การแปรผันตามฤดูกาลของประชาคมพิโคไฟโตแพลงก์ตอน นาโนไฟโตแพลงก์ตอน และไมโครไฟโตแพลงก์ตอนบริเวณเกาะสีชัง จังหวัดชลบุรี

นางสาวอณัศยา ดีสุข

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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SEASONAL VARIATIONS OF PICOPHYTOPLANKTON, NANOPHYTO-PLANKTON AND MICROPHYTOPLANKTON COMMUNITIES AT SICHANG ISLAND, CHONBURI PROVINCE

Miss Anassaya Deesuk

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Marine Science
Department of Marine Science
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Thesis Title	SEASONAL VARIATIONS OF PICOPHYTOPLANKTON, NANOPHYTO-PLANKTON AND MICROPHYTOPLANKTON COMMUNITIES AT SICHANG ISLAND, CHONBURI PROVINCE
By	Miss Anassaya Deesuk
Field of Study	Marine Science
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อณัศยา ดีสุข : การแปรผันตามฤดูกาลของประชาคมพิโคไฟโตแพลงก์ตอน นาโนไฟโตแพลงก์ตอน และไมโครไฟโตแพลงก์ตอนบริเวณเกาะสีชัง จังหวัดชลบุรี (SEASONAL VARIATIONS OF PICOPHYTOPLANKTON, NANOPHYTO-PLANKTON AND MICROPHYTOPLANKTON COMMUNITIES AT SICHANG ISLAND, CHONBURI PROVINCE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. อัจฉราภรณ์ เปี่ยมสมบูรณ์, 84 หน้า.

เกาะสีชัง จังหวัดชลบุรีตั้งอยู่ทางตะวันออกของอ่าวไทยตอนใน ได้รับอิทธิพลจากลมมรสุมทั้งลมมรสุมตะวันออกเฉียงเหนือและลมมรสุมตะวันตกเฉียงใต้ อิทธิพลจากลมมรสุมส่งผลต่อการเปลี่ยนแปลงปัจจัยสิ่งแวดล้อมรวมถึงคุณภาพของน้ำทะเลซึ่งกระทบต่อการเปลี่ยนแปลงประชาคมของแพลงก์ตอนพืช การศึกษาแพลงก์ตอนพืชส่วนใหญ่จะเน้นการศึกษาในกลุ่มไมโครไฟโตแพลงก์ตอน แต่ยังขาดข้อมูลในส่วนของพิโค- และนาโนไฟโตแพลงก์ตอนซึ่งมีขนาดเล็กเนื่องจากข้อจำกัดทางด้านเครื่องมือในการศึกษา จึงเป็นที่มาของการศึกษาในครั้งนี้ โดยการศึกษาการแปรผันตามฤดูกาลของประชาคมพิโค-, นาโน-, และไมโครไฟโตแพลงตอนจาก 10 สถานีรอบเกาะสีชัง ในช่วงฤดูมรสุมและระหว่างฤดูมรสุม โดยพิโค-และ นาโนไฟโตแพลงก์ตอนจะศึกษาโดย flow cytometry ส่วนไมโครไฟโตแพลงตอนศึกษาด้วยกล้องจุลทรรศน์แบบเลนส์ประกอบ รวมไปถึงความชุกชุม มวลชีวภาพในรูปคลอโรฟิลล์ *เอ* และสารอาหารอนินทรีย์ ผลการศึกษาพบว่าประชาคมของแพลงก์ตอนพืชแบ่งออกเป็น 4 กลุ่มตามฤดูกาลที่ศึกษา โดยมีปัจจัยหลักที่เกี่ยวข้องคือ อุณหภูมิ, ความเค็ม, และสารอาหาร ซึ่งแตกต่างกันอย่างชัดเจนในแต่ละฤดู มวลชีวภาพของพิโค-และ นาโนไฟโตแพลงก์ตอนเป็นองค์ประกอบหลักของประชาคมแพลงก์ตอนยกเว้นในฤดูมรสุมตะวันออกเฉียงเหนือซึ่งมีไมโครไฟโตแพลงก์ตอนกลุ่มไดอะตอมเป็นองค์ประกอบหลัก นอกจากนี้ สามารถแยกกลุ่มของพิโค- และ นาโนไฟโตแพลงก์ตอนออกเป็น 4 และ 5 กลุ่ม ได้แก่ Synechococcus และ picoeukaryote อย่างละ2 กลุ่ม และ cryptoophyte, coccolitophores, prasinophyte, และ nanoeukaryote 2 กลุ่ม ตามลำดับ โดยพบ Synechococcus เป็นกลุ่มเด่น ประชาคมพิโค-และนาโนไฟโตแพลงก์ตอนมีความหนาแน่นมากที่สุดในช่วงฤดูมรสุมตะวันออกเฉียงเหนือตรงข้ามกับความหนาแน่นของไมโครไฟโตแพลงก์ตอน

ภาควิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อนิสิต .....

สาขาวิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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ANASSAYA DEESUK: SEASONAL VARIATIONS OF PICOPHYTOPLANKTON, NANOPHYTO-PLANKTON AND MICROPHYTOPLANKTON COMMUNITIES AT SICHANG ISLAND, CHONBURI PROVINCE. ADVISOR: ASSOC. PROF. AJCHARAPORN PIUMSOMBOON, Ph.D., 84 pp.

Sichang Island is in the eastern part of the Inner Gulf of Thailand where is primarily influenced by two monsoon winds, the northeast monsoon and the southwest monsoon. The effect of this factor lead to changes in phytoplankton community. However, most studies have been focused on microphytoplankton community not pico- and nanophytoplankton due to their small size and study methods. Therefore, seasonal variations of pico-, nano-, and microphytoplankton around Sichang Island were observed. The samplings were conducted at 10 stations around Sichang Island in two monsoon and two intermonsoon periods. Pico- and nanophytoplankton were analyzed by flow cytometry whereas microphytoplankton were analyzed by a compound microscope. Phytoplankton abundance, size-frationated of chlorophyll *a* and inorganic nutrient concentrations were analyzed. The community structure of pico- nano- and microphytoplankton could be divided into four groups dominated with diatoms and Synechhococcus which were related to the main factors; temperature, salinity, and nutrient concentrations which clearly differed between seasons. Pico- and nanophytoplankton were observed as major contributor to phytoplankton biomass in all seasons except in the northeast monsoon where microphytoplankton was dominated. Flow cytometry resolved 4 groups of picophytoplankton and 5 groups of nanophytoplankton. They were high in density during the northwest monsoon in contrast to microphytoplankton density.

Department:	Marine Science	Student's Signature	.....
Field of Study:	Marine Science	Advisor's Signature	.....
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CHAPTER I
INTRODUCTION

Picophytoplankton, nanophytoplankton, and microphytoplankton ranges from 0.2 – 2 µm, 2 -20 µm, and 20 - 200 µm, respectively. They are producer in grazing food and microbial food web (Azam et al. 1983). The small size of pico- and nanophytoplankton makes them have more ability to uptake dissolved materials than larger phytoplankton or microphytoplankton (Marañón, 2015). Thus, these tiny cells dominated phytoplankton primary production especially in the oligotrophic region where the nutrients are limited. Recently, there has been growing interest in microbial food web which mostly involves in small phytoplankton. Nowadays, climate change leads to changing in environmental factors and monsoon patterns and they strongly affected the plankton communities including cell size structure in marine food webs (Acevedo-Trejos et al., 2015). In Thailand, there were little studies of small-sized phytoplankton communities whereas there have been focused only on microphytoplankton (20 - 200 µm) and zooplankton. Some information in picophytoplankton and nanophytoplankton have been reported in an estuary (Gunbua et al., 2012) and mangrove forest (Tarangkoon, 2002), rarely in coastal clear water of Thailand.

Objective

The aim of this study is to determine seasonal variation of picophytoplankton, nanophytoplankton, and microphytoplankton communities around Sichang Island.

Scope of the study

To study seasonal pattern of picophytoplankton, nanophytoplankton, and microphytoplankton assemblages over one year in 2016, the samplings were conducted at 10 stations around Sichang Island in two monsoons periods (the northeast monsoon and the southwest monsoon) and two intermonsoons periods (the first intermonsoon and the second intermonsoon). Seawater samples were collected to enumerate pico- and nanophytoplankton and microphytoplankton using flow cytometry and compound microscope. Phytoplankton abundance, size-frationated of chlorophyll *a* and Inorganic nutrient concentration were analyzed to study relationship with physiochemical parameters. Similarity and ordination methods were used to compare seasonal variations of phytoplankton communities.

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Expected outcome

The change in diversity, abundant and distributional patterns of picophytoplankton, nanophytoplankton, and microphytoplankton data will use to determine how this change may affect marine biological resources around Sichang Island.

Literature reviews

Phytoplankton are small phototrophic organisms which use sunlight and nutrients to convert carbon through photosynthesis process into organic matters which are the basis resource for all living creatures throughout food chains. Because of the ability to produce their own food, they are function as producer in aquatic ecosystem like higher plants and algae. They have to live at the surface layer of water or euphotic zone (upper 200 meters) from tropical oceans to beneath snow-covered Arctic ice sheet (Assmy et al., 2017) where penetrating sunlight is enough for photosynthesis.

Before 1980s, studies on phytoplankton aimed at microalgae of size larger than 20 µm which can be collected by net sampling (Ingebrigtsen et al., 2017). However, after Pomeroy (1984) marine scientists started to aware of microalgae living as plankton that had size range smaller than 20 µm down to the size of less than 1 µm, same size as bacteria. Nowadays, phytoplankton can be categorized into 3 groups based on their sizes. These include picophytoplankton, nanophytoplankton, and microphytoplankton (Sieburth et al., 1978) which ranges from 0.2 – 2 µm, 2 -20 µm, and 20 - 200 µm, respectively (Figure 1). They are producer in grazing food web. They are also related to microbial food web (Azam et al., 1983) because some microbes can utilize the organic matter exudates by phytoplankton and turn to inorganic nutrients which is vital ingredients for photosynthesis of phytoplankton

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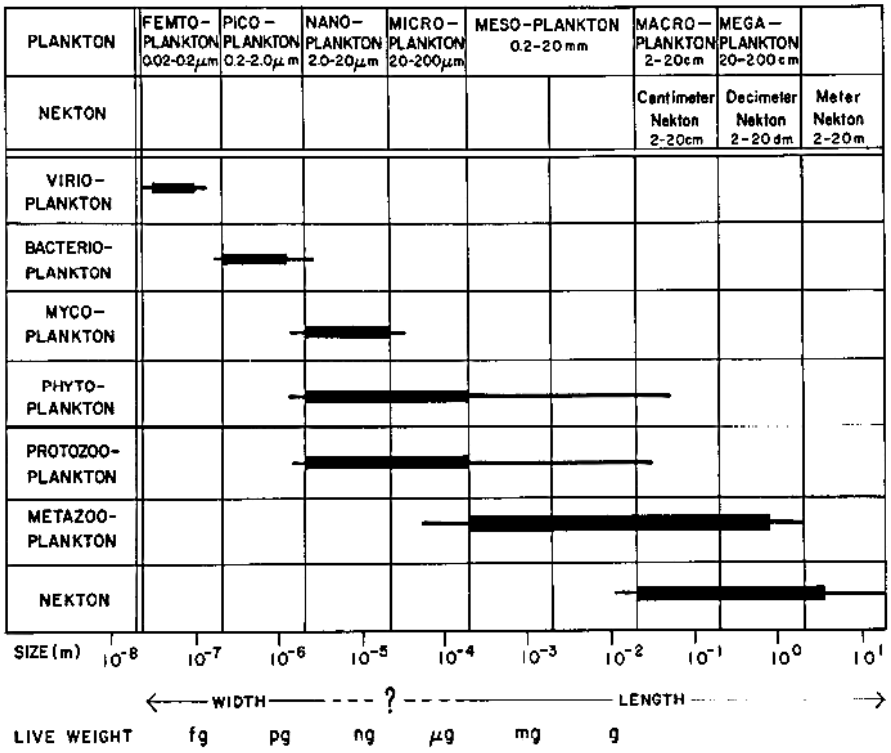

Figure 1 Size range of plankton
(Sieburth et al., 1978)

Pico- and nanophytoplankton

Picophytoplankton have been reported as important producer in the world's oceans in term of biomass in both open oceans and coastal waters (Campbell et al., 1994). They are frequently distinguished into three populations which can be observed by flow cytometry technique (Vaulot et al., 2004). The first two groups are prokaryotic coccoid unicellular cyanobacteria genus *Synechococcus* (Johnson and Sieburth, 1979) and *Prochlorococcu*s. They can be separated based on their size and fluorescence characteristics and the others is photosynthetic picoeukaryotes. Because of their very small size (mostly 1 μm in diameter), they had been overlooked previously until the fluorescence microscope was introduced to study of marine phytoplankton. The cell was studied by filtering onto the black membrane filter and examined under epifluorescense microscope. Then, the blue light excite through the objective lens passes to the cell sample. Their autofluorescence from photosynthetic pigments can be detected whereas bacteria and detritus unable to auto-fluoresce (Fogg, 1986).

Three groups of picophytoplankton will be described in detail in the following paragraphs.

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Synechococcus

In euphotic zone, *Synechococcus* frequently contributed up to half or more of the photosynthetic biomass in almost all marine environment (Miller and Wheeler, 2012). The small cell contains chlorophyll *a* as a primary photosynthetic pigments and phycobilliproteins as its asscessory pigments (Waterbury et al., 1979). Most of cyanobacteria consist of three types of phycobilliproteins; phycocyanin, allophycocyanin and allophycocyanin B. But some strains contain phycoerythrin. Phycocyanin and phycoerythrin are the major phycobilliproteins in *Synechococcus* which affected the cell color (Figure 2). Their pigments can fluoresce in the orange wavelength when excited by blue light. The marine *Synechococcus* groups mostly contain phycoerythrin that will present reddish-orange to olive-green depend on ratio of phycoerythrin to phycocyanin (Olson et al., 1988; Waterbury et al., 1986) as showed in Figure 3.

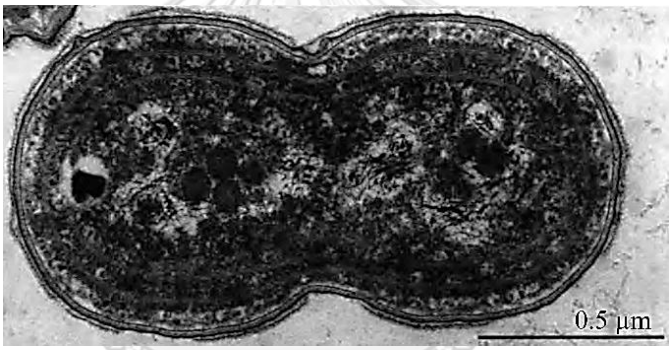

Figure 2 *Synechococcus*
(Waterbury et al., 1986)

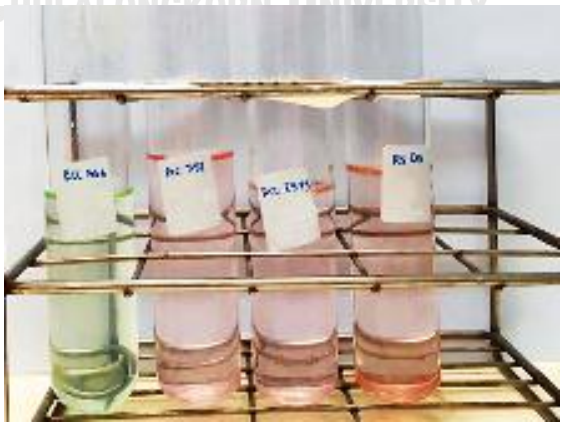

Figure 3 *Synechococcus* strains occur in different color (three tube in the left side). All strains received from the Roscoff Culture Collection (RCC), Roscoff, France.

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Prochlorococcus

The discovery of *Prochlorococcus*, a new group of picoplankton, has changed our understanding in marine food web (Chisholm et al., 1988). It is the smallest prochlorophyte, 0.5 – 0.7 µm in diameter (Figure 4), which is the most abundant on the earth (Partensky et al., 1999a) especially in oligotrophic, tropical, and subtropical oceans (Biller et al., 2014; Campbell and Vaulot, 1993). It can be found as deep as 200 m, and sometimes reaches abundance greater than 10^5 cells per ml (Partensky et al., 1999b). This organism is closely related to *Synechococcus* in genetic analysis, many phenotypic and ecological traits (Biller et al., 2014)

Prochlorococcus ia unicellular or colonial bacteria which lackind membrane-bound plastids (Reynolds, 2006b).has divinyl chlorophyll *a* and divinyl chlorophyll *b* as primary photosynthetic pigments differ from other plants (Goericke and J., 1992) which present chlorophyll *a* as main photosynthetic pigment. Moreover, it lacks phycobillisomes which found in almost cyanobacteria. Dissimilar to *Synechococcus*, *Prochlorococcus* uses divinyl chlorophyll *a* and divinyl chlorophyll *b* binds with prochlorophyte chlorophyll-binding protein (Pcb) as the main light-harvesting antenna complex including monovinyl chlorophyll *b* as asscessory pigments in photosynthetic process instead of phycobillisomes. This unique characteristic of its pigments increase the absorption of blue light which make it dominant in deep waters (Chisholm et al., 1988; Goericke and J., 1992).

Both *Prochlorococcus* and *Synechococcus* can distinguish by cell size, photosynthetic pigment and unique adaptation to environmental condition such as light and temperature (Moore et al., 1995). Moreover, they are different in seasonal pattern (Olson et al., 1990).

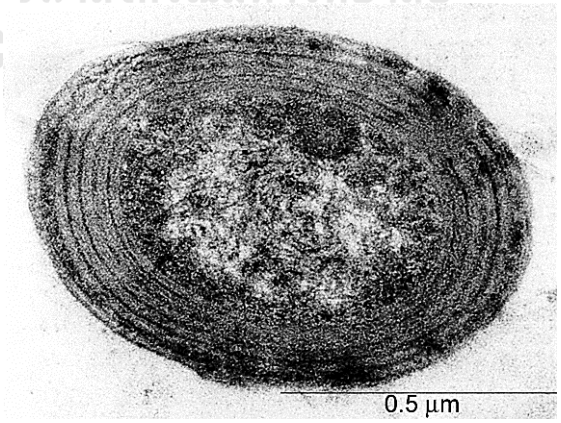

Figure 4 *Prochlorococcus*
(Partensky et al., 1999b)

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Photosynthetic picoeukaryotes

The reported of very small photosynthetic eukaryotes were thoroughly studied but in a detail description. It was begun around 70 years ago since the electron microscope was introduced to study spermatozoid of *Fucus* (Manton and Clarke, 1950). There was a report in water samples from Iceland to the Caribbean Sea (Johnson and Sieburth, 1982). In spite of similar in morphology, these picoeukaryotes consist of different organisms which mostly compose of flagellated algae, prasinophytes, at the coastal area and haptophytes, chrysophytes, and pelagophytes in the open sea regions.

Nanophytoplankton

Nanophytoplankton recorded as a large proportion of phytoplankton biomass (Tarran et al., 2001). Members of nanophytoplankton were mostly diverse in taxonomy which consist of unicellular gold or yellow-brown flagellates (2 divisions; Haptophyta and Chrysophyta), cyanobacteria, and diatoms.

Haptophyta contains a single class, Prymnesiophyceae, is well-known as coccolithophorids. It is spherical or ovoid cell with the size ranges from 5 – 20 µm. It build surface covering with $CaCO_3$ (calcite) plates or coccoliths which surround its cell to form coccosphere (De Vargas et al., 2007; Jeffrey et al., 1997; Tyrrell and Young, 2009). The high density of coccolithophorids generates white water event or large bloom which commonly cause by *Emiliana*.

Crysophyceae consists of two classes, Chrysophyceae and Raphidophyceae. The members of Chrysophyceae are silicoflagellates (order Dictyochales) and nanoplanktonic chrysophyes (order Parmales). They are coccoid or ovoid sphere with the size range from 20-200 µm and 2-5 µm in silicoflagellates and nanoplanktonic chrysophyes, respectively. Raphidophyceae is called as chloromonads. The well-known marine species is *Chattonella* which were found.

