

# Dynamics of Microalgae Along the Coastal Areas of Sooyoung Bay, Busan, South Korea

Binod Prasad<sup>1</sup>, General Thiyam<sup>1</sup>, Dong-Gyu Lee<sup>1</sup>, Moo-Sang Kim<sup>2</sup>, Man-Gi Cho<sup>1\*</sup>

<sup>1</sup>Department of Bio-chemical Engineering, Dongseo University, San69-1, Churye-2-Dong, Sasang-Gu, Busan, 617-716, Republic of Korea

<sup>2</sup>DUTUJAL, Department of Bio-chemical Engineering, Dongseo University, San 69-1 Churye-2-Dong, Sasang-Gu, Busan, 617-716, Republic of Korea

**Abstract** Microalgae are one of the major, sustaining components of ecosystem processes and are responsible for biogeochemical reactions that drive our climate changes. Despite this, many marine microalgae are poorly described and little is known of their abundance and distribution along the coastal areas of Sooyoung Bay, Busan, South Korea. The present study has been conducted from November, 2011 to August, 2009 with the objective to provide an overview of the taxonomy diversity and abundance of microalgae along the coastal areas of the Sooyoung Bay. Water samples were collected from different sites, which were located by using a GPS tracker. Chlorophyll fluorescence of the water samples were measured by using ToxY-PAM dual-channel yield analyzer. The chlorophyll fluorescence values were relatively higher during the spring and summer and even in the region near to the sea port. Similarly the abundance of microalgae was higher near the port but diversity index had lower values. The temperature and pH values were same at all the sites. However, only the temperature varied during the sampling period, with higher values during summer and lower in winter. From the preliminary results, the following class of microalgae were found; Bacillariophyceae, Dinophyceae, Silicoflagellate and Cryptophyceae. With a future ongoing work, microalgae are being isolated to establish single cell culture and for identification using light microscopic observations, photography and molecular approaches.

**Key words :** Microalgae, Diversity, Chlorophyll fluorescence

## Introduction

The term microalgae define a heterogeneous group of organism. They are unicellular at the microscopic scale and perform oxygenic photosynthesis, although some algae forming groups or chains which may be visible by eyes. Because this is rather a phenotypical then a taxonomically valid classification, they are very diverse in terms of phylogeny. They encompass prokaryotic forms such as cyanobacteria, and eukaryotic organism such as green and red algae. Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications (Pulz and Gross, 2004). Microalgae are usually found in damp places or bodies of water and thus are

common in terrestrial as well as aquatic environments. Marine microalgae play a very important role in many biogeochemical processes that sustain the biosphere apart from contributing a major portion of primary production and support food webs in waters. It also provides a variety of goods and services that are essential to mankind's existence, including food production, assimilation of waste and regulation of the global climate.

Understanding and preserving biodiversity is one of the most important global challenges and will continue to be an important scientific issue in the coming years. Unicellular eukaryotic microalgae are the product of over 3 billion years of evolution, and are highly diverse groups of organism known (Falkowski et al., 2004). Continual discovery of new species in the ocean sug-

\* Corresponding author

Phone: +82-51-320-2662, Fax: +82-51-320-1547

E-mail: mgcho@gdsu.dongseo.ac.kr

gests that there are likely many more microalgae lineages to be recognized. Environmental factors and population densities fluctuate in time and space (Litchman and Klausmeyer 2001), and because of the close coupling of physical forcing and biota, environmental fluctuations are expected to significantly affect communities in aquatic systems. The major environmental factors affecting seasonal dynamics and succession of microalgae populations are water temperature, light, wave motion, and salinity and nutrient concentrations (Boaventura et al., 2002; Cecere & ). Water temperature is an important factor controlling the algal growth in natural environments (Lund 1949; Talling 1955). Temporal variability due to interactions among physical, chemical and biological variables results in the structure and function of the microalgae community, and is of fundamental importance to aquatic environments. Furthermore, high temporal variability results in frequent organization of the relative abundance and species composition of phytoplankton (Reynolds et al. 2000). Moreover, wide ranges of salinity and water temperature may be important in the frequent appearance of phytoplankton species throughout the year in the oceans (Hoshiai et al. 2003). The global environment is experiencing rapid and accelerating changes, especially by human activities which are also altering the distribution and movements of nutrient elements and hence altering the life forms along the coastal regions. Reolke et al. 1999 reported changes in nutrient availability can alter the species composition of the primary producers. Furthermore, increased concentrations of nutrient also increases phytoplankton diversity to levels far greater than those under limited nutrients (Grover 1989).

In recent years, microalgae have gained much attention due to their high nutritional value, high-value chemicals such as pigments and vitamins, high growth rate as compared to higher plants, and the ability to utilize light energy. In addition, microalgae can biosynthesize, metabolize, accumulate and secrete a great diversity of primary and secondary metabolites, many of which are valuable substances with potential applications in the food, pharmaceutical and cosmetics industries (Yamaguchi, 1997). They can be used to enhance the nutritional value of food and animal feed, due to their well balanced chemical composition. Further, microalgae cultivation is easy by using sunlight energy and can be carried out in open or covered ponds

or closed photobioreactors, based on tubular, flat plate or other designs. Furthermore, microalgae provide advantage for usage of unfertile lands, inefficient for agriculture, for biodiesel production instead of using productive lands for food production. However, among 800,000 microalgae species estimated to exist, only a few thousands strains are kept in collections; a few hundred are investigated for chemical content, already yielding about 15000 novel compounds and just a handful is cultivated in industrial quantities (Olaizola, 2003).

