoplankton to bacteria suddenly increased, which was considered spring bloom (Carstensen and Conley, 2004). As a result of metagenomic analysis, chlorophyta was dominant in April. Especially, *O. tauri* was most abundant whose proportions was 63.89 %. *O. tauri* is picophytoplankton, which contains the protein binding Chl *a*, b and the additional chlorophyll like c

(Chrétiennot-Dinet et al., 1995; Rodríguez et al., 2005). Chl *c* was isolated from *Ostreococcus* sp. (Álvarez et al., 2013). *Ostreococcus* sp. was found in several areas including the Mediterranean Sea (Dupuy et al., 2000; Vaquer et al., 1996), Long Island Sound, New York (O'Kelly et al., 2003), the English Channel (Guillou et al., 2004; Romari and Vaulot, 2004), the Arabian Sea (Brown et al., 2002), the San Pedro Channel in the North Pacific Ocean (Countway and Caron, 2006) and Pacific Ocean (Derelle et al., 2006).

In Mediterranean Sea, Thau Lagoon was important oyster farming area in Europe, which was similar to Jaran bay. We identified difference between the level of phytoplankton and Chl *a* In the area, proportions of *O. tauri* was high. In this area, the growth rate of oyster was high even though the concentration of Chl *a* was low. Picoplankton was abundant in oyster breeding area where was dominant by *O. tauri* (Vanquer et al. 1996, Phonis et al., 2006). This can be explained by several possible explanations. First, the pico-sized phytoplankton was not retained by gills of oyster because of a relatively low grazing pressure. *O. tauri* has the ability to use solar radiation efficiently and exist in high level without photodestruction even though light intensity is high such as in Thau Lagoon waters.

Another possible explanation about the difference in growth rate may be due to the adverse environmental condition. In top 20 OTUs in April, we found

Sulfur-Oxidizing sp. in the sample of St.4. This is anoxgenic heterotrophs, and they exist in anaerobic condition. They oxidized substrates, sulphate as the terminal electron acceptor (Friedrich, 1997). Presence of sulfur-oxidizing microbes indicates the hypoxia in the station. In fact, hypoxia warning was announced in June 2017 (NIFS, http://www. Nifs.go.kr). Through this record, the existence of *Sulfur-Oxidizing* sp. could suggested that the environmental condition was short of dissolved oxygen from April.

When this study compared seasonal variation and regional variation in microbial communities by NGS, the proportion of phyla remarkably changed in seasonal variation. In contrast, regional variation showed similar patterns. Also, they were similar in dominant species, their patterns and the proportion of them. So the similarity was high between St.1 and St.5. This result showed that changes in community structures in Jaran bay may have come from the seasonal factors not from the regional factors. As the conclusion, we were able to know that the microbial communities may provide an important information about the environmental conditions and a long-term surveys should be made to understand the various environmental events occur including algal bloom, hypoxia, and thermal stress and following effects on the ecosystem in Jaran bay.

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국문요약

미생물 군집은 환경조건을 보여주며, 이는 생물과 물리·화학적 환경요소들의 복잡한 상호작용의 결과이다. 자란만 내 환경조건을 알아보기 위하여, Miseq platform 을 이용하여 자가영양 식물플랑크톤과 종속영양 박테리아 군집의 시간적 변화와 공간적 변화를 연구하였다. 해수 샘플은 총 5개의 정점에서 2016년 12월부터 2017년 8월까지 매월 1회 채집되었다. 정점 4에서는 배고장으로 인하여 3월에 조사를 수행하지 못하였다. 전체의 미생물 군집은 16S universal primer set 로 분석이 되었으며, 반면에 식물플랑크톤 군집구조분석에는 Yoon 등과 Kang 에 의해 고안된 23S universal primer set 가 쓰였다. 정점별 월별로 측정된 수온은 5.1℃에서 29.4℃ 사이의 범위에 있었고, 염분은 28.5 psu 에서 33.3 psu 의 범위에 있었다. 클로로필 *a* 의 농도는 0.1015 μgL^{-1}에서 6.4974 μgL^{-1}까지의 범위에 있었다. qPCR 을 통해 전체 미생물군집에 대한 식물플랑크톤의 비를 측정하였으며, 그 수치는 2월에 가장 낮고 4월에 가장 높았다. NGS 데이터는 4월에 식물플랑크톤의 비가 증가한 주요인은 pico-size 의 녹조식물문의 *Ostreococcus tauri* 이었다. 그리고 5월에 정점1과 정점2에서 분석된 NGS 데이터는 두 정점 사이에서 서로 매우 비슷한 경향을 보이고 있었으며, 식물플랑크톤의 비의 차이는 *O. tauri* 가 정점5에 비하여 정점1에서 상대적으로 더 낮았기 때문인 것으로 생각된다. 이 결과를 통하여, 자란만 내 군집구조의 변화가 공간적 요소가 아닌 시간적 요소에 의하여 많은 영향을 받는다는 것을 알 수 있었다.

결론적으로, 우리는 미생물군집이 환경조건에 대하여 중요한 정보를 제공할 수 있다는 것을 알 수 있었으며, 자란만 내 생태계에서 일어나는 조류의 대증식, 빈산소수괴 그리고 고수온과 같은 다양한 환경적 현상들을 이해하기 위해서는 장기적인 연구가 전적으로 필요한 실정이다.

감사의 글

학위를 하는 동안 많은 도움을 주셨던 모든 분들께 감사의 인사를 전합니다. 저에게 다양한 관점에서 연구를 할 수 있도록 많은 가르침을 주신 김현우 교수님께 감사드립니다. 또 논문에 대하여 적극적으로 조언을 해주신 오철웅 교수님, 박경동 박사님께도 감사드립니다. 학업과 직장을 병행하느라 부족함이 많았는데, 많은 응원과 배려를 해주셨던 실험실 식구들과 저희 회사 식구들에게도 감사하단 말씀을 전하고 싶습니다. 마지막으로, 언제나 저를 믿어주시고, 저보다 더 제 걱정해주시는 가족들 그리고 항상 응원해주는 친구들에게도 감사의 인사를 전합니다.