Microphytoplankton

Cyanobacteria

Cyanobacteria are blue-green algae contain chlorophyll *a* and phycobilins as accessory pigments. Their life form are unicellular, filamentous, or coenobia (Reynolds, 2006b). The cyanobacteria are classified into Division Cyanophyta (lately Cyanobacteria) consist of 3 order, Order Chroococcales, Order Oscillatoriales, and Order Nostocales. The first order is mostly unicellular living form or coenobia but never filamentous such as *Chroococcus* and *Merismopedia.* The second order is uniseriate filamentous cyanobacteria such as *Trichodesmium, Pseudanabaena*, and *Spirulina.* The others is unbranch-filamentous cyanobacteria mostly found in the fresh water and dilute

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seas. They may be facultatively differentiated into heterocysts such as *Anabaena* and *Anabaenopsis.*

Diatoms

Diatoms are characterized by their exoskeleton and cell wall made of biogenic silica and organic layer called frustule or theca (Kraberg et al., 2010; Tomás, 1996). They have the basic architecture of two theca, epitheca and smaller hypotheca, fitting in to the other like a petri dishes and bound by one or more band among the theca called girdle bands or cingulum. The valves have a unique grooves patterns, perforations, and process patterns which can be used to identify a cell to species level (Reynolds, 2006b; Tomás, 1996)

Diatoms can be classified into Division Heterokontophyta, Phylum Bacillariophyta, Class Bacillariophyceae. They are single cells, filaments and coenobia which the pattern of this characteristics is one of the important feature to identify the diatoms (Kraberg et al., 2010). Other features are the inter-linkage of cells and the shape or the apertures form are also needed. They contain chlorophyll *a* and *c* including fucoxanthin, diaxoxanthin, and diadinoxanthin as accessory pigments. The diatoms consist of two order, Order Biddulphiales (centric diatoms) and Order Bacillariales (pennate diatoms). In centric diatoms, the valves are radially symmetrical, cylindrical and sometimes separated by girdle bands such as *Thalassiosira, Chaetoceros, Rhizosolenia,* and *Skeletonema.* In pennate diatoms, they are boat-like halves and bilaterally symmetric which have elongate valves parallel to the raphes. Cytoplasm leaks along the raphes, make the cell movement (Miller and Wheeler, 2012). The common genera were *Thalassionema, Nitzschia, and Navicula*.

Dinoflagellates

Dinoflagellates mostly unicellular with two flagella of unequal orientation and length. They are classified into Division Dinophyta. The cells contain chlorophyll *a* and *c* including betacarotene, peridinin, and fucoxanthin as accessory pigments. There are two morphological types, desmonkont and dinokont. The first type have two dissimilar flagella emerge from the anterior part of cell while the other the two flagella are inserted ventrally; one flagellum is transverse in a groove, the cingulum, and the other is simpler flagellum and housed in lontitudinal groove, the sulcus. Dinoflagellates can be divided into three broard groups: the unarmoured Gymnodiniales and the armored peridiniales and Dinophysales (Miller and Wheeler, 2012; Tomás, 1996). The genera commonly found in coastal water is *Ceratium, Noctiluca, Dinophysis,* and *Protoperidinium*.

Some member of dinoflagellates, *Noctiluca scintillans*, has chlorophyll-containing endosymbiont *Pedinomonas noctilucae* (Sweeney, 1971) in cell. In coastal area, dinoflagellates are responsible of red tide in the surface layer of water as irregular

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patches. The color of seawater become greenish or reddish color depend on species from one to another dinoflagellates. The common species which cause the red tide are *Noctiluca scintillans, Ceratium furca*, and *Dinophysis caudata.* The last one can cause Diarrhetic Shellfish Poisoning (DSP), a human intoxication by the consumption of shellfish (Yasumoto et al., 1985). *Noctiluca scintillans* is also a heterotrophic species feeding on phytoplankton,copepod as well as eggs of copepod and fish (Nakamura, 1998; Nikishina et al., 2011). They might affect on copepod *Acartia clausi* which is important food for planktivorous fishes like anchovy and sand-eel. Hence, *N. scintillans*'s predation might restrict the availability of food for commercial fishes (Sekiguchi and Kato, 1976).

Silicoflagellates

Silicoflagellates are silica-secreting marine microplankton which constitute a small component of microphytoplankton (Boney, 2009; Haq, 1998). They can be classified into Phylum Chrysophyta (golden algae), Class Dictyochophyceae. They are unicellular or colony forming flagellates containing extraordinary siliceous scales containing chlorophyll *a* and *c*, including fucoxanthin as accessory pigments. The genus commonly found in coastal water is *Dictyocha* (Boney, 2009; Wang et al., 2016).

Factors affecting phytoplankton community structure

In aquatic ecosystem, there are physio-chemical forcing and biological processes which couple together which cause temporal and spatial heterogeneity of phytoplankton (Steele, 1985). The major factors influencing phytoplankton community were described in detail in the following paragraphs.

Temperature

Temperature is an important factor for picophytoplankton rather than nanophytoplankton especially *Prochlorococcus* which were recorded in very low density or cannot detected (<23 cell/ml) if the water temperature was exceed than 26 ^{o}C (Pan et al., 2007) while *Synechococcus* and picoeukaryotes were usually more abundance and presented in higher concentration than *Prochlorococcus* in eutrophic coastal water (Pan et al., 2005; Partensky et al., 1999a).

Salinity

Salinity is one of abiotic factors that affected the distribution of phytoplankton. The change in salinity cause the water fluxes in the cell. In phytoplankton, turgor pressure were occurred depend on the types of cells (Bisson and Kirst, 1995) and may change in distributions of, and variations in, phytoplankton communities from green algae to cyanobacteria which have more tolerate in higher osmotic stress (Chakraborty

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et al., 2011). High salinity often occurred in nutrient-poor water which also more favor environment for picophytoplankton due to the high surface/volume ratio such as in Levantine Basin (Quéré Corinne et al., 2005).

Nutrients

Nutrients are basic requirement of phytoplankton. Some are directly needed and affected to the abundances of phytoplankton such as oxygen, hydrogen, and nitrogen, some are required in smaller amounts such as phosphorus and calcium, or as vital traces such as silicon and iron (Reynolds 2006b). However, the amounts of nutrients relate to demand and resource supply in each phytoplankton groups. In the result, their amounts will control the appearance of phytoplankton growth dynamic (Reynolds 2006b) until characterize community structure which associated with different environmental drivers (Reynolds 2006a). Small phytoplankton like *Prochlorococcus* was dominated in the tropical and subtropical oligotrophic water while large phytoplankton were dominated in high nutrient concentration water (Agawin et al., 2000; Chisholm et al., 1988; Marañón et al., 2001).

Another nutrients composition is carbonate ion. Decreasing of $[CO_3^{2-}]$ caused the declining of coccoliths mass and calcification in coccolithophorids (Beaufort et al., 2011).

Light

Prochlorococcus has different light-harvesting antenna complex systems compared with *Synechococcus*. Thus, *Prochlorococcus* responses to the absorption of blue light that dominant in deep water. This adaptation related to niche partitioning of both organism (Biller et al., 2014). In low light intensity, the proportion of pico- and nanophytoplankton are lower than heterotrophic pico- and nanoplankton. The predation of them and cell lysis by virioplankton (virus) in picophytoplankton is a factors that controls the abundance and assemblage of species composition (Fuhrman, 1999). Moreover, light intensity and fluxes of nutrients is combine effect to control the abundance and distributions of phytoplankton blooms (Falkowski, 1994)

Study of pico- and nanophytoplankton

The identification of microphytoplankton usually using morphology which observe by light microscope. The density of microphytoplankton can be counted and identified by using Sedwidge-Rafter slide. In order to study pico- and nanophytoplankton, it is very difficult to identify by light microscope because of limitation of their tiny size especially *Prochlorococcus* and *Synechococcus*.

The traditional method for counting nanophytoplankton is filter-transfer-freeze (FTF) technique (Hewes and Holm-Hansen, 1983) while picophytoplankton can be

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recognized and enumerated by using epifluorescence microscope (Waterbury et al., 1979). The sample are filtered onto the black membrane filter which they often fixed first with glutaraldehyde or formaldehyde, then filtered covered with a drop of immersion oil and a cover slip, and determined in the epifluorescence microscope (Johnson and Sieburth, 1979). The phytoplankton cell generally auto-fluoresce from green to red wavelength when excited by blue light whereas bacteria and detritus unable to auto-fluoresce (Fogg, 1986). It is extremely useful to discriminated groups of picophytoplankton. Phycoerythrin-containing cells appear orange and other appear red (Li, 1986).

Flow cytometry

In the present, there is a suitable method to examine the pico- and nanophytoplankton which automatically distinguishing and counting the large number of cells is flow cytometry (Olson et al., 1988). It relies on autofluorescence like an epifluorescence microscope but easier to identifiy the phytoplankton cell which were too dim (Marie et al., 2000). The sample is allowed to flow through a narrow tube fitted with photomultipliers cell by cell and categorize in respect to size and fluorescence response based on composition of pigment in cell. It is more rapid and precise, and less tedious than microscopy (Li, 1986; Miller and Wheeler, 2012; Olson et al., 1993) thus there are widespread of using flow cytometry in oceanographers, for example, study of pico- and and nanoplankton abundance, biomass and community structure (Tarran and Bruun, 2015; Zhang et al., 2008; Zhao et al., 2016).

Recent studies in Thailand

The studies in Thailand have been only focused on microphytoplankton (20 - 200 µm) and zooplankton while reported in picophytoplankton and nanophytoplankton were slightly describes. All informations studied in estuaries (Gunbua et al., 2012; Phromthong, 1999) and mangrove forest (Tarangkoon, 2002) which using epifluorescence microscope and filter-transfer-freeze (FTF) technique (Hewes and Holm-Hansen, 1983) to study cell abundance including size-fractionated chlorophyll *a* determination (Keith et al., 2002) to study biomass. However, the biomass of pico- and nanophytoplankton determination by using size-fractionated chlorophyll *a* technique were higher than in microphytoplankton. The populations of small size phytoplankton were dominated by heterotrophic picoplankton in similar studied at Bangpakong estuary which nanophytoplankton biomass were reached the highest proportion at 84% of primary producer (Gunbua et al., 2012) and there were nanoflagellates and nanocyanobacteria as dominant groups. The study at Pakpoon and Bandon bay also found the pico- and nanophytoplankton were higher than microphytoplankton (Piumsomboon et al., 1999; Plongon and Salaenoi, 2015). Another reported in Andaman Sea found *Synechococcus* biomass contained 72 – 74 % of total

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phytoplankton biomass (Nielsen et al., 2004). The study on microphytoplankton in the inner Gulf of Thailand found the diatoms dominated the populations followed by dinoflagellates which the dominant genera were *Chaetoceros, Rhizosolenia, Nitzschia, Noctiluca* and *Ceratium* while dominant cyanobacteria were *Trichodesmium* and *Richelia* (Boonyapiwat, 1999; Chumnantana, 2006).

The study on pico-, nano- and microphytoplankton will create important basis data which helps us to understand the roles of phytoplankton and fulfill the knowledge on small size of phytoplankton in the coastal ecosystem around Sichang Island.

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CHAPTER II
MATERIALS AND METHODS

Study area

Sichang Island is an island approximately 12 kilometers offshore of Sriracha district, Chonburi province. The island locates in the eastern part of the Inner Gulf of Thailand with a total area of 7.9 square kilometers. The climate pattern in this area is influenced by two monsoon winds, the northeast and the southwest monsoons (Loo et al., 2015). The northeast monsoon starts from November to March with the dry and cold mild air from Siberia while the southwest monsoon which begins in May and lasts to September brings the warm moist air from Indian Ocean together with rain fall over the area. These monsoons prevail the oceanographic conditions including temperature, salinity, and current circulation in the Inner Gulf of Thailand including Sichang Island (Buranaprathepprat et al., 2006).

Methods

1. Sample collection

The samplings were conducted at 10 stations around Sichang Island in January, April, July and November of 2016 (Table 1and Figure 5). These sampling periods represented 4 seasons; the northeast monsoon or dry season, the first intermonsoon, the southwest monsoon or wet season, and the second intermonsoon, respectively.

Table 1 Sampling stations

Station	Latitude	Longitude
SC 1	N 13˚ 10' 54.78"	E 100˚ 48' 20.22"
SC 2	N 13˚ 09' 53.10"	E 100˚ 48' 47.16"
SC 3	N 13˚ 09' 08.64"	E 100˚ 49' 17.04"
SC 4	N 13˚ 07' 38.16"	E 100˚ 48' 53.46"
SC 5	N 13˚ 08' 02.82"	E 100˚ 43' 18.66"
SC 6	N 13˚ 08' 43.08"	E 100˚ 48' 06.72"
SC 7	N 13˚ 09' 39.00"	E 100˚ 47' 54.12"
SC 8	N 13˚ 06' 59.34"	E 100˚ 48' 47.28"
SC 9	N 13˚ 06' 53.76"	E 100˚ 48' 14.64"
SC 10	N 13˚ 07' 18.24"	E 100˚ 48' 26.40"

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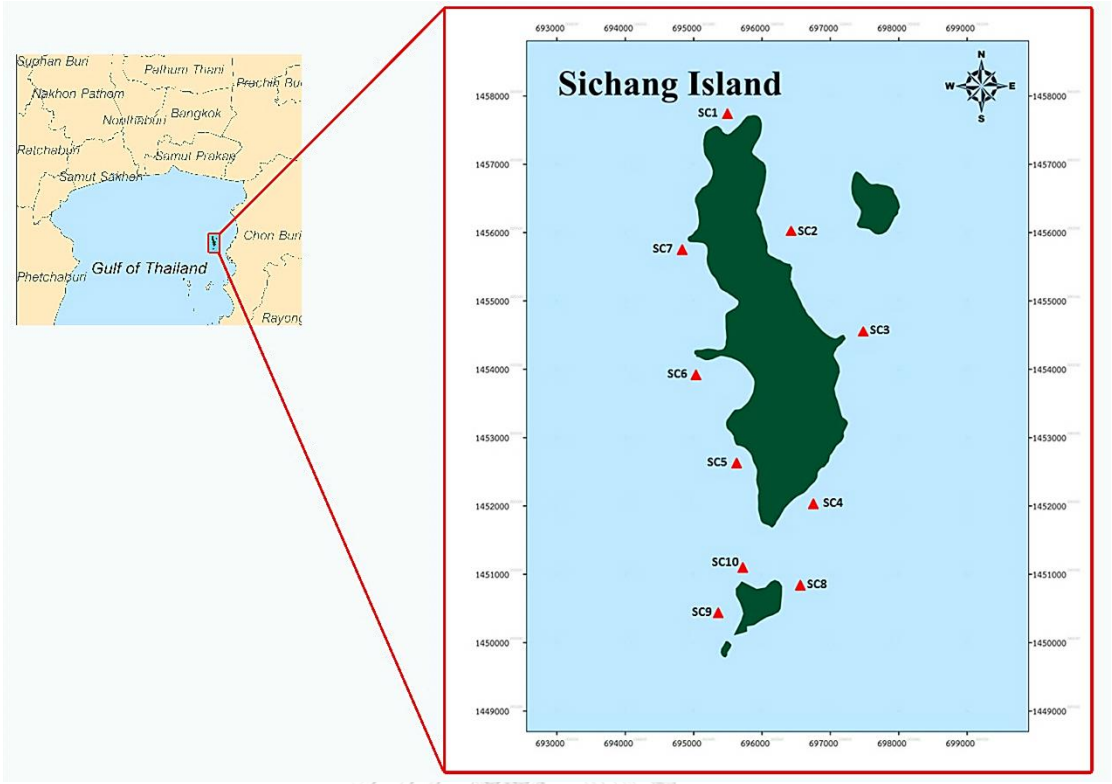

Figure 5 Sampling stations around Sichang Island

1.1. Physio-chemical parameters

Physio-chemical parameters of seawater were measured *in situ* at depth of 0.5, 1.0, and every 1.0 meter interval down to 1 meter above from the bottom. Depth were measured using a depth sounder (Speedtech). Light intensity and water transparency were measured by a light meter with spherical quantum sensor LI-193 SA (LI-COR) and secchi disc, respectively. Reading of temperature, salinity, dissolved oxygen and pH were obtained from multi-parameter water quality sonde (YSI XL600).

1.2. Water samples for Chlorophyll a and nutrient analyses

At each station, an aliquot of 1 L of seawater from two depths (0.5 m from surface and mid-depth due to total depth at each station) was taken in duplicate using a Van dorn water sampler, pre-screened with a 200 µm mesh net to remove zooplankton and large particles and immediately frozen at - 20˚ C for chlorophyll *a* and dissolved inorganic nutrient other than ammonia-nitrogen analysis.

For ammonia-nitrogen analysis, 60 ml of seawater was collected in duplicate at each depth, kept in polyethylene bottles and immediately frozen at - 20˚ C for further analysis (Parsons et al., 1984).

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1.3. Phytoplankton sampling

At each station, 20 L of seawater from the same depths as water sample for chlorophyll *a* were collected in duplicate using a centrifugal pump. Seawater samples were filtered through a 20 µm mesh net. Retained phytoplankton samples were stored in a 180 ml pre-cleaned plastic bottle and preserved with mixed solution of Lugol' solution: formalin: sodium thiosulphate (Sherr and Sherr, 1993) for study of microphytoplankton abundance.

The 20 µm filtrate fraction was kept in a 15 ml conical tube (Nunc) fixed with 1% glutaraldehyde (final concentration) and kept on dry ice in dark before being transferred to a -80˚ C freezer (Vaulot et al., 1989) for the study on abundance of picophytoplankton and nanophytoplankton (Tarran and Bruun, 2015) by a Cytomics FC 500 MPL flow cytometer (Beckman Coulter, USA).

2. Analyses of dissolved inorganic nutrients

Each samples, 500 ml subsample was filtered through Whatman GF/C glass-fiber filter to remove suspended particles. The filtrate was transferred to 500 ml bottle and immediately frozen at - 20˚ C for dissolved inorganic nutrients (nitrite-nitrogen, nitrate-nitrogen, phosphate-phosphorus, and silicate-silica-silicon) analyses. The samples were analyzed within one week after collection using colorimetric methods (Parsons et al., 1984).

Ammonia-nitrogen concentration was analyzed using the alternative method (Parsons et al., 1984).

3. Study of phytoplankton community structure

3.1. Size-fractionated chlorophyll a

For size-fractionated chlorophyll *a* determination, each seawater sample was separated into 3 fractions. The first 100 ml was filtered onto a Whatman GF/F glass-fiber filter for the measurement of total phytoplankton chlorophyll *a* (Chl_{total}). The second aliquot of 100 ml pre-filtered with a 20 µm-mesh net was filtered onto another Whatman GF/F glass-fiber filter for the determination of <20 µm phytoplankton chlorophyll *a* ($Chl_{pico\text{-}nano}$). The other fraction also pre-filtered with a 20 µm-mesh net before filtered onto a 3 µm nucleopore membrane filter to measure chlorophyll *a* fraction from 3-20 diameter phytoplankton (Chl_{nano}). All filtered samples were kept in cover with aluminum foil and immediately frozen at -20˚ C until analysis.

The filters were extracted with 10 ml of 90% acetone solution and placed in a refrigerator for 24 hours. The samples were centrifuged at 2000 rpm for 10 minutes

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and measured with Trilogy Laboratory Fluorometer (Turner Designs) equipped with U.S.EPA. Method 445.0 (Arar and Collins, 1997).

The concentration of chlorophyll *a* in each size-fraction was calculated by the following formula:

microphytoplankton chlorophyll *a* concentration = $Chl_{total} - Chl_{pico\text{-}nano}$
nanophytoplankton chlorophyll *a* concentration = Chl_{nano}
picophytoplankton chlorophyll *a* concentration = $Chl_{piconano} - Chl_{nano}$

When Chl_{total} = total chlorophyll *a* fraction
$Chl_{pico\text{-}nano}$ = <20 µm phytoplankton chlorophyll *a* fraction
Chl_{nano} = 3-20 diameter phytoplankton chlorophyll *a* fraction

3.2. Abundance and diversity on a microphytoplankton

Preserved phytoplankton sample was enumerated under a compound microscope (Olympus BX51 model) in a Sedwidge-Rafter slide and identified to genera level based on morphology (Round et al., 1990; Tomás, 1996). Cell count was converted to density in the unit of cell/L (except cyanobacteria, *Trichodesmium*, was counted in the unit of trichomes/L) using the following formula.

$$\text{Phytoplankton cell density (cell/l)} = \frac{A \times B}{C}$$

When A = number of cell in 1 ml (cell)
B = sample volume in bottle (ml)
C = total volume of seawater filtered (l)

Shannon - Weiner diversity index and Pielou's evenness index were performed by using PRIMER 6 (Clarke and Warwick, 2001) to measure diversity and evenness of microphytoplankton, respectively.

3.3. Abundance of picophytoplankton and nanophytoplankton by flow cytometry

The principle of flow cytometer consists of three part. The first is laser beam disperse the cells. Second, they emit fluorescence after the laser excitation. The last is the photomultipliers collected the data and sent to the computer and then they are processes by software (Gasol and Morán, 2016).

In general, phytoplankton can be discriminated from heterotrophic bacteria and non-living particles using fluorescing pigments. The common pigments are chlorophyll and phycobilliproteins (phycoerythrin, and phycocyanin). They are

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well excited by 488 nm excitation laser and fluoresce at and 570 nm (orange) and 690 nm (red) (Marie et al., 2010), respectively.