Thus, keeping in view the importance of microalgae and effect of environment and human activities on its community, in this present study we aimed to survey on diversity of marine microalgae along the coastal areas of Sooyoung Bay, South Korea. This diversity study may create an essential background to assess the marine biodiversity and its role in marine ecosystem.

## Materials and methods

### Study area and sampling

The study was conducted off the south east coast of South Korea at Sooyoung Bay, where human activities are extreme. In total, 21 sampling stations were designated along different transects belonging to inshore, middle shore, and offshore areas of the coast (Fig. 1). The sampling sites were located by using GPS-740-51ch. Water samples were collected monthly from the surface of the sea from November 2008 to August 2009. Each sample was divided into two bottles; one was used for the analysis of Chlorophyll fluorescence, temperature and pH, and the other was used for quantitative and qualitative analyses of microalgae.

### Chlorophyll fluorescence Measurement

Chlorophyll fluorescence values of the water samples were carried out with the ToxY-PAM dual-channel yield analyzer (WALZ, Germany). The ToxY-PAM employs a pulse-modulated fluorescence for measuring the chlorophyll fluorescence yield, which may vary between a quasidark level ( $F_0$ ), when all PS II reaction centers are open, and a maximal level ( $F_m$ ), when all PS II reaction centres are closed. The latter state can be induced transiently in a non-invasive way by a short (ca. 0.5 s) pulse of saturating light (saturation pulse). An illuminated sample displays a fluorescence yield,  $F$ , between  $F_0$  and  $F_m$ . The maximum fluorescence yield that can be induced by a saturation pulse in an

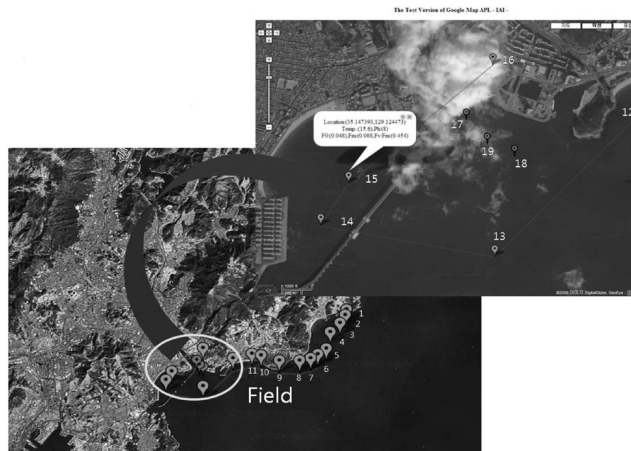


Fig. 1. Sampling sites as located by using GPS.

illuminated sample is normally lowered with respect to  $F_m$  by nonphotochemical quenching, and is denoted  $F_m$ . The so called Yield or Gentyparameter Yield =  $Y = (F_m - F_0)/F_m = F_v/F_m$ , is a measure of the effective quantum yield of energy conversion at PS II reaction centers. Open the program in computer to measure the photosynthetic parameters

For effect measurements, Aliquots of sample (4 ml) was covered with aluminum foil and incubated for dark adaptation. After 5 min of dark adaptation, 1 ml of sample was transferred into Quartz cuvette and placed into the S chamber of the analyzer. The photosynthetic response was monitored on the Toxy Win chart The fluorescence was measured by turning on the light as shown on the program file after the  $F_0$  value is constant (stable). Each sample was measured three times to get a mean value of the chlorophyll fluorescence.

## Temperature and pH

The temperature of the water samples from different sites were measured on spot by using thermometer. The pH of the samples measured in lab using pH meter (Mettler Toledo AG, Switzerland).

## Microalgae analysis

Water samples of only five stations were used for quantitative and qualitative analysis of microalgae. These analyses were carried out by NLP company, Busan, South Korea,

## Result and discussion

Marine biodiversity and new lineages have con-

tinually been discovered. However, in this study we attempt to explore the diversity of marine microalgae along the south east coast of Korea. A measurement of chlorophyll fluorescence of the water samples using Toxy-PAM and microalgae analysis both quantitatively and qualitatively was carried. The chlorophyll fluorescence was carried out as an index for the presence of photosynthetic microorganisms in the sea water. Furthermore, the effect of human activities, and temperature and pH of the water as an indicator for nutrient variability was also studied.

The physical characteristics, especially the changing patterns of water temperature were nearly the same from all the sites (Fig. 2). The invariability in the temperature at different sites can attributable to the effective mixing of the sea water. The temperature was significantly lower from the start of sampling date i.e., beginning of winter November 2008 to the end of spring May 2009. While, the water temperature of the surface layer was high from the beginning of summer (May) to the end of summer (August), and no significant difference was observed among stations. Like water temperature, the pH from winter to spring was similar in the surface layers due to effective vertical mixing (Fig. 2). The pH in summer was slightly higher, but non-significant in some of the sites.

The abundance and species composition of the marine microalgae varied strongly with season. The presence of photosynthetic microorganisms varied throughout the