Preliminary study was conducted with five phytoplankton cultures in order to set a suitable protocol for samples. The two cultures consist of two strains of picocyanobacteria; *Synechococcus* RS 9917 (greenish color) and *Synechococcus* WH 8018 (pinkish-orange color). The third was cryptophyte; *Rhodomonas salina*. The forth was prasinophyte; *Tetraselmis sp.* The others was diatom; *Chaetoceros sp.* (Table 2 and Table 3). *Synechococcus* represents picocyanobacteria group which contain chlorophyll and phycobilliproteins (phycoerythrin, and phycocyanin). The *Synechococcus* RS 9917 is contain more phycocyanin: phycoerythrin ratio that made turn it to greenish color than the others, WH 8018, which shows pinkish-orange color. *Rhodomonas salina* represents cryptophyte group which contains both chlorophyll *a* and phycobilliproteins. *Tetraselmis* sp. represents prasinophytes which contains chlorophyll *a* and *b*.

Both *Synechococcus* were received from the Roscoff Culture Collection (RCC), Roscoff, France. *Rhodomonas salina* was kindly provided by Saskia Ohse and Dr. Alexandra Kraberg (Alfred Wegener Institute, Helgoland, Germany). *Tetraselmis* sp. and *Chaetoceros* sp. were received from Marine ecological laboratory, Department of Marine Science, Faculty of Science, Chulalongkorn University.

The typical setting for pico and nanophytoplankton on Cytomics FC 500 MPL flow cytometer are using: forward scatter (FSC), side scatter (SSC), orange fluorescence (FL2=575 nm), red fluorescence (FL4=675 nm) all parameter were set on logarithmic amplification. All cultures were mixed and analyzed with Cytomics FC 500 MPL flow cytometer as showed in Figure 6.

Table 2 List of cultures as fluorescence references for pico and nanophytoplankton analysis on Cytomics FC 500 MPL.

Class	Taxon	Size (µm)	Main pigments	Fluorescence emission
Cyanophyceae	*Synechococcus* (RS 9917)	1	Chlorophyll *a* and phycobilliproteins	Orange
Cyanophyceae	*Synechococcus* (WH 8018)	1	Chlorophyll *a* and phycobilliproteins	Orange
Cryptophyceae	*Rhodomonas salina*	10	Chlorophyll *a*, c_2, carotenoids, and phycobilliproteins	Orange-red
Prasinophyceae	*Tetraselmis* sp.	10	Chlorophyll *a*, *b*, and carotenoids	Red
Bacillariophyceae	*Chaetoceros* sp.	10	Chlorophyll *a*, c_2, carotenoids, and fucoxanthin	Red

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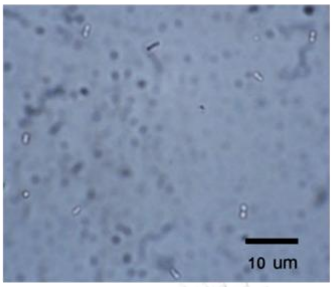
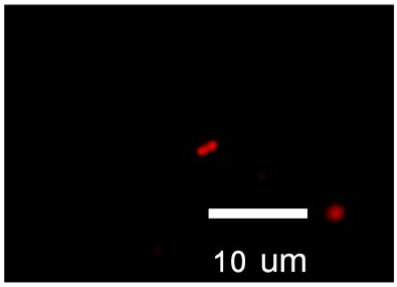
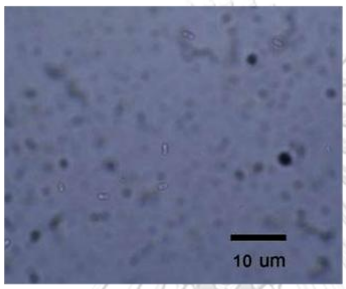
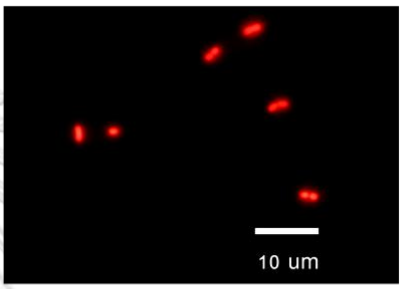
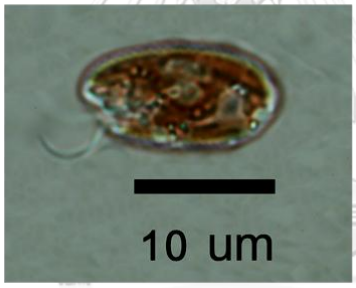
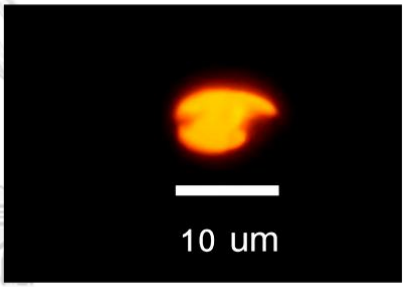
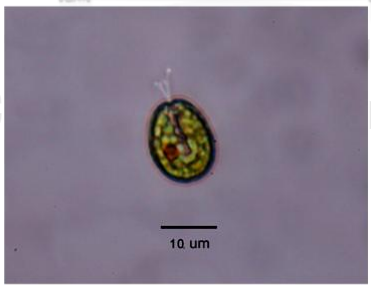
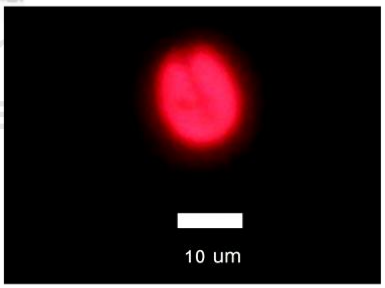
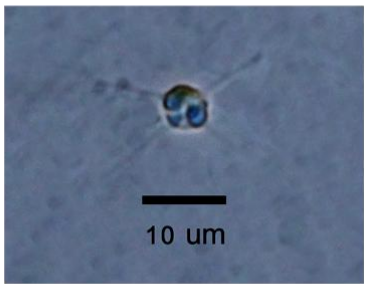
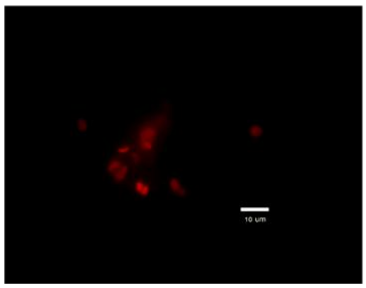
Table 3 List of phytoplankton cultures for pico and nanophytoplankton analysis on Cytomics FC 500 MPL.

cultures	Cultures in visible light	Cultures in blue light
Synechococcus (RS 9917)		
Synechococcus (WH 8081)		
Rhodomonas salina		
Tetraselmis sp.		
Chaetoceros sp.		

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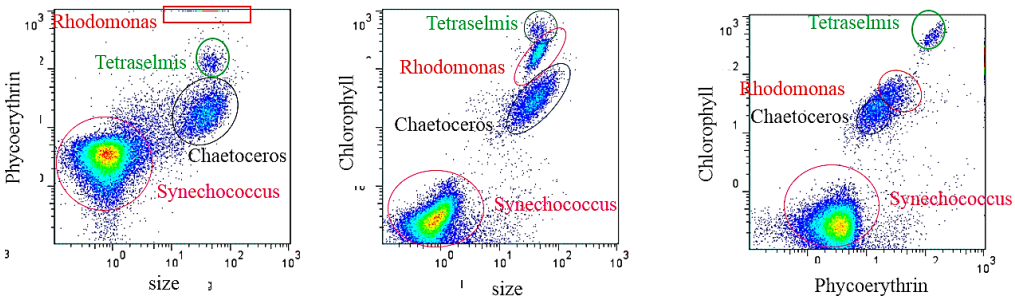

Figure 6 Cytograms obtained from a mixture of four phytoplankton cultures references

For natural samples, frozen samples were thawed at room temperature before the analysis by a Cytomics FC 500 MPL flow cytometer (Beckman Coulter, USA) equipped with 488 nm laser. The different subpopulations of pico- and nanophytoplankton were discriminated using the combination of parameters including cell size by both forward scatter and side scatter (FSC and SSC) as well as their auto-fluorescence by FL 4 chlorophyll fluorescence and FL 2 phycoerythrin fluorescence (Marie et al., 2010). A 2.0 µm yellow-green fluorescent microspheres (FluoSpheres carboxylate-modified microspheres, Invitrogen Molecular Probes) at a known concentration was added to use as an internal reference. Distilled water were used to weighted and reweighted before and after sample acquisition for volume calibration (Gasol and Morán, 2016; Li, 1986). Data were collected as logarithmic mode and list-mode files for further analyzed with FlowJo software (FlowJo, LLC., USA).

4. Statistical analysis

Picophytoplankton, nanophytoplankton, and microphytoplankton abundance were tested for normality and were then tested for significant differences between stations and seasons using the analysis of variance (ANOVA). The non-parametric test, Kruskal-Wallis test was applied to non-normal data. All analyses were performed by IBM SPSS Statistics for Windows, Version 22

4.1. Similarity and multidimensional scaling ordination of microphytoplankton

To study the structure of phytoplankton communities around Sichang Island between seasons, abundance of all phytoplankton groups were transformed with log(x+1) prior to normalize distribution to balance dominant and rarer genera. The degree of similarity were then computed using Bray-Curtis similarities which range from 0% (totally dissimilar) to 100% (totally similar). Samples were grouped using cluster analysis showed by dendrogram and non - metric multidimensional scaling (nMDS) were also performed by PRIMER 6 (Clarke and Warwick, 2001).

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4.2. Spatial and temporal variables of environmental parameter.

PCA is an ordination explained by the high - dimensional variance which are projected onto best fitted plane or principle component (PC) where the highest covariance lies on. This helps to determine the true relationship between different samples with respect to environmental parameters or phytoplankton taxa through the direction of vectors. To study the correlation of physio - chemical parameters (temperature, salinity, dissolved oxygen, and nutrients) on phytoplankton community, all parameters were normalized prior to adjust into same comparable measurement scales and prevented the larger measurement completely dominated the PC's (Clarke and Warwick, 2001). The data were then computed using Euclidean distances before displaying as PCA plot and overlaying with MDS plot of microphytoplankton density. All steps were performed by PRIMER 6.

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CHAPTER III
RESULTS

Physio-chemical characteristics of Sichang Island

The range and average values of temperature, salinity, dissolved oxygen, pH and light intensity of seawater at each seasons, which measured *in situ* during the sampling periods, were summarized below in Figure 7 and Table 4. The values of physio – chemical parameters were generally showed seasonal differences between seasons ($p < 0.05$) with no significant differences between stations ($p > 0.05$).

1. Temperature

Water temperature during the first intermonsoon and the southwest monsoon season were slightly higher than the northeast monsoon and the second intermonsoon season (the northeast monsoon and the intermonsoon II) with no significant differences ($p > 0.05$) between stations (Table 4). Water temperature was highest in the southwest monsoon ranging from 29.87 to 30.25 ˚C, and an average value of 30.13 ± 0.10 ˚C. The lowest temperature was recorded in the northeast monsoon with the range of 29.23 to 29.79 ˚C, and an average value of 29.41 ± 0.17˚C.

2. Salinity

Average value of salinity varied in the similar trend as temperature being higher during the southwest monsoon season than the northeast monsoon season with no significant differences ($p > 0.05$) between stations. The highest salinity occurred in the southwest monsoon ranging from 32.52 to 33.21 psu, and an average value of 32.98 ± 0.19 psu and the lowest salinity occurred in the northeast monsoon which ranged from 31.55 to 31.61 psu, and an average value of 31.57±0.02 psu.

3. Dissolved oxygen

Average dissolved oxygen showed significant different ($p<0.01$) between seasons with no significant differences between stations. During the southwest monsoon, average dissolved oxygen were lower and more fluctuated than in the northeast monsoon. The highest value occurred in the intermonsoon II ranging from 6.02 to 6.71 mg/l, and an average value of 6.34 ± 0.20 mg/l and the lowest value occurred in the southwest monsoon which ranged from 3.06 to 7.33 mg/l, and an average value of 5.12 ± 1.02 mg/l.

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4. pH

The minimum value of pH was recorded in the intermonsoon II ranging from 7.86 to 8.23, and an average value of 8.03 ± 0.12. The maximum value was recorded in the southwest monsoon which ranged from 8.37 - 8.65, and an average value of 8.55 ± 0.08, followed by the intermonsoon I and the northeast monsoon which ranged from 7.90 to 8.33 and 8.27 to 8.44, and the average values of 8.25 ± 0.13 and 8.39 ± 0.05, respectively.

5. Light

Light intensity in surface water was highest in the first intermonsoon (the intermonsoon I) which showed an average value of 1,746.62 μmol m^{-2} s^{-1} followed by the southwest monsoon which showed an average value of 1,387.64 μmol m^{-2} s^{-1}, northeast monsoon which showed an average value of 1,078.04 μmol m^{-2} s^{-1} and second intermonsoon which showed an average value of 800.85 μmol m^{-2} s^{-1} (Figure 8). Sunlight was attenuated by the water so the light intensity was decreased with depth in all stations. In all seasons, light intensity at the bottom were higher than 1% PAR.

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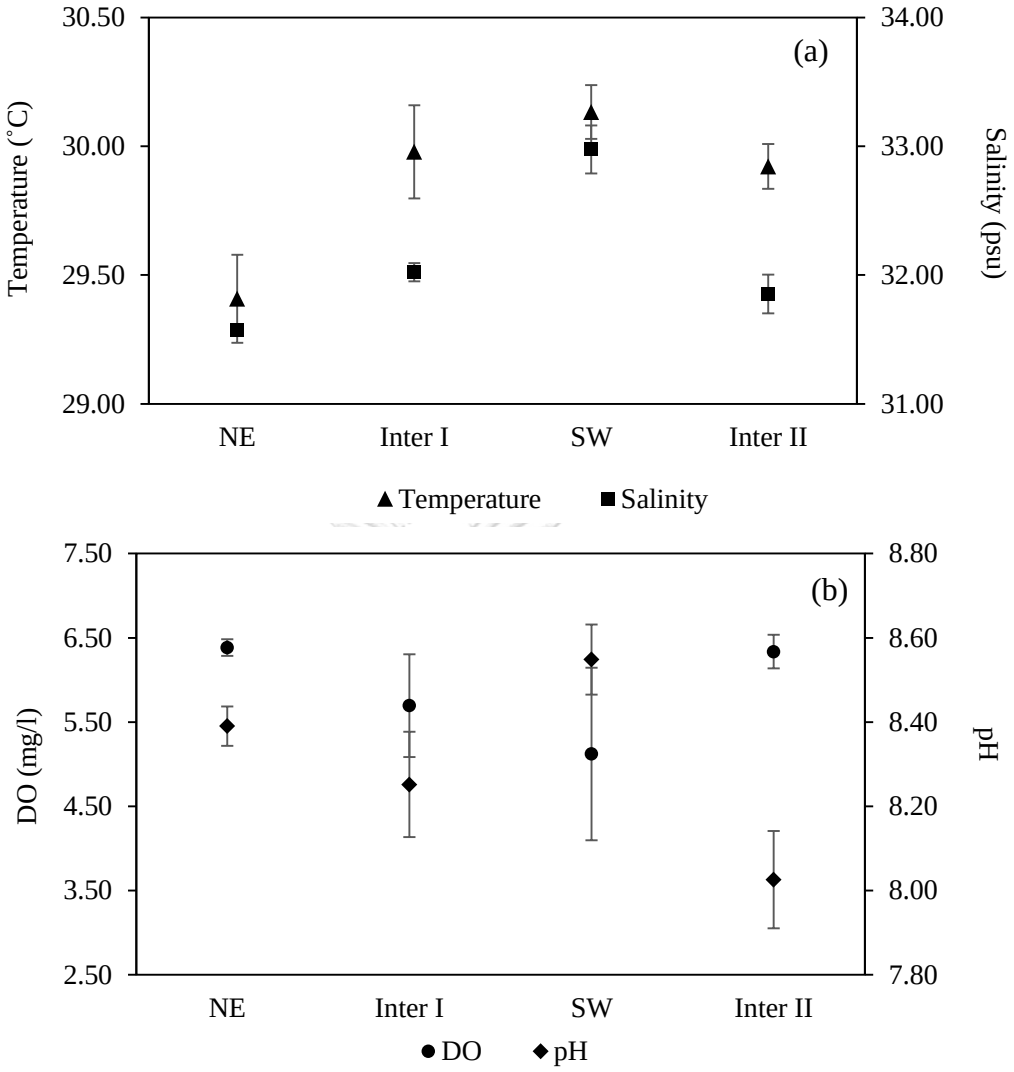

Figure 7 Average temperature and salinity (a), and average dissolved oxygen (DO) and pH (b) between seasons (value±SD). NE=northeast monsoon, Inter I=intermonsoon I, SW=southwest monsoon, and Inter II=intermonsoon II.

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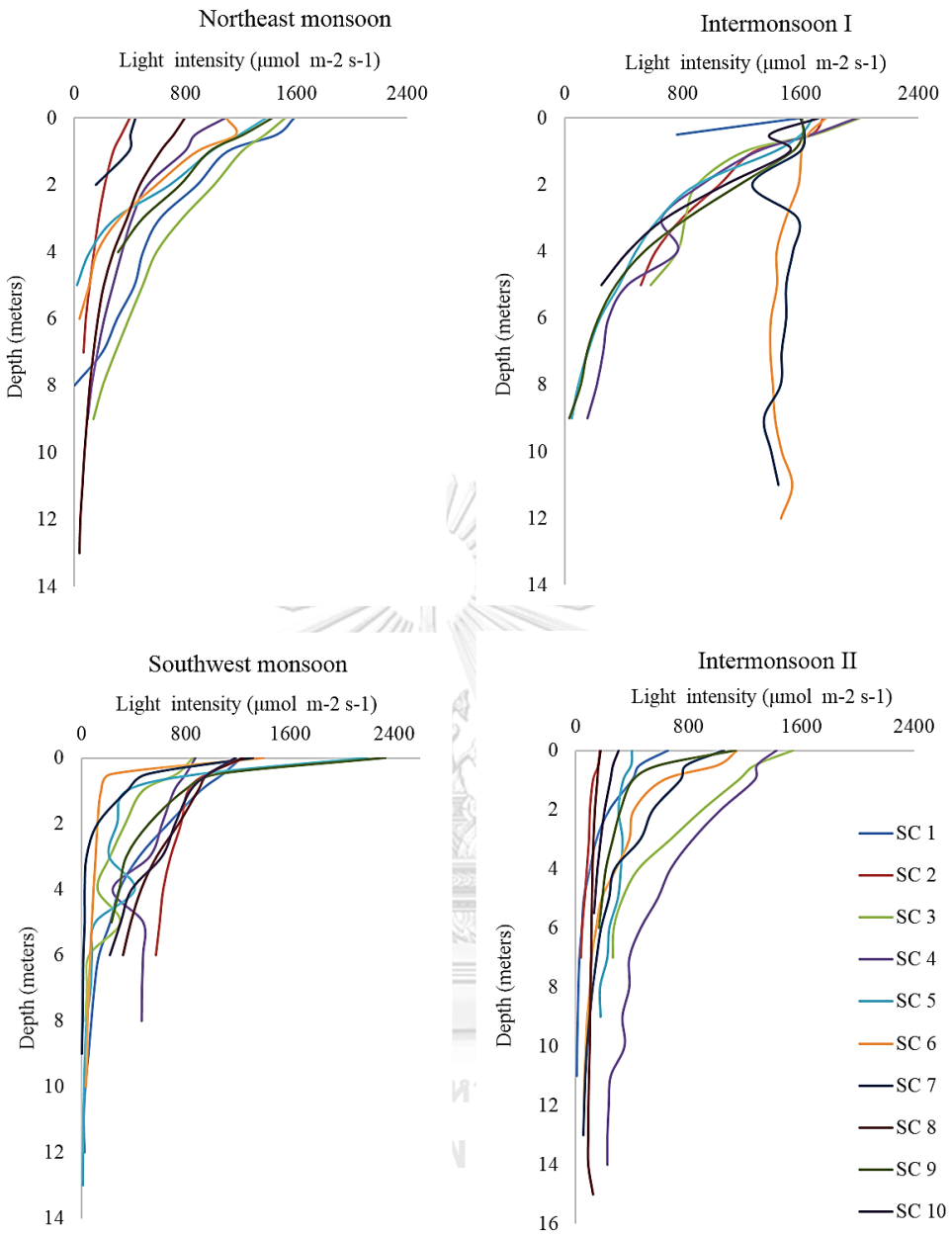

Figure 8 Light attenuation versus depth (meters) in each sampling seasons.

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Table 4 Physio-chemical parameters and nutrient concentrations around Sichang Island (range and average value with standard deviation).

	Northeast monsoon		First intermonsoon		Southwest monsoon		Second intermonsoon	
Physio-chemical parameters	**Range**	**Average ± SD**	**Range**	**Average ± SD**	**Range**	**Average ± SD**	**Range**	**Average ± SD**
Depth (m.)	5.00-15.80	9.66±3.02	6.20-14.50	9.99±2.67	5.20-15.00	10.42±3.30	6.00-15.00	10.58±3.39
Temperature(˚C)	29.23-29.79	29.41±0.17	29.70-30.27	29.98±0.18	29.87-30.25	30.13±0.10	29.83-30.08	29.92±0.09
Salinity (psu)	31.55-31.61	31.57±0.02	31.84-32.08	32.02-0.07	32.52-33.21	32.98±0.19	31.61-32.01	31.85±0.15
DO (mg/l)	6.22-6.55	6.38±0.10	5.05-7.14	5.70±0.61	3.60-7.33	5.12±1.02	6.02-6.71	6.34±0.20
pH	8.27-8.44	8.39±0.05	7.90-8.33	8.25±0.13	8.37-8.65	8.55±0.08	7.86-8.23	8.03±0.12
Ammonia-nitrogen(µM)	0.06-0.44	0.26±0.11	1.45-2.79	1.95±0.44	1.78-6.49	2.87±1.41	0.03-0.15	0.10±0.05
Nitrite-nitrogen (µM)	0.04-0.10	0.07±0.02	0.01-0.06	0.03±0.02	0.12-0.29	0.20-±0.05	0.03-0.14	0.09±0.03
Nitrate-nitrogen (µM)	0.07-0.61	0.22±0.16	0.03-0.42	0.14±0.12	0.19-0.78	0.44±0.21	0.05-0.45	0.20±0.13
Phosphate-phosphorus (µM)	0.03-0.26	0.07±0.13	0.41-0.75	0.58±0.10	0.89-2.51	1.30±0.46	0.01-0.11	0.07±0.03
Silicate-silicon (µM)	4.81-5.32	5.00±0.13	1.04-16.48	5.54±5.04	17.16-19.22	17.91±0.83	7.19-10.70	9.34±1.40

Nutrients

Nutrient concentrations varied between seasons (Table 4 and Figure 9). However there were significant different between stations (Figure 10; 11; 12 and Table 4). Dissolved inorganic nitrogen (DIN) concentration was highest during the southwest monsoon periods than the northeast monsoon period. Phosphate-phosphorus and silicate-silicon concentrations showed the similar pattern as DIN. Silicate-silicon concentrations in The intermonsoon I varied in the range of 1.04 - 16.48 µM, with the average values of 5.54 ± 5.04 µM than other seasons while DIN and phosphate-phosphorus concentrations were more highly fluctuated in the southwest monsoon which ranged from 2.18 - 7.31 µM and 0.89 - 2.51 µM, with the average values of 3.51 ± 1.55 µM and 1.30 ± 0.46 µM, respectively.

1. Dissolved inorganic nitrogen (DIN)

Average dissolved inorganic nitrogen (DIN) concentration were higher in the southwest monsoon (2.12 ± 0.48 µM, in the intermonsoon I and 3.51 ± 0.55 µM in the southwest monsoon) than in the northeast monsoon (0.55 ± 0.21 µM in the northeast monsoon and 0.40 ± 0.11 µM in the intermonsoon II). Ammonia-nitrogen concentrations were lower in the northeast monsoon than in the southwest monsoon while nitrate concentrations tended to inversely increased with ammonia-nitrogen concentrations (Figure 10).

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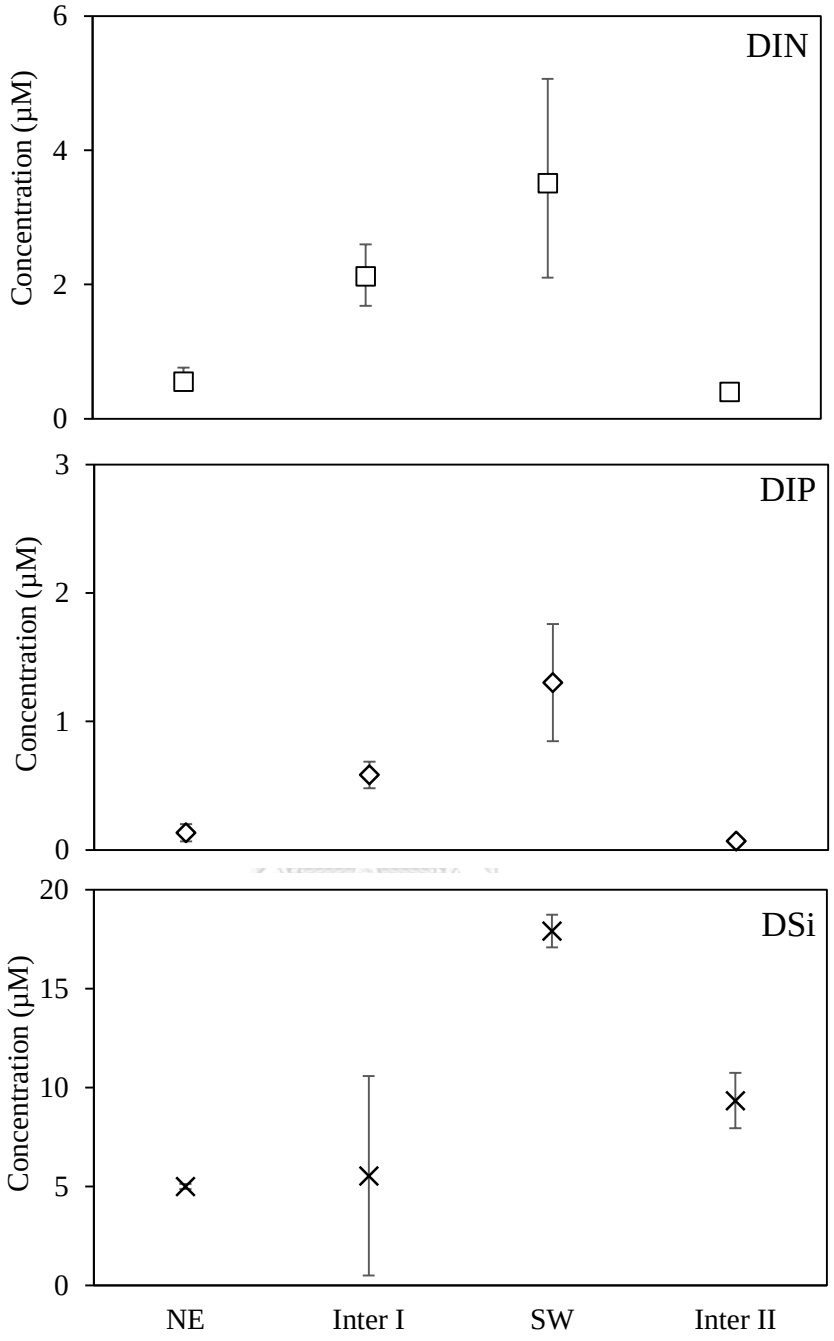

Figure 9 Average dissolved inorganic nitrogen (DIN), phosphate-phosphorus (DIP), and silicate-silicon (DSi) concentrations (value±SD) collected around Sichang Island for 4 seasons. NE=northeast monsoon, Inter I=intermonsoon I, SW=southwest monsoon, and Inter II=intermonsoon II.

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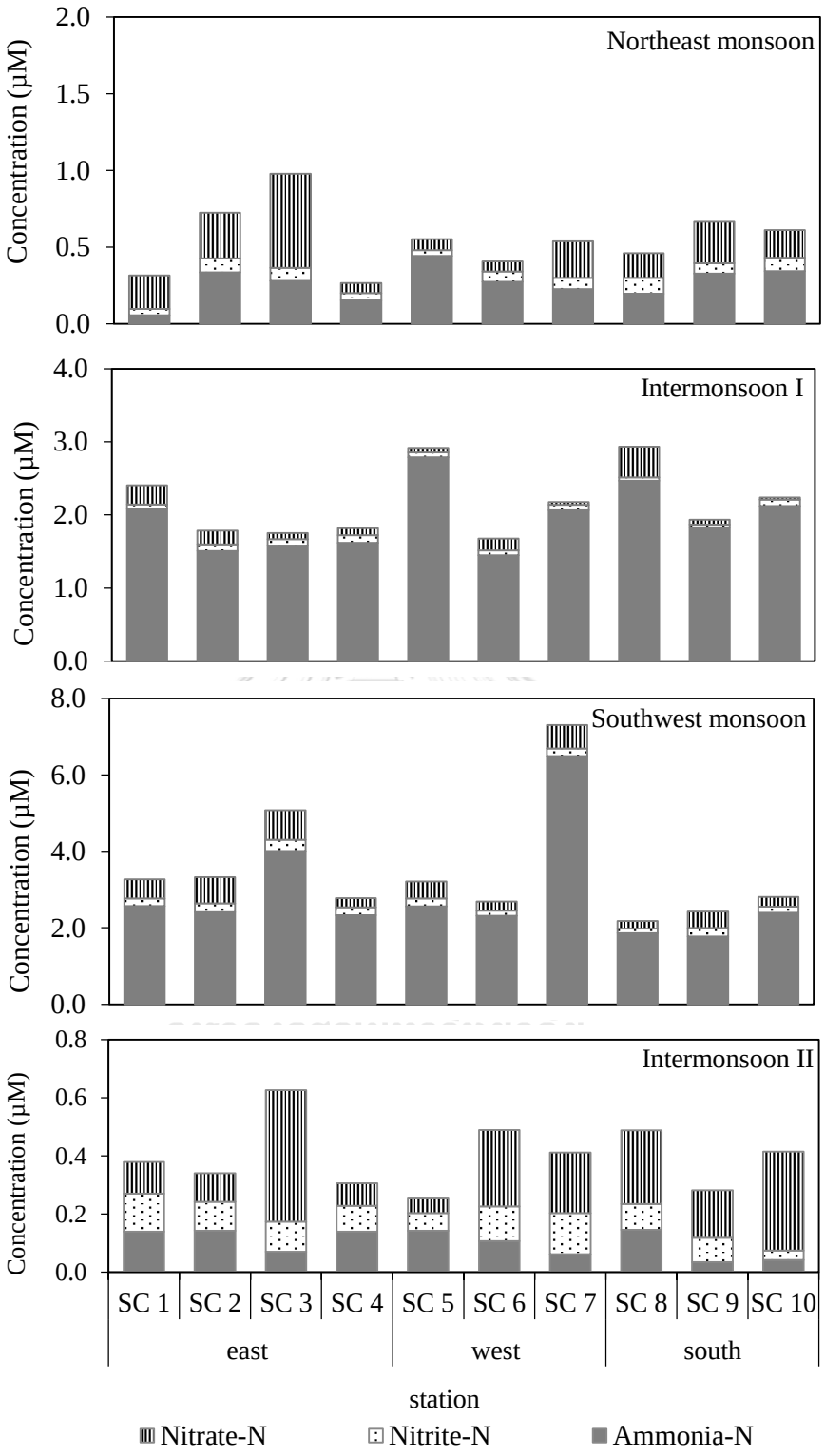

Figure 10 Dissolved inorganic nitrogen concentrations (value±SD) from Sichang Island.

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2. Phosphate-phosphorus

Average phosphate-phosphorus concentrations were higher in the southwest monsoon (0.58 ± 0.10 µM in the intermonsoon I and 1.30 ± 0.46 µM in the southwest monsoon) than in the northeast monsoon (0.07 ± 0.13 µM in the northeast monsoon and 0.07 ± 0.03µM in the intermonsoon II) showed in Figure 11.

3. Silicate-silicon

Average silicate-silicon were higher in the southwest monsoon (17.91 ± 0.83 µM) followed by the intermonsoon II (9.34 ± 1.40 µM), the inter monsoon I (5.54 ± 5.04 µM), and the northeast monsoon (5.00 ± 0.13 µM). In the intermonsoon I, the lowest values were noticed in the station SC 3 – SC 6 (the southeast and the southwest parts of the island) while the higher value were found in station SC 1 (the northern part of the island). In the other seasons, silicate-silicon concentrations between stations were much smaller differences (Figure 12).

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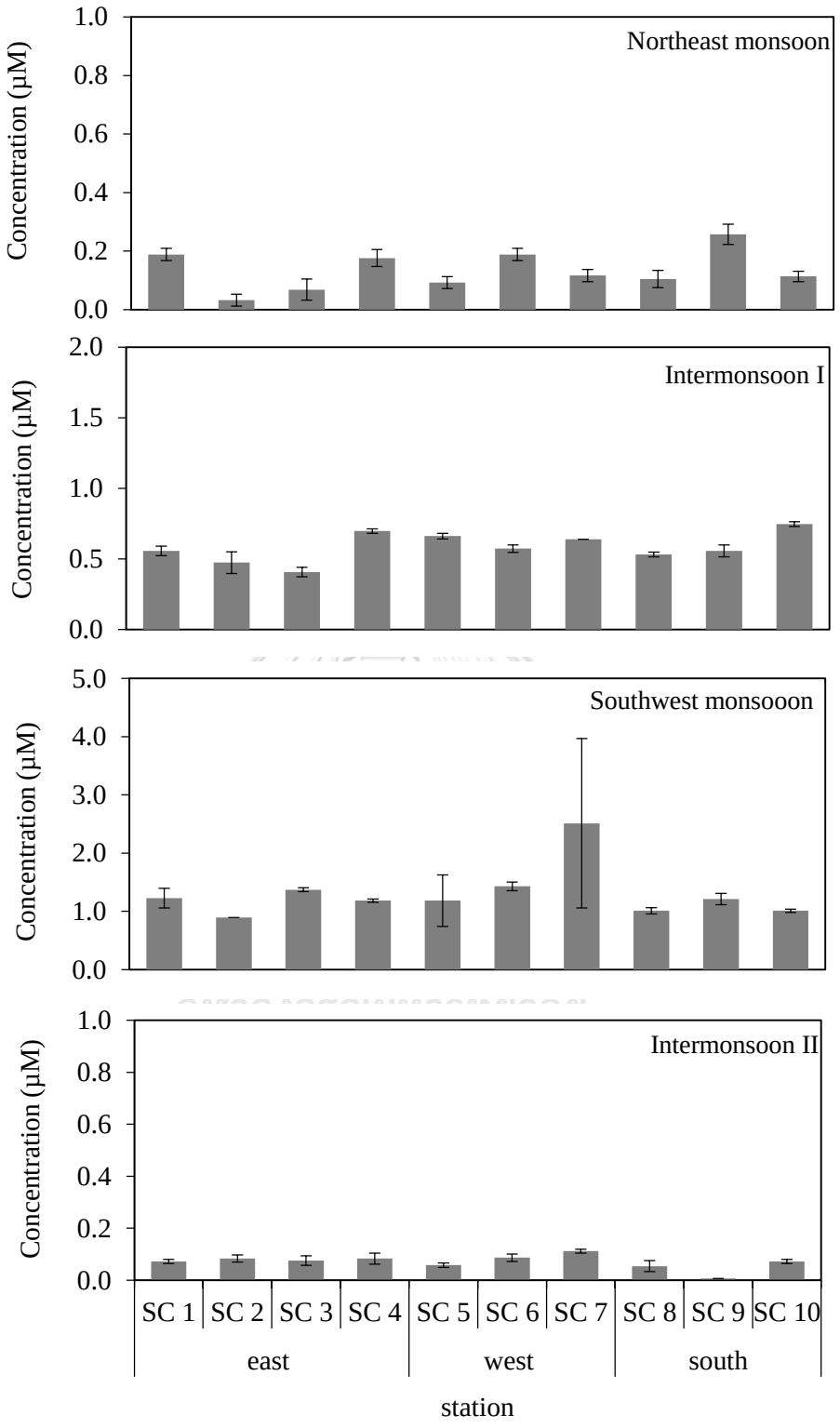

Figure 11 Phosphate-phosphorus concentration (value±SD) collected around Sichang Island.

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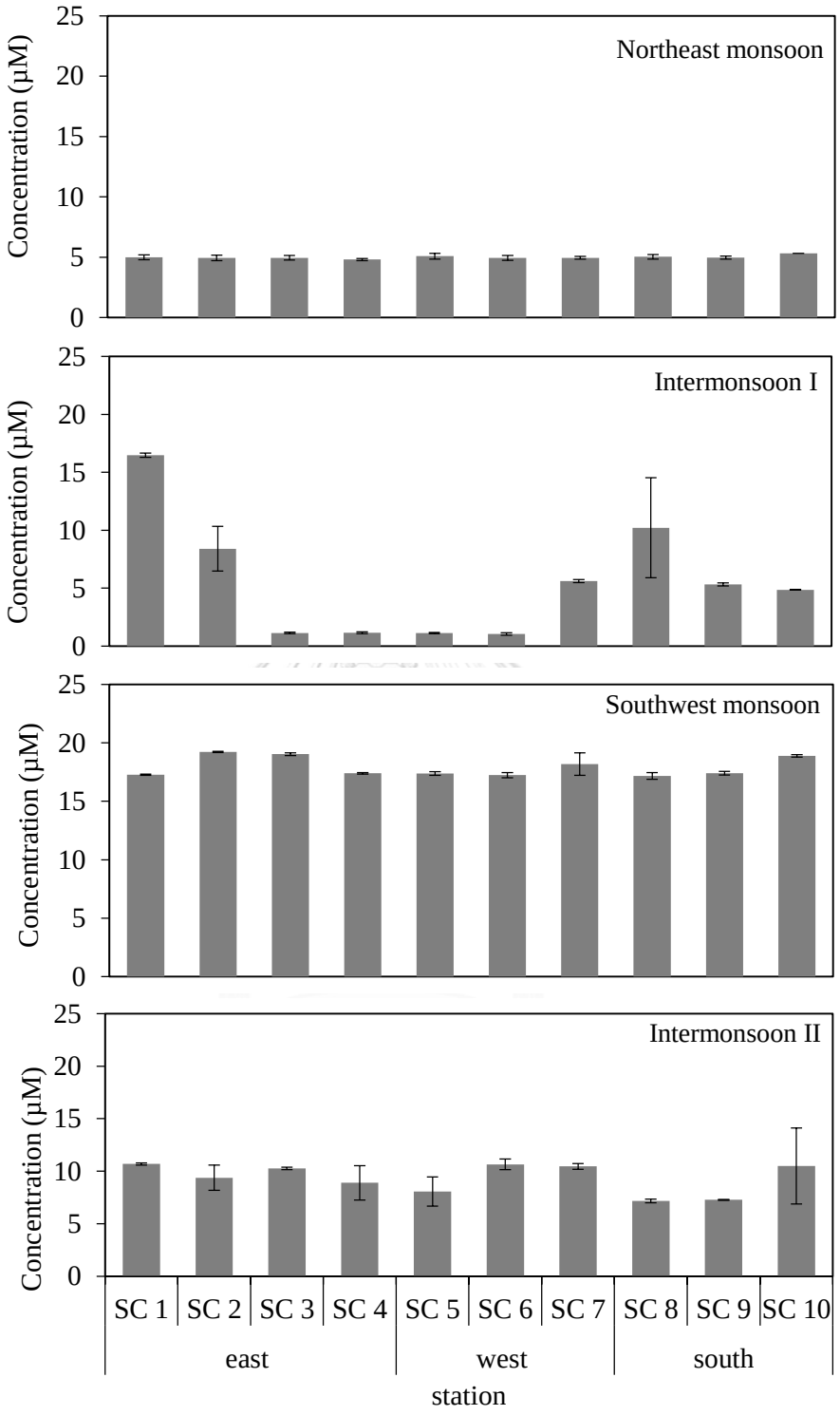

Figure 12 Silicate-silicon concentration (value±SD) collected from Sichang Island.

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4. Redfield ratios

The ratios of DIN: DIP in all periods were mostly lower than lower than 16 which the values were in the range of 2.69 ± 0.64 to 8.88 ± 10.75 (Table 5). The highest value was recorded during the second intermonsoon with average values of 8.88 ± 10.75, followed by the northeast monsoon, the first intermonsoon and the southwest monsoon, with average values of 6.50 ± 6.71, 3.70 ± 0.90, and 2.69 ± 0.64, respectively. Since all DIN: DIP ratio were less than Redfield ratio of 16:1, this indicated that Nitrogen was the limiting nutrient at Sichang Island.

DIN: DSi ratios were also noticed that they were in the range of 0.04 ± 0.01 to 0.89 ± 0.82. The highest value was recorded during the first intermonsoon with average values of 0.89 ± 0.82, followed by the southwest monsoon, the northeast monsoon, and the second intermonsoon, with average values of 0.19 ± 0.08, 0.11 ± 0.04, and 0.04 ± 0.01, respectively. The DIN: DSi ratios were less than 1, this showed that silica was not limiting nutrient.

Table 5 Ratios of inorganic nutrients

Periods	DIN:DIP (±SD)	DIN:DSi (±SD)
Northeast monsoon	6.50 ± 6.71	0.11 ± 0.04
First intermonsoon	3.70 ± 0.90	0.89 ± 0.82
Southwest monsoon	2.69 ± 0.64	0.19 ± 0.08
Second intermonsoon	8.88 ± 10.75	0.04 ± 0.01

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Phytoplankton community

1. *Total chlorophyll a and size-fractionated chlorophyll a*

Average total chlorophyll *a* concentration were higher in the intermonsoon I and the southwest monsoon than in the northeast monsoon and the intermonsoon II) which ranged from 0.62 µg/l in the intermonsoon II to 44.75 µg/l in the southwest monsoon during the blooming of *Noctiluca scintillans* (Figure 13).

In general, picophytoplankton and nanophytoplankton fractions were dominated in terms of chlorophyll *a* concentration in the intermonsoon II (87%), the southwest monsoon (67%), and the intermonsoon I (63%) while in the northeast monsoon, it was dominated by microphytoplankton (52%) as showed in Figure 13.

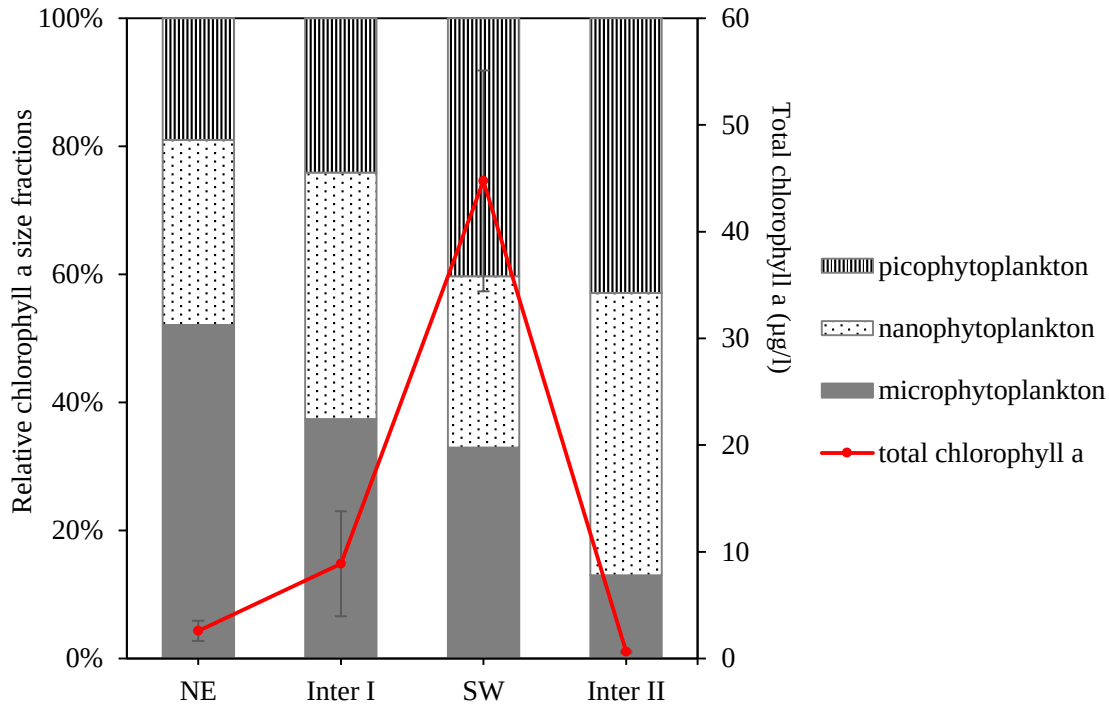

Figure 13 Total chlorophyll a concentrations (value±SD) in red line and relative chlorophyll a size fractions in stack bar graph in 4 seasons.

NE=northeast monsoon, Inter I=intermonsoon I, SW=southwest monsoon, and Inter II=intermonsoon II.

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The highest total chlorophyll *a* concentrations were detected in the southwest monsoon which range from 7.75 – 267.55 µg/l with the average concentration of 44.75 ± 78.89 µg/l. The blooming of dinoflagellate *N. scintillans* caused the maximum peak in the station SC 7 (Figure 14). In the northeast monsoon, the total chlorophyll *a* concentrations were range from 0.94 – 4.46 µg/l with the average concentration of 2.60 ± 0.99 µg/l. The higher values were found near the west coast of the Island (station SC 5 – SC 7) than those encountered at the other stations (Figure 14) while in the intermonsoon I, the values were range from 3.60 -19.15 µg/l with the average concentration of 8.88 ± 4.91 µg/l (Figure 3-8). The higher values in this month were recorded at the northeast coast (SC 1- SC 3). The lowest values were recorded in the intermonsoon II which range from 0.46 – 0.81 µg/l with the average concentration of 0.62 ± 0.10 µg/l (Figure 14). Picophytoplankton and nanophytoplankton fractions were dominated in terms of chlorophyll *a* concentration in all stations and contributed more than 80% of total chlorophyll *a* in the intermonsoon II showed in Figure 15 (with an exception in the northeast monsoon) chlorophyll *a* concentration was mostly dominated by microphytoplankton.

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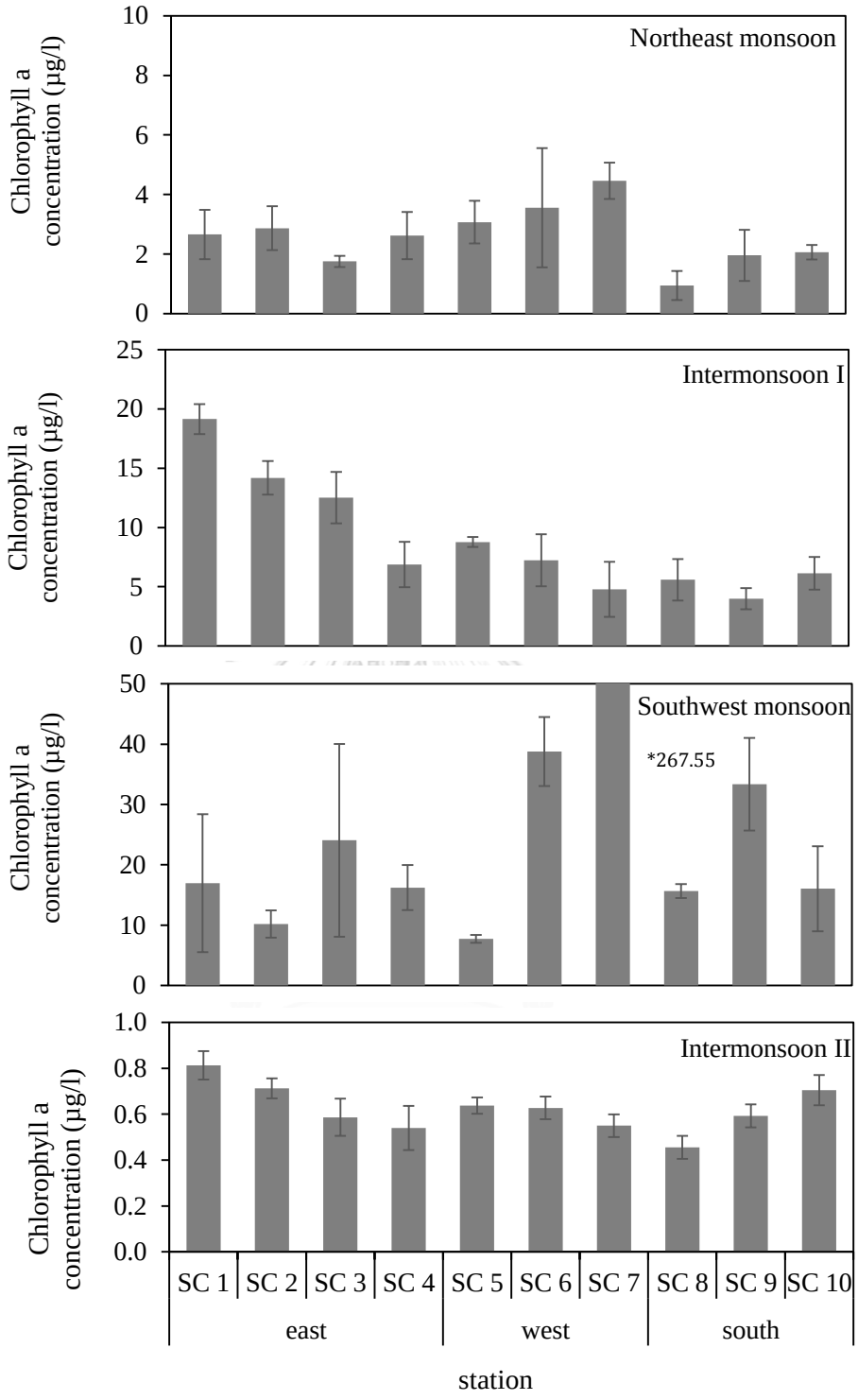

Figure 14 Total chlorophyll a concentration (value±SD) of phytoplankton from Sichang Island.

*Average chlorophyll *a* concentration at station SC 7 in the southwest monsoon was 267.55 µg/l caused by the blooming of *N. scintillans*

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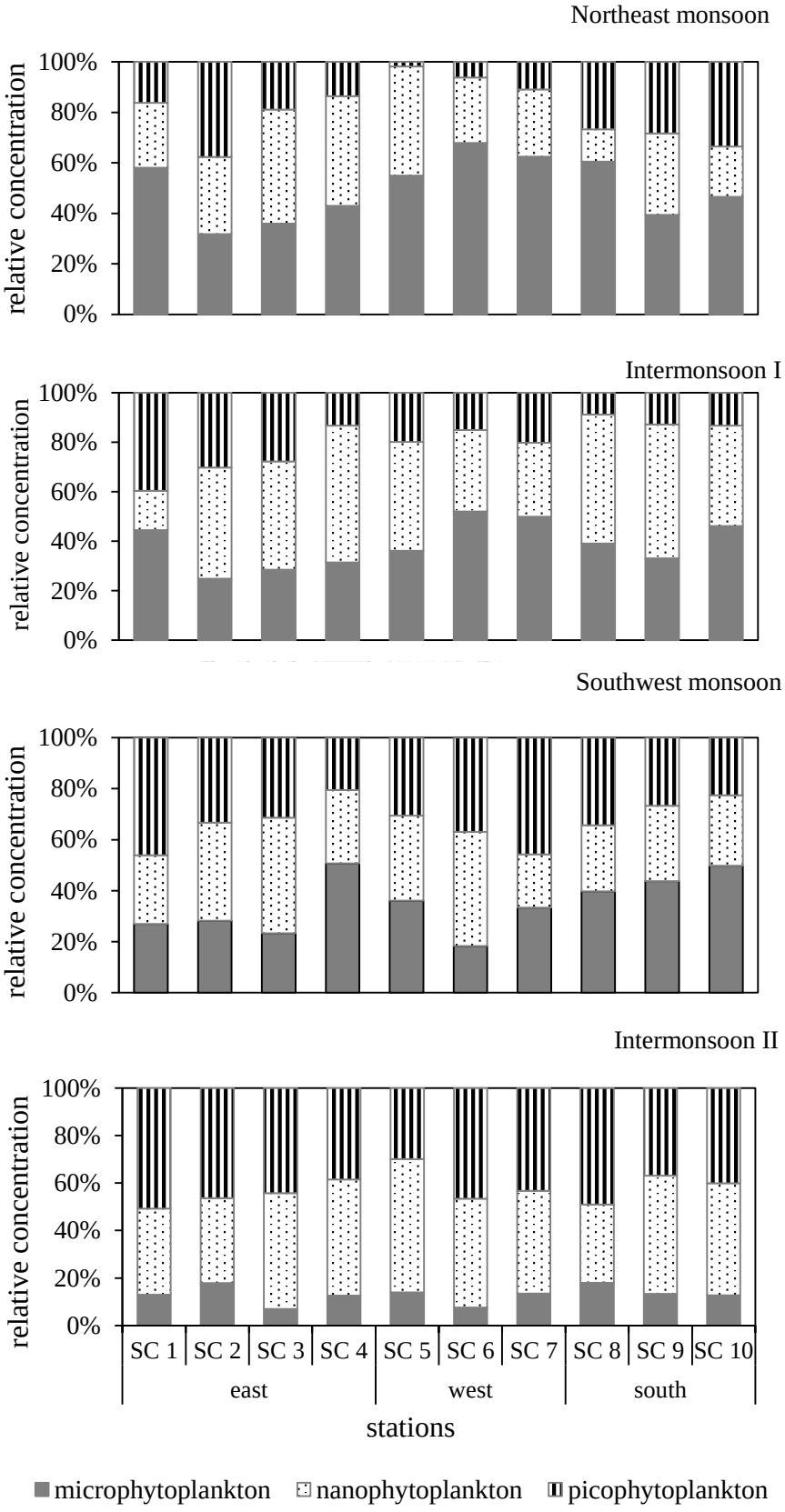

Figure 15 Relative chlorophyll *a* size fractions around Sichang Island.

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2. Abundance of microphytoplankton

Microphytoplankton groups that were observed during the study consisted of four groups; cyanobacteria (Class Cyanophyceae), diatom (Class Bacilariophyceae), dinoflagellate (Class Dinophyceae), and silicoflagellate (Class Dictyochophyceae). Figure 16 showed the average microphytoplankton density which reached to the highest cell density in the intermonsoon I (4.89 ± 1.33 x 10^4 cell/l) followed by the intermonsoon II (3.0 ± 2.66 x 10^4 cell/l), the southwest monsoon (2.53 ± 1.81 x 10^4 cell/l) and the northeast monsoon (2.24 ± 0.91 x 10^4 cell/l), respectively. There are no significant different between stations but can be noticed when compared between seasons ($p<0.05$). The dominant microphytoplankton group throughout the study was the diatoms which represented 92.6%, 95.5%, 54.4%, and 93.6% in the northeast monsoon, the intermonsoon, the southwest monsoon, and the intermonsoon II., respectively, while the silicoflagellate was found in the lowest density along the study (Figure 16). In the southwest monsoon, the proportion of dinoflagellate was higher than the other seasons, represented 43.5% due to the blooming of *Noctiluca scintillans*.

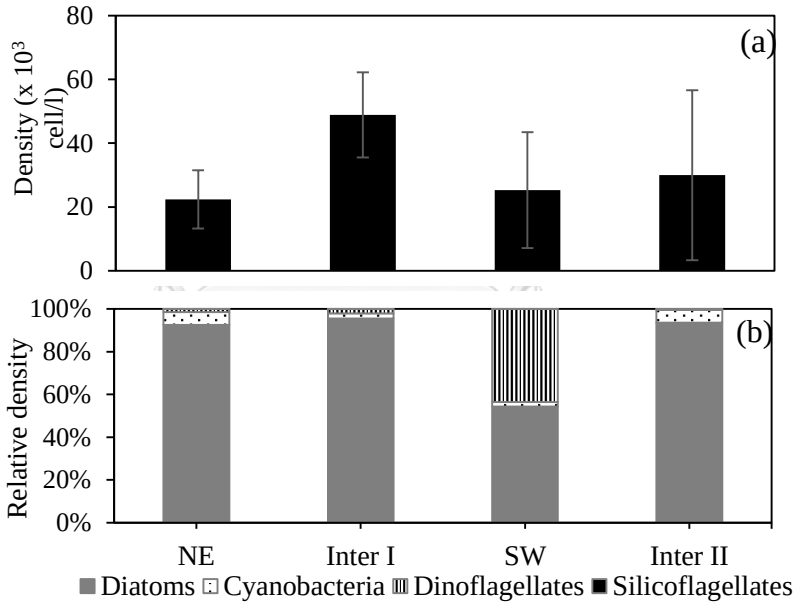

Figure 16 Average density (value±SD) in (a) and relative density (b) of microphytoplankton groups from Sichang Island.
NE=northeast monsoon, Inter I=intermonsoon I, SW=southwest monsoon, and Inter II=intermonsoon II.

In the northeast monsoon and the intermonsoon I, total cell density showed low variable and were dominated by diatoms while in the southwest monsoon, the total cell density was founded in high variability and the proportion of dinoflagellates showed sharply increased in particular near the west coast, SC 6 and SC 7, of Sichang Island (Figure 16) and reached the total cell density up to 7.02 x 10^4 cell/l and 4.05 x 10^4 cell/l, respectively. In the intermonsoon II, the maximum cell density (9.68 x 10^4 cell/l) was

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found in the east coast (SC 2) and the lowest cell density (4.41 x 10^3 cell/l) was found in the west coast (SC 7) showed in Figure 17.

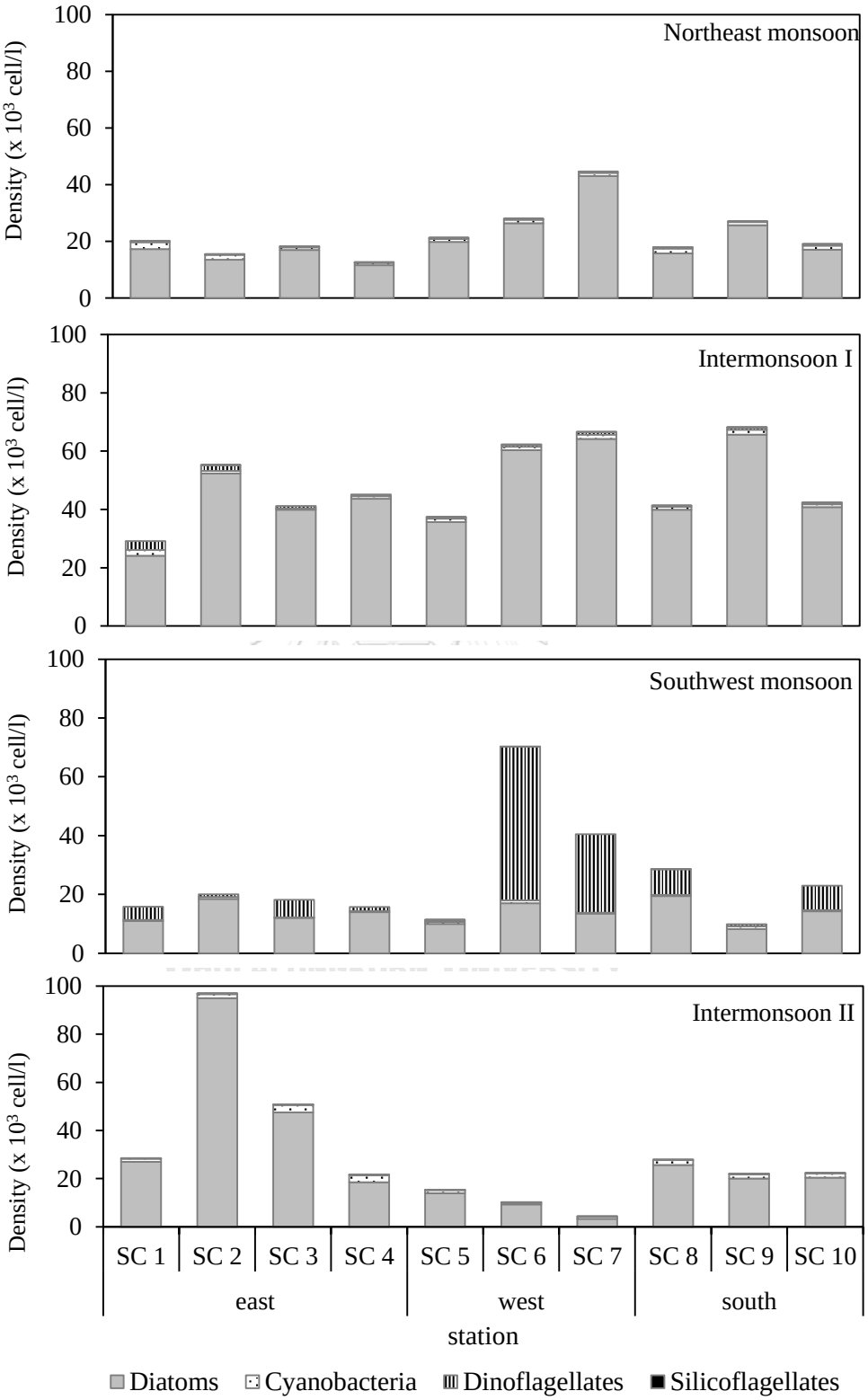

Figure 17 Average density of phytoplankton in 10 stations collected around Sichang Island for 4 seasons.

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3. Diversity and evenness of microphytoplankton

A total eighty-three genera of microphytoplankton consisted of five genera from cyanobacteria, twenty genera of dinoflagellates, one genera of silicoflagellate, and fifty-seven genera of diatoms were identified in four periods of time which were summarized in Table 6.

The diatoms genus *Chaetoceros*, *Pseudonitzschia* and cyanobacteria genus *Pseudanabaena* were generally found in high density (> 1000 cell/L) in all stations and seasons (Table 6). The dinoflagellates were found in low density (< 10% of total cell density in all seasons) except *Noctiluca scintillans* which found in high density and reached to its maximum capacity up to 41.4% of total cell density in the southwest monsoon. However, *N. scintillans* was not be found in the intermonsoon II.

Pseudanabaena and *Trichodesmium* were dominant genera in cyanobacteria groups along the study. In the northeast monsoon and The intermonsoon II, diatoms were dominated by *Chaetoceros* (7.28×10^3 and 2.34×10^4 cell/l) and *Pseudonitzschia* (2.27×10^3 and 1.86×10^3 cell/l) while in the intermonsoon I were dominated by *Pseudonitzschia* (2.92×10^4 cell/l) and *Thalassionema* (7.49×10^3 cell/l) and in the southwest monsoon were dominated by *Eucampia* (9.11×10^3 cell/l) and *Chaetoceros* (1.66×10^3 cell/l) as showed in Figure 18.

Dinoflagellate group were dominated by *Protoperidinuim* in the northeast monsoon (1.9×10^2 cell/l), the intermonsoon I (4.1×10^2 cell/l), and the intermonsoon II (75 cell/l) except in the southwest monsoon which *N. scintillans* was found in high density (1.05×10^4 cell/l). The abundance of *N. scintillans* can turned sea water into greenish color.

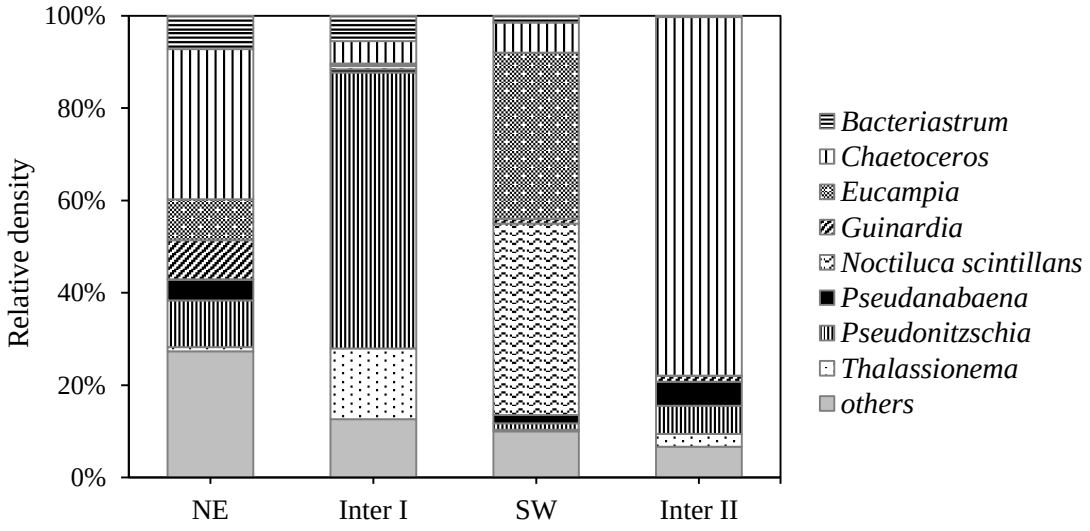

Figure 18 Proportion of dominant microphytoplankton genera in 10 stations collected around Sichang Island for 4 seasons.

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Table 6 List of microphytoplankton genera from Sichang Island during the study collected around Sichang Island for 4 seasons.

Group	Genera	Presence/Absence			
		NE	Inter I	SW	Inter II
Division Cyanophyta		++++	++++	++++	++++
Class Cyanophyceae					
Subclass Oscillatoriophycidae					
Order Chroococcales					
Family Chroococcaceae	*Chroococcus*	+	+	+	-
Order Oscillatoriales					
Family Microcoleaceae	*Trichodesmium**	++	++	+	++
Order Spirulinales					
Family Spirulinaceae	*Spirulina*	-	-	-	+
Subclass Nostocophycidae					
Order Nostocales					
Family Nostocaceae	*Richelia*	-	+	-	-
Subclass Synechococcophycidae					
Order Synechococcales					
Family Pseudanabaenaceae	*Pseudanabaena*	+++	++	++	+++
Division Chromophyta					
Class Dinophyceae					
Order Prorocentrales					
Family Prorocentraceae	*Prorocentrum*	+	+	+	+
Order Dinophysiales					
Family Amphisoleniaceae	*Amphisolenia*	-	+	-	-
Family Dinophysiaceae	*Dinophysis*	+	+	++	+
	Ornithocercus.	-	-	+	+
Order Gymnodiniales					
Family Gymnodiniaceae	*Gymnodinium*	+	+	+	-
	Gyrodinium	-	-	-	+
Order Noctilucales					

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Group	Genera	Presence/Absence			
		NE	InterI	SW	InterII
Family Noctilucaceae	*Noctiluca scintillans*	+	++	++++	-
Order Gonyaulacales					
Family Ceratiaceae	*Ceratium*	+	++	++	+
Family Goniodomaceae	*Alexandrium*	+	+	+	-
	Gambridiscus	+	-	-	-
	Pyrodinium	-	-	+	-
Family Oxytoxaceae	*Oxytoxum*	+	+	+	-
Family Pyrophacaceae	*Pyrophacus*	+	+	+	+
Order Peridiniales					
Family Calciodinellaceae	*Scrippsiella trocoidea*	+	+	+	+
Family Diplopsalidaceae	*Diplopsalis*	+	+	+	+
	Diplopelta.	-	-	-	+
Family Peridiniaceae	*Peridinium*	+	+	+	+
Family Podolampadaceae	*Podolampas*	+	+	-	-
Family Protoperidiniaceae	*Protoperidinum*	++	++	++	+
Class Dictyochophyceae					
Order Dictyochales					
Family Dictyochaceae	*Dictyocha*	+	+	+	+
Class Bacillariophyceae					
Order Biddulphiales					
Suborder Coscinodiscineae					
Family Thalassiosiraceae	*Cyclotella*	+	++	+	+
	Lauderia	++	+	++	++
	Planktonella	-	-	-	+
	Skeletonema	+	+	-	-
	Thalassiosira	++	++	++	++
Family Merosiraceae	*Melosira*	+	-	-	+
	Paralia	+	+	+	+
	Stephanopyxis	-	-	-	+

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Group	Genera	Presence/Absence			
		NE	InterI	SW	InterII
Family Coscinodiscaceae	*Coscinodiscus*	+	++	++	+
Family Leptocylindraceae	*Corethron*	+	+	+	+
Family Hemidiscaceae	*Actinocyclus*	+	+	+	+
	Hemidiscus	+	-	-	+
	Pseudoguinardia	++	+	+	+
Family Asterolampraceae	*Asterolampra*	+	-	-	+
	Asteromphalus	+	+	+	+
Family Heliopeltaceae	*Actinoptychus*	+	-	-	-
Suborder Rhizosoleniineae					
Family Rhizosoleniaceae	*Rhizosolenia*	++	++	++	++
	Guinardia	+++	++	++	++
	Dactyosolen	+++	+	+	+
Family Probosciaceae	*Proboscia*	+	-	-	-
Suborder Biddulphiineae					
Family Hemiaulaceae	*Cerataulina.*	+	+	+	+
	Climacodium	-	-	-	+
	Eucampia	+++	++	+++	+
	Hemiaulas	++	++	+	+
Family Cymatosiraceae					
Family Biddulphiaceae	*Biddulphia*	-	-	-	+
Family Chaetoceratoceae	*Bacteriastrum*	+++	+++	++	+
	Chaetoceros	+++	+++	+++	++++
Family Lithodesmiaceae	*Bellerochea*	+	-	-	+
	Ditylum	+	+	+	+
	Helicotheca	+	+	+	+
Family Eupodiscaceae	*Odontella*	+	+	+	+
	Triceratium	+	+	+	+
Order Bacillariales					
Suborder Fragilariineae					
Family Fragilariaceae	*Diatoma*	+	-	-	++++

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Group		Genera	Presence/Absence			
			NE	InterI	SW	InterII
	Family Fragilariaceae	*Diatoma*	+	-	-	++++
		Fragillaria	+	+	+	+
	Family Thalassionemataceae	*Lioloma*	+	+	+	+
		Thalassionema	++	+++	+	++
		Thalassiothrix	+	++	+	+
	Family Tabellariaceae	*Tabellaria*	+	-	-	-
	Family Licmophoriaceae	*Licmophora*	+	-	-	+
Suborder Bacillariineae						
	Family Achnanthes	*Achnanthes*	-	-	+	-
	Family Lyrellaceae	*Cocconeis*	+	+	+	+
		Lyrella	+	+	+	+
	Family Naviculaceae	*Diploneis*	+	+	+	+
		Haslea sp	+	+	-	-
		Meunier	+	+	+	+
		Navicula	+	+	+	+
		Pleurosigma/ Gyrosigma	++	+++	+	++
		Trachyneis	+	+	+	+
	Family Catenulaceae	*Amphora*	+	+	+	+
	Family Bacillariaceae	*Bacillaria*	++	+	+	++
		Cylindrotheca	+	+	+	+
	Family Bacillariaceae	*Pseudonitzschia*	+++	++++	++	+++
		Nitzschia	+	++	+	+
	Family Entomoneidaceae	*Entomoneis*	+	+	+	+
	Family Surirellaceae	*Surirella*	+	+	+	+
		Campyrodiscus	-	-	-	+
Order Plagiogrammales						
	Family Plagiogrammaceae	*Dimeregramma*	-	-	-	+

Note: - not detected
+ ≤ 100 cell/l
++ 101 - 1000 cell/l
+++ 1001 - 10000 cell/l
++++ > 10000 cell/l
*counted as trichomes

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Number of phytoplankton genera was highest in the northeast monsoon (70 genera) followed by the intermonsoon II (68 genera), the intermonsoon I (62 genera), and the southwest monsoon (60 genera).

The average Shannon diversity (H') and Pielou's eveness (J') was highest in the northeast monsoon followed by the southwest monsoon, the intermonsoon I and lowest in the intermonsoon II, showed in Figure 19.

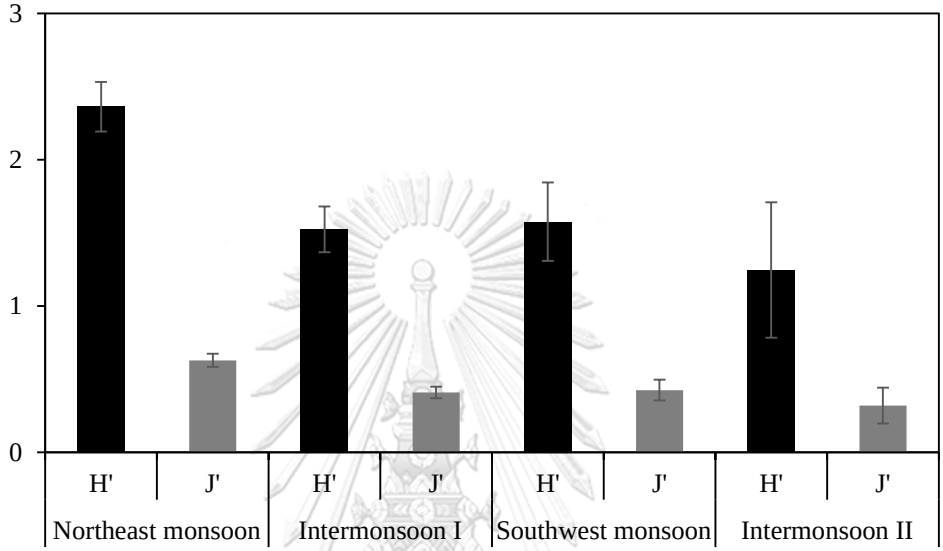

Figure 19 Average Shannon diversity (H') and Pielou's evenness (J') of microphytoplankton (value±SD) collected around Sichang Island.

4. Abundance of picophytoplankton and nanophytoplankton by flow cytometry

Picophytoplankton observed during this study consisted of four groups while nanophytoplankton were divided into five groups based on their size and fluorescence characteristics. Figure 20 showed the average total pico- and nanophytoplankton density which picophytoplankton reached the highest cell density in the northeast monsoon ($1.18 \times 10^6 \pm 2.94 \times 10^5$ cell/ml) followed by the intermonsoon I ($4.65 \pm 1.39 \times 10^5$ cell/ml), the intermonsoon II ($1.05 \pm 0.81 \times 10^5$ cell/ml) and the southwest monsoon ($2.22 \times 10^4 \pm 0.43 \times 10^4$ cell/ml), respectively. Nanophytoplankton showed the similar trend as picophytoplankton reaching the highest cell density in the northeast monsoon ($5.74 \pm 1.36 \times 10^5$ cell/ml) followed by the intermonsoon I ($1.32 \pm 0.48 \times 10^5$ cell/ml), the intermonsoon II ($2.22 \pm 0.68 \times 10^4$ cell/ml) and the southwest monsoon ($1.29 \pm 0.50 \times 10^4$ cell/ml), respectively.

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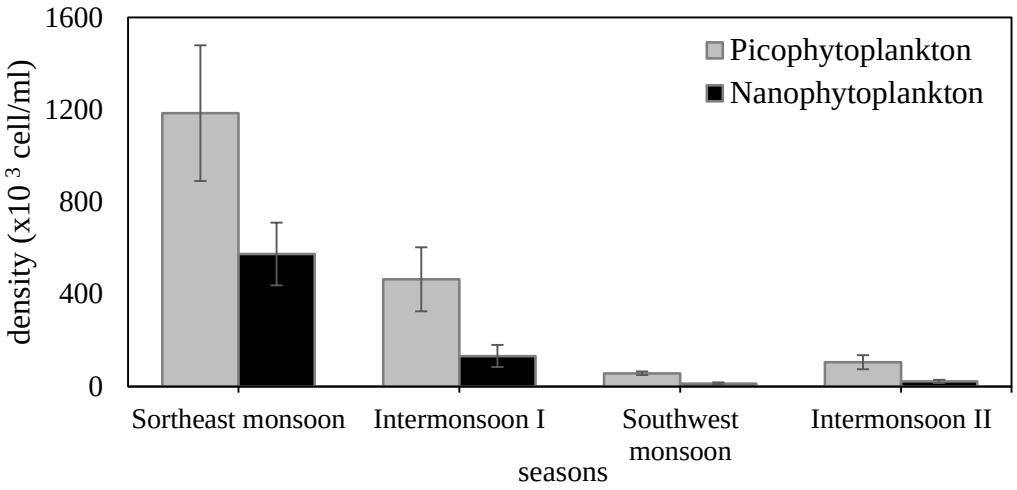

Figure 20 Average density of picophytoplankton and nanophytoplankton (value±SD) collected around Sichang Island.

The average cell density of picophytoplankton were more fluctuated between stations in the northeast monsoon and the intermonsoon I which range from 8.20 – 17.56 x 10^5 cell/ml and 2.85 – 6.90 x 10^5 cell/ml, with the average values of 11.84 ± 2.94 x 10^5 cell/ml and 4.65 ± 1.38 x 10^5 cell/ml, respectively, than in the southwest monsoon and the intermonsoon II which range from 5.07 – 7.63 x 10^4 cell/ml and 0.73 – 16.76 x 10^5 cell/ml, with the average values of 5.97 ± 0.71 x 10^4 cell/ml and 1.05 ± 0.31 x 10^5 cell/ml, respectively

Nanophytoplankton density showed the same pattern as picophytoplankton that in the northeast monsoon was highest followed by the intermonsoon I, the intermonsoon II, and the southwest monsoon which the cell density range from 5.74 – 8.61 x 10^5 cell/ml, 1.32 – 2.12 x 10^5 cell/ml, 1.34 – 3.76 x 10^4 cell/ml, and 0.71 – 2.47 x 10^4 cell/ml, with the average values of 3.85 ± 1.36 x 10^5 cell/ml, 0.70 ± 0.48 x 10^5 cell/ml, 2.22 ± 0.68 x 10^4 cell/ml, and 1.35 ± 0.51 x 10^4 cell/ml, respectively (Figure 21).

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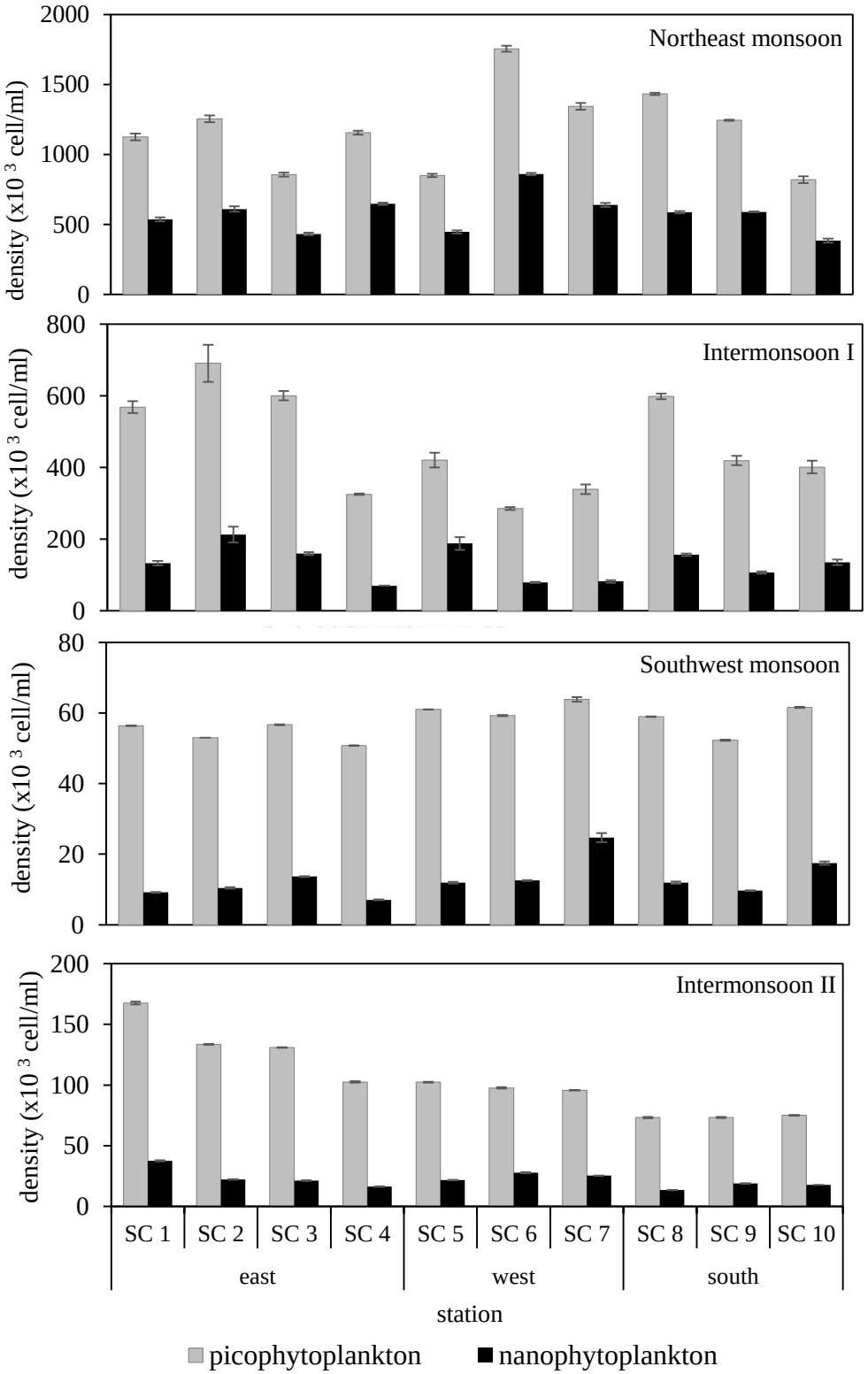

Figure 21 Average density of pico- and nanophytoplankton (value±SD) in 10 stations collected around Sichang Island for 4 seasons.

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5. Diversity of pico- and nanophytoplankton

A 2.0 µm µm yellow-green fluorescent microspheres (FluoSpheres carboxylate-modified microspheres Invitrogen Molecular Probes) and cultured cell (*Synechococcus* RS 9917, *Synechococcus* WH8018, and *Rhodomonas salina* from the Roscoff Culture Collection (RCC), Roscoff, France) were used as references for size and fluorescence characteristics compared with the natural samples (Figure 22-23). The group of cell with similar size and fluorescence were detected into same position on the graph.

As shown in Figure 22, 2.0 µm were used to separate picophytoplankton (left region) from nanophytoplankton (right region). Each region was then analyzed into specific groups depend on red or orange fluorescence characteristic compare with culture cells Figure 23. The populations can be discriminated are as follows: syn1is *Synechococcus* RS 9917, syn2 is *Synechococcus* WH8018, and rhodo is *Rhodomonas salina*).

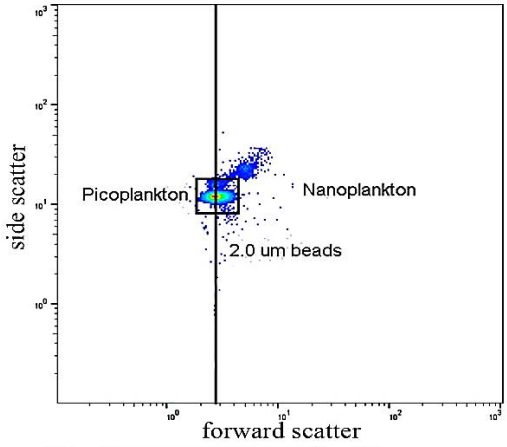

Figure 22 The 2.0 µm µm yellow-green fluorescent microspheres (FluoSpheres carboxylate-modified microspheres) was added to use as an internal reference.

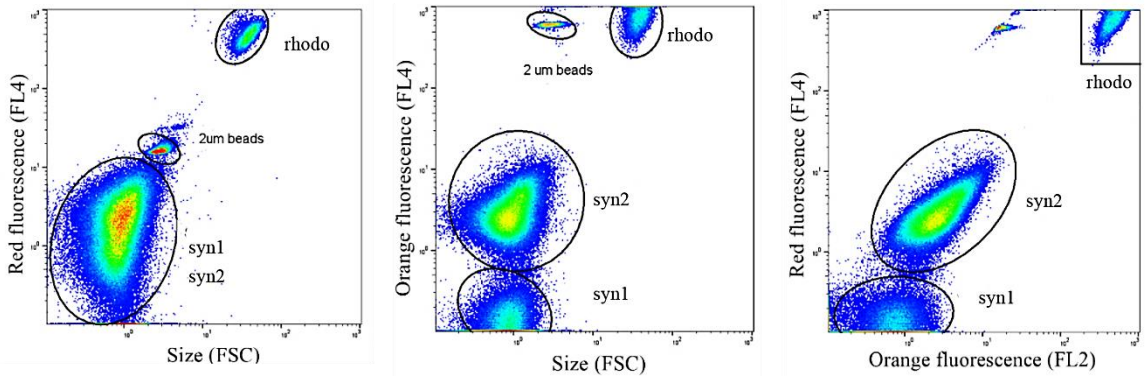

Figure 23 Examples of cultured cells observed under blue light excitation by flow cytometry to use as fluorescence references.

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Four groups of picophytoplankton were found along the study consisted of 2 type of *Synechococcus* and 2 groups of picoeukaryote while nanophytoplankton was differentiated into five groups which were cryptophyte and 4 types of nanoeukaryote. Their total cell density and proportion of each seasons was showed in Figure 24- 26 and Table 7.

*Synechococcus*1 dominated picophytoplankton community which reached the proportion up to 99.32% in the northeast monsoon, 98.73% in the intermonsoon I, 93.55% in the southwest monsoon, and 92.07% in the intermonsoon II followed by *Synechococcus*2 with its increasing in density in the southwest monsoon (6.18%) and the intermonsoon II (7.55%). Picoeukaryote1 and Picoeukaryote2 were recorded in low ratio (<1.00%).

Nanophytoplankton groups along 4 seasons (the northeast monsoon, the intermonsoon I, the southwest monsoon, and the intermonsoon II) were dominated by nanoeukaryote1 (98.32% and 97.53%, 74.57%, and 75.00%, respectively) while the nanoeukaryote2, 3, and 4 were noticed in low proportion (<1.30%) in the northeast monsoon and the intermonsoon I. However, there were increasing in the proportion of nanoeukaryote2 and nanoeukaryote3 in the southwest monsoon (13.57% and 10.50%) and the intermonsoon II (20.41 % and 3.46%). Cryptophyte was found in low ratio along the study (<0.30%)

The dominant group of picophytoplankton along the study was *Synechococcus*1 which reached the cell density more than 90% in all month followed by *Synechococcus*2 which higher in the southwest monsoon and the intermonsoon II than in the northeast monsoon and the intermonsoon I. The 2 types of picoeukaryote found in low ratio except in The southwest monsoon in station SC 7 that picoeukaryote2 was recorded in higher density than other stations.

Nanoeukaryote1 was dominated the total cell density showed >95% in the northeast monsoon and the intermonsoon I and >70% in the southwest monsoon and the intermonsoon II (except station SC 7 in the southwest monsoon). Nanoeukaryote2 and nanoeukaryote3 were increased the proportion in the southwest monsoon and the intermonsoon II. In the intermonsoon I, the ratio of nanoeukaryote4 become greater than other seasons which found in low density in the same direction as cryptophyte.

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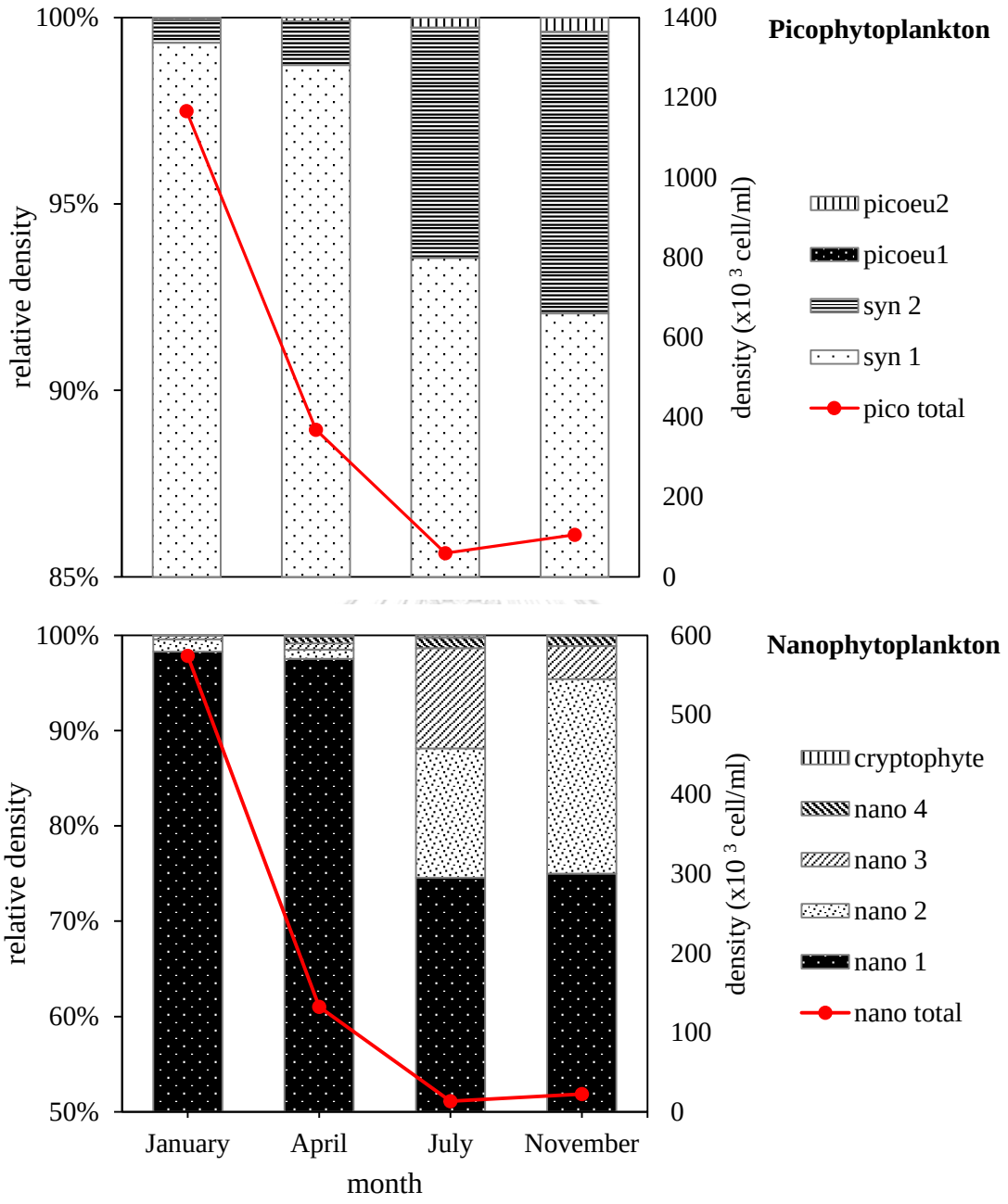

Figure 24 Relative density and total density of picophytoplankton and nanophytoplankton groups collected from Sichang Island.

*minimum value of y axis begin at 85% in relative density of picophytoplankton and 50% in relative density nanophytoplankton.

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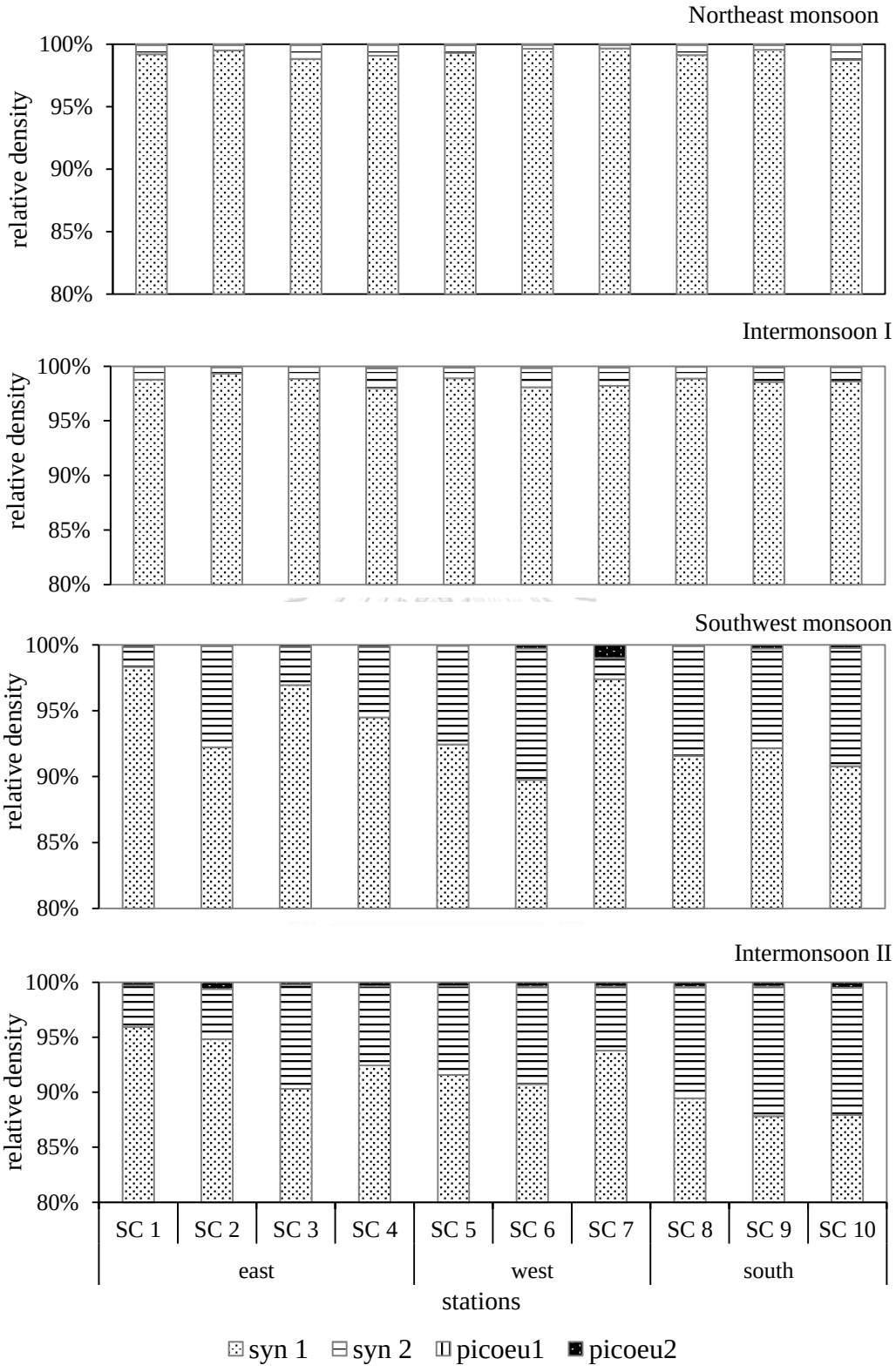

Figure 25 Relative density of picophytoplankton groups from Sichang Island.
*minimum value of y axis begin at 80% in all relative density of picophytoplankton.

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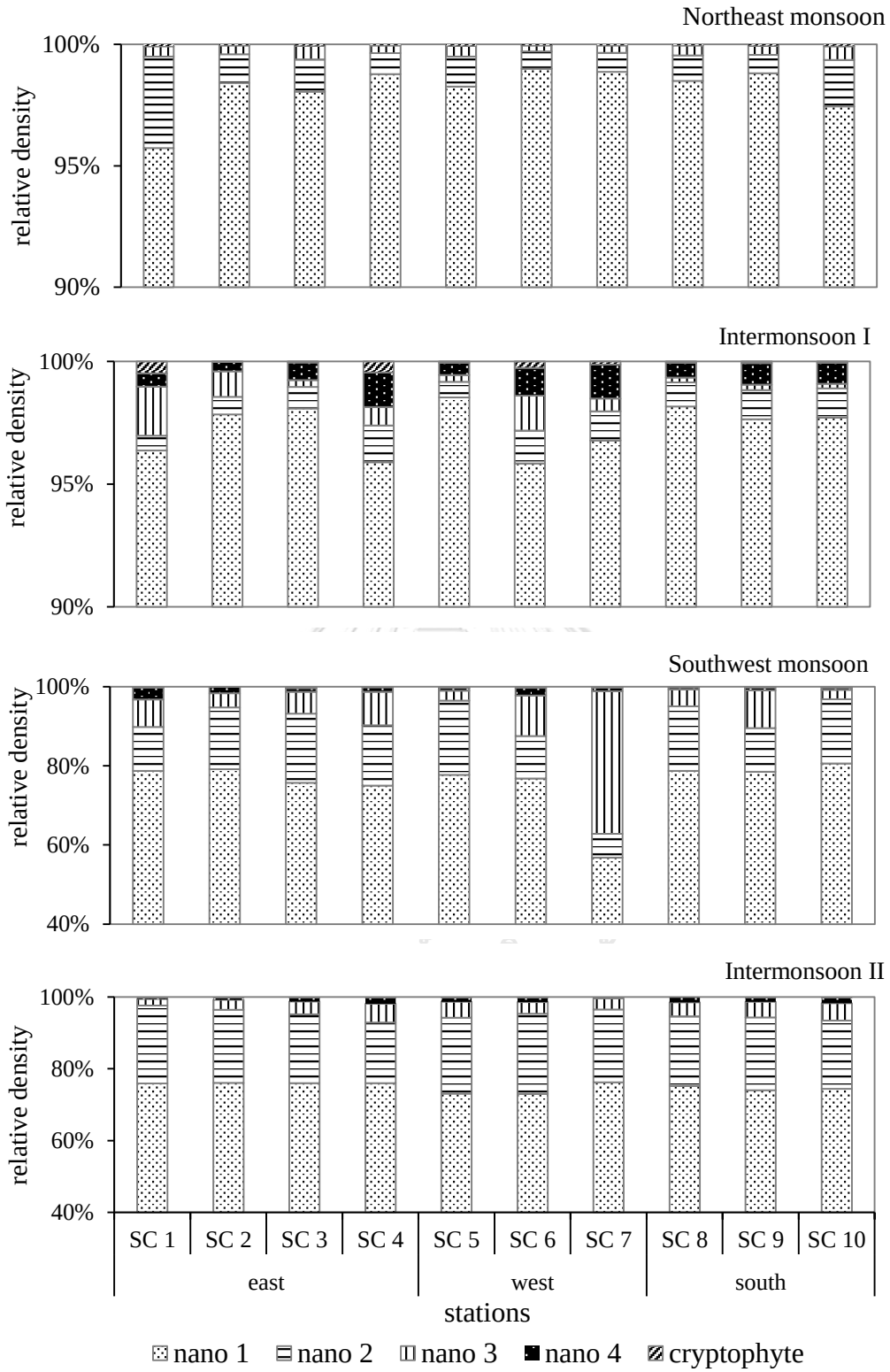

Figure 26 Relative density of nanophytoplankton groups from Sichang Island.
*minimum value of y axis begin at 90% in the northeast monsoon and the intermonsoon I and 40% in the southwest monsoon and the intermonsoon II in relative density of nanophytoplankton.

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Table 7 Size and fluorescence characteristics of samples from the northeast monsoon, the intermonsoon I, the southwest monsoon, and the intermonsoon II, the intermonsoon I, the southwest monsoon, and the intermonsoon II.

Picophyto-plankton (<2 µm)	Fluorescence characteristics	Nanophyto-plankton (2-20µm)	Fluorescence characteristics
*Synechococcus*1	Orange	Cryptophyte	The biggest size compared with others Orange-red
*Synechococcus*2	Higher orange than *Synechococcus*1	Nanophytoplankton1	Low red
Picoeukaryote1	Red	Nanophytoplankton2 *(coccolithophores)*	Low red Higher granules or rough surface than Nanophytoplankton1
Picoeukaryote2	Lower red than Picoeukaryote1	Nanophytoplankton3 *(prasinophyte)*	Red
		Nanophytoplankton4	Lower red than Nanophytoplankton3 but higher red than Nanophytoplankton1 and 2

6. Spearman rank correlation between microphytoplankton density and environmental parameters

Correlation between microphytoplankton density and environmental parameters were tested by using Spearman rank test correlation as shown in Table 8 and Table 9. Total microphytoplankton density showed no significant differences between all of physio - chemical parameters but positively correlated with DIN concentration ($p<0.05$). Cyanobacteria density were significantly negatively correlated with temperature, salinity, pH, DIN, and phosphate-phosphorus concentration but positively correlated with DO ($p<0.01$). Dinoflagellate density were significantly positively correlated with temperature, salinity, pH, DIN, and silicate-silicon concentration while there were negatively correlated with DO ($p<0.01$). Diatom density were significantly negatively correlated with pH ($p<0.01$) and phosphate-phosphorus concentration ($p<0.05$).

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Table 8 Spearman rank test of microphytoplankton density and physio-chemical parameters.

		Temperature	Salinity	DO	pH	Cyano density	Dino density	Diatom density	Total density
Spearman's rho	Temperature	1							
	Salinity	.785**	1						
	DO	-.499**	-.686**	1					
	pH	0.176	.319*	-.371*	1				
	Cyano_density	-.326*	-.544**	.443**	-.567**	1			
	Dino_density	.574**	.652**	-.563**	.669**	-.577**	1		
	Diatom_density	-0.088	-0.229	0.059	-.384*	.405**	0.013	1	.
	Total_density	0.148	0.026	-0.08	-0.14	0.2	0.305	.875**	1

****Correlation is significant at the 0.01 level (2-tailed).**

***Correlation is significant at the 0.05 level (2-tailed).**

Table 9 Spearman rank test of microphytoplankton density and nutrients.

		DIN	Phosphate	Silicate	Cyano density	Dino density	Diatom density	Total density
Spearman's rho	DIN	1						
	Phosphate	0.026	1					
	Silicate	.584**	-.388*	1				
	Cyano_density	-.431**	-.420**	-0.162	1			
	Dino_density	.777**	0.309	.503**	-.577**	1		
	Diatom_density	0.182	-.330*	0.054	.405**	0.013	1	
	Total_density	.379*	-0.11	0.132	0.2	0.305	.875**	1

****Correlation is significant at the 0.01 level (2-tailed).**

***Correlation is significant at the 0.05 level (2-tailed).**

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7. Community structure of microphytoplankton

A comparison of the abundance of microphytoplankton were analyzed on each data from the distance sampling technique. Four major groups of samples [the first group (A), second group (B), third group (C) and forth group (D)] were defined from the hierarchial clustering at 74% similarity level. Under the analysis of the non-metric multidimentional scaling (nMDS) plot, it was indicated that the clear separation of microphytoplankton assemblage in all seasons were present (Figure 27). A two-dimension stress of 0.16 indicated a reliable ordination. The community structure of microphytoplankton were summarized in Table 10.

8. Community structure of pico- and nanophytoplankton

Pico- and nanophytoplankton were divided into four major groups including the first group (A), second group (B), third group (C) and forth group (D) and summarized in Table 11. Results from this study showed that Pico- and nanophytoplankton were defined the hierarchial clustering at 90% similarity level. The first group (A) in all stations at the northeast monsoon and the second group (B) at the intermonsoon I consisted of all population. Interestingly, only population in station SC 7 at the southwest monsoon was observed in the third group (C). In the fourth group (D), all stations, excepting SC 7 at the southwest monsoon were exhibited, whereas the southwest monsoon and the intermonsoon II in this group were seen in all stations (Figure 28).

According to the non-metric multidimentional scaling (nMDS) plot, it was clearly showed that the separation of pico- and nanophytoplankton assemblaged at 90% similarity level. As well, a two-dimensional stress of 0.05 indicated a reliable ordination.

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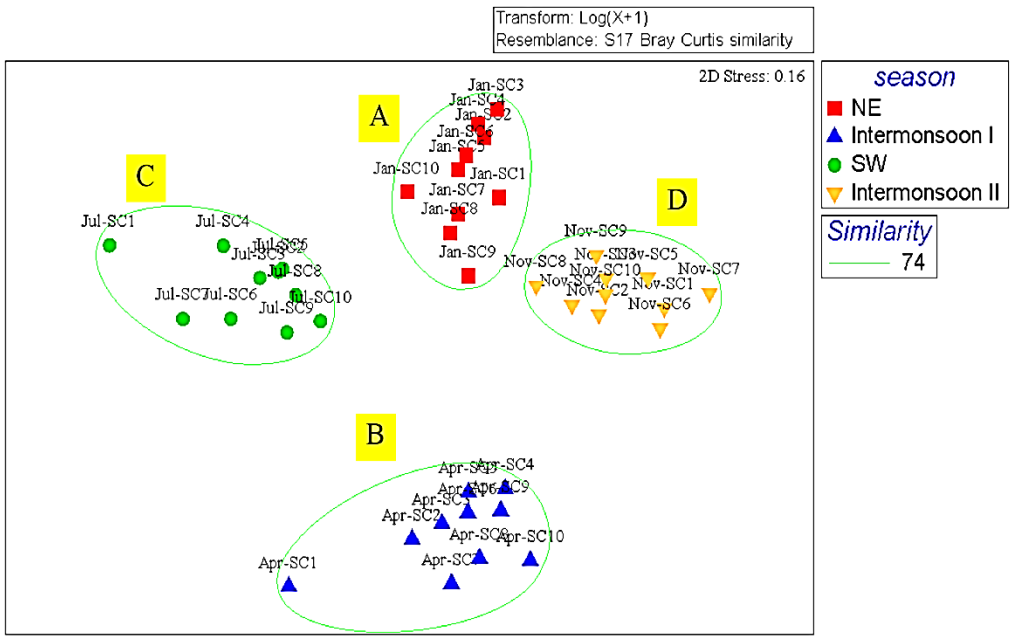

Figure 27 nMDS ordination plots of microphytoplankton assemblages based on microphytoplankton abundance data. The circle is 74% of similarity showed the main four groups depend on each month

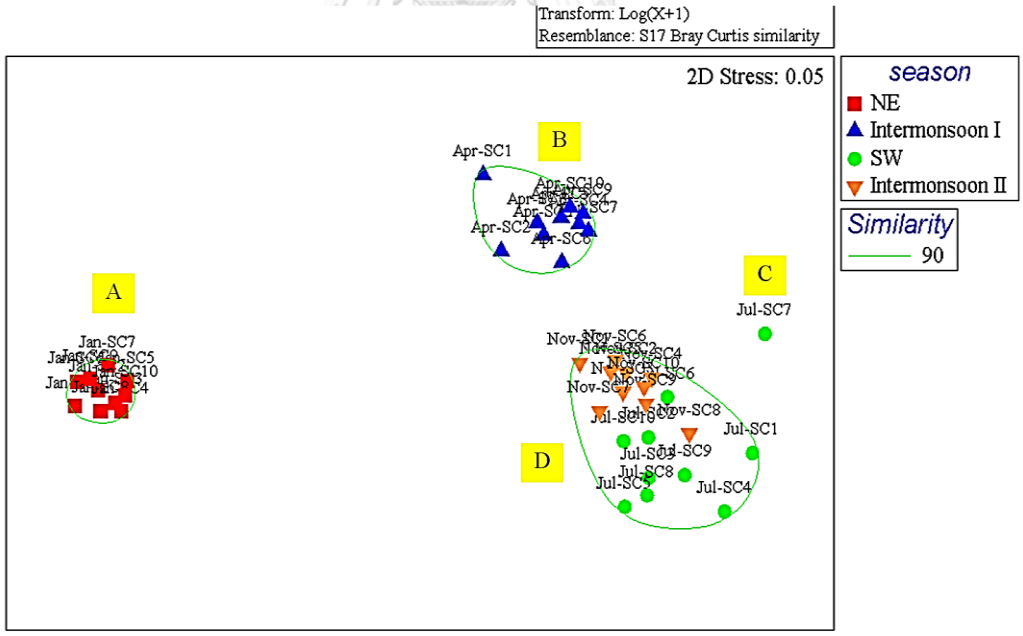

Figure 28 nMDS ordination plots of pico- and nanophytoplankton assemblages based on pico- and nanophytoplankton abundance data. The circle is 94% of similarity showed the main four groups depend on each seasons.

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9. Spatial and temporal variables of environmental parameters

The vectors in the Principle component analysis (PCA) plot (Figure 29) of physio - chemical parameters and nutrients showed the first two components cumulative explained 73% of data variation. Salinity, temperature, and phosphate-phosphorus concentration was defined on component 1 (PC1 axis) which were clearly positively correlated with all stations in the southwest monsoon (coefficient: 0.53, 0.41, and 0.41 respectively). The first component explained the unique characteristic of the highest salinity, temperature, and phosphate-phosphorus concentration in the southwest monsoon which clearly discriminating this month from the northeast monsoon and the intermonsoon II. PC 1 axis also showed an inverse relationship with dissolved oxygen (coefficient: -0.44) which reached the highest value in the northeast monsoon and the intermonsoon II but low in the southwest monsoon. The second conponent showed an inverse relationship of silicate-silicon concentration, DIN, and pH (coefficient: -0.65, -0.48, and -0.34 respectively) that separating the intermonsoon I from The southwest monsoon and the intermonsoon II.

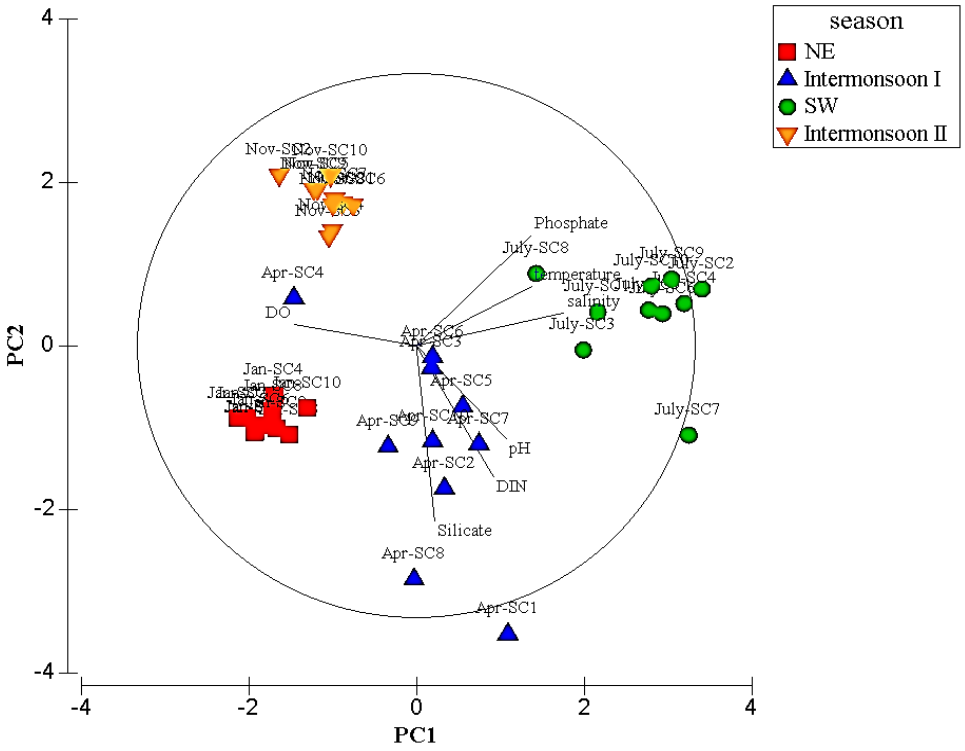

Figure 29 Principle component analysis (PCA) illustrated the relationships between stations with similar environmental characteristics.
The data showed the four main regions divided by seasons. Vectors visualize the fitted physio - chemical variables (temperature, salinity, dissolved oxygen (DO), pH, dissolved inorganic nitrogen (DIN) phosphate-phosphorus and silicate-silicon).

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Table 10 Community structure of microphytoplankton community between 4 periods

Periods	Environmental condition	Microphytoplankton community structure
Northeast monsoon	• Low temperature and salinity • High dissolved oxygen (DO) • low nutrients	• The community was dominated by diatom. • Highest in diversity • Dominant genera are *Chaetoceros, Pseudonitzschia, Guinardia.*
First intermonsoon	• High temperature • High variation in physiochemical factors and nutrients	• The community was dominated by diatom. • The abundance become highest. • Dominant genera are *Pseudonitzschia, Thalassionema, Chaetoceros*
Southwest monsoon	• High temperature and high salinity • High nutrients (DIN, phosphate, and silicate) • Low DO	• There is occurrence of *Noctiluca scintillans* blooming in the western part of Sichang island • Dominant genera are *Noctiluca scintillans, Eucampia, Chaetoceros.*
Second intermonsoon	• Low DIN • High dissolved oxygen (DO) • Low nutrients	• The community was dominated by diatom. • The abundance and diversity become lowest. • Dominant genera are *Chaetoceros, Pseudonitzschia, Psudanabaena.*

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Table 11 Community structure of pico- and nanophytoplankton community between 4 periods

Periods	Environmental condition	Pico- nanophytoplankton community structure
Northeast monsoon	• Low temperature and salinity • High dissolved oxygen (DO) • low nutrients	• The abundance was highest • Dominant groups were *Synechococcus*1, Nanoeukaryote 1
First intermonsoon	• High temperature • High variation in physiochemical factors and nutrients	• Dominant groups were *Synechococcus*1, Nanoeukaryote 1 and 4
Station SC 7 in the southwest monsoon	• High temperature and high salinity, low DO • High nutrients (DIN, phosphate, and silicate)	• The abundance become lowest. • Dominant groups were *Synechococcus*1, Nanoeukaryote 1 and 3
Southwest monsoon (except SC 7) and the second intermonsoon	• Low DIN	• Dominant groups were • *Synechococcus*1 and 2, Nanoeukaryote 1

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CHAPTER IV
DISCUSSION AND CONCLUSION

Seasonal variations of microphytoplankton community structure at Sichang Island

In the first group (A), microphytoplankton community was dominated by diatoms followed by cyanobacteria. Moreover, this stable condition created the most diverse in microphytoplankton community. Continuously, the diatoms were still dominated the community in the group (B) but the cyanobacteria was found in lower proportion than the previous group. The high salinity and low dissolved oxygen during the southwest monsoon in group (C) were observed in consistent with the changes in composition of microphytoplankton community. The coastal water of Sichang Island is more rapid interchange in resources with river discharge or new resources from Bangpakong river mount and Sriracha district together with the bottom sediment because of the shallow water. The southwest monsoon wind may bring the warm moist air from Indian Ocean into this area (Loo et al., 2015) and causes the turbulent. This situation may be related to increased variation in abundance and diversity (Reynolds, 2006a). Physio-chemical associate with nutrients enrichment leads the shift from diatom to dinoflagellate (Smayda, 1980) or large diatom to nanophytoflagellates (Hare et al., 2007). During the southwest monsoon which dinoflagellate, *N. scintillans* was bloomed. The occurrence of *N. scintillans* in the low DO or hypoxia region where favor for their blooming more than other groups of phytoplankton may disrupt the traditional diatom assemblage (do Rosario Gomes et al., 2014).

The high salinity and temperature including nutrients concentration may favor for the growth of *N. scintillans* and its photosynthetic endosymbiont, *Pedinomonas noctilucae* (Sweeney, 1971), similar to the study in the upper Gulf of Thailand which the abundance of *N. scintillans* was found in high density during the southwest monsoon period in May to September and low density during the northeast monsoon in the intermonsoon II to February (Rujinard et al., 2008). *N. scintillans* was first recorded in 1957 and have been increasing occurrence in term of density and frequency which cause the red tide particularly in the eastern part in the inner Gulf of Thailand (Lirdwitayaprasit, 2003). Some studies reported that physical processes such as winds, tides, and currents related with the blooming of *N. scintillans* (Zhang et al., 2017). River discharge due to the prevailing clockwise circulation of the water in the inner Gulf of Thailand during southwest monsoon period also affected the increasing of nutrients (Buranapratheprat et al., 2006; Rujinard et al., 2008). This area is under the strong effect from the Bangpakong river discharge (Buranapratheprat et al., 2006) and nutrients from the mainland, Sriracha district (approximately 12 kilometers from Sichang island).

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Moreover, its physiology in nutritional status may be the combined effect which controlled its buoyancy of *N. scintillans* (Uhlig and Sahling, 1990) to float in the surface layer to reach sunlight for photosynthesis and influenced their blooming. Moreover, a special ability of its chlorophyll-containing endosymbiont, *P. noctilucae,* which can fix carbon more efficiently than others phytoplankton under low oxygen concentration (do Rosario Gomes et al., 2014) supporting this present study that during southwest monsoon period showed the lowest DO which related with the high density of *N. scintillans*.

The results of the blooming of *N. scintillans* also leaded to chemical impact on producing NH_4 toxicity (Smayda, 1997) consistent with this present data that the highest values of ammonia-nitrogen concentrations around Sichang Island were recorded during the southwest monsoon. Interestingly, there were reports that *N. scintillans* grazed on large diatom, *Coscinodiscus* (Tada et al., 2000) and invertebrate egg such as copepod egg; *Acartia clausi* and *Calanus euxinus* (Nikishina et al., 2011) and planktivorous fishes egg like anchovy and sand-eel (Sekiguchi and Kato, 1976).

Diatom genus *Chaetoceros* and cyanobacteria genus *Trichodesmium* was the common genera which can found in all seasons but showed the variation in density. However, there were some genera of cyanobacteria (*Spirulin*a), dinoflagellate (*Gyrodinium*), and diatom (*Campyrodiscus* and *Dimeregramma*) was noticed only in the second intermonsoon. It may cause by sharply changed from the highest salinity in the southwest monsoon to the lowest salinity in the second intermonsoon. There were some report showed the good tolerability through wide *range* of salinity in *Spirulin*a (Almahrouqi et al., 2015). This period was found in the high number of genera but it showed the lowest diversity and evenness. It caused by the dominating of particular genus, *Chaetoceros,* which was consisting 78% of the total composition. The chain-forming diatoms were noticed that they were preference prey items of *Gyrodinium* (Hiroaki et al., 2006) which can be found only in the second intermonsoon consistent with the high abundance of *Chaetoceros* during on this periods.

Seasonal variations of pico- and nanophytoplankton

Pico- and nanophytoplankton communities around Sichang Island showed a clear seasonal variation which can be distinguish into four communities; the northeast monsoon, the intermonsoon I, the southwest monsoon in station SC 7, and the southwest monsoon and the intermonsoon II (Table 15).

With increasing temperature and salinity, there is a significantly decrease in the pico- and nanophytoplankton density especially in the southwest monsoon which the highest temperature and salinity were recorded in consistent with the lowest density of both pico- and nanophytoplankton. Moreover, the abundance were decrease despite nutrients were increase which similar trend of distribution pattern of pico- and

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nanophytoplankton in Changjiang Estuary (Pan et al., 2007; Xiuren et al., 1988) in contrast to microphytoplankton community which sharply increased in consistent with increasing of DIN especially in cyanobacteria and dinoflagellates groups.

Influence of environmental factors on phytoplankton community

In the present study, microphytoplankton communities from Sichang Island showed a clear seasonal variations, which could be distinguished into four communities based on seasons; the first group (A), second group (B), third group (C) and forth group (D). The physio-chemical factors clearly differed among the group. During the northeast monsoon period, the physio-chemical parameters were quietly stable due to their low variations, when compared to other seasons. This condition can be caused by the northeast monsoon wind which brings the cold mild air to this area (Loo et al., 2015). Previous observation reported that the activity of the microphytoplankton community is influenced from that physical parameters, as namely designed "seasonal cycle of the microphytoplankton" (Margalef, 1967).

Overall of the study, the nutrients of the first group (A), was depleted in the northeast monsoon, which was associated with the lowest temperature and salinity. At the same time, the microphytoplankton community were dominated by diatom, *Chaetoceros* and some cyanobacteria, in particular to *Psudanabaena*. The lowest density of the populations in this group was documented. This feature was the initial stage (Smayda, 1980), it was dominated by diatoms as similar to this research during the northeast monsoon. Continuously, the study confirmed that the temperature, salinity, and nutrients were dramatically increased at the first intermonsoon. The community were also still dominated by diatoms, but the microphytoplankton density were rapidly increased, compared to previous group. As a results, it was determined the third group (C) at the southwest monsoon that temperature, salinity, and nutrients reached as the highest values. Interestingly, *Noctiluca scintillans* was considered to be a dominant species and found in high density. It is possible that the appearance of *N. scintillans* might be related to DO. This situation was similar to the study in the Arabian Sea due to the spread of hypoxia which favored *N. scintillans* than other microphytoplankton (do Rosario Gomes et al., 2014). In all parameters including nutrients (excepting DO), temperature and salinity of forth group (D) were depleted. As supported by Smayda's study who studied the seasonal variation of the microphytoplankton community at Naragansett Bay, Rhode Island, USA. (Smayda, 1980). Another report was noticed in Bulgarian Black Sea during the summer which the diatoms became low in density but the dinoflagellates were dominated the microphytoplankton community (Velikova et al., 1999).

It is confirmed to support that the microphytoplankton community from Sichang Island may be associated to environmental factors.

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Although other factors like silicate-silicon and pH were correlated with the abundance of phytoplankton community, the temperature, salinity, and phosphate-phosphorus concentration were defined clearly as the most important environmental factors influencing the community structures of both microphytoplankton and pico- and nanophytoplankton based on principle component analysis which these three factors were highly affected the community.

Pico- and nanophytoplankton significantly differed which could be distinguished into four communities based on seasons; the first group (A), second group (B), third group (C) and forth group (D)]. However, the abundance of pico- and nanophytoplankton showed the opposing trend that highest density were noticed in low temperature, salinity, dissolved inorganic nitrogen (DIN) and silicate-silicon concentration. Interestingly, there are only population in station SC 7 was noticed in the group (C). It may cause by the high density of nanoeukaryote3 which supposed to be *Pedinomonas noctilucae*, the endosymbiont in *N. scintillans*. Because of the characteristic of this endosymbiont is green flagellate nanophytoplankton contains chlorophyll *a* and *b* (Jeffrey et al., 1997).

Based on the concept that the good growth condition of phytoplankton relative with atomic ratios of nutrients (Redfield, 1958). The change in DIN and DIP concentration is one of the important factors to determine the variations in phytoplankton community structure (Xu et al., 2010). In N and P ratios should be 16:1. In this study, DIN: DIP ratios in all periods mostly lower than the Redfield's ratio (lower than 16) It reflected the N-limited condition in this area indicating the primary production around Sichang Island was decreased by the lack of nitrogen based on the Redfield's ratio (Redfield, 1958). The high of DIN:DSi ratios (>1) may affect the diatom growth because of silicate-silicon limitation (Xu et al., 2008). The increasing ratio of DIN:DSi in the first intermonsoon period compared to the northeast monsoon period may cause by increasing of DIN or a reduction in silicate-silicon supply due to diatom utilization. It was noticed by a higher abundance of diatom more than twice in density in the first intermonsoon period than in northeast monsoon period.

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Conclusion

Seasonal variations of phytoplankton community around Sichang Island were controlled by the monsoonal alterations in physio-chemical factors and nutrient concentrations. Physio-chemical associate with nutrients enrichment leads the shift from diatom during the first intermonsoon period to dinoflagellate in the southwest monsoon period. Then in the second intermonsoon period, the population were restored community structure back to diatom-dominated. Phytoplankton populations which are in the similar characteristic and their responses to environmental factors may be used as biological indicator to indicate the change in water quality and environmental stress. The change in phytoplankton communities were basis data which may reflect types of the food web in term of size structure and diversity.

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APPENDIX

VITA

Miss Anassaya Deesuk was born in 7 February 1991 at Samutsakhon, Thailand. She received her bachelor's degree of Science in Department of Marine Science from Faculty of Science, Chulalongkorn University in 2009. She graduated study for Master's degree of Marine Science in Department of Marine Science, Faculty of Science, Chulalongkorn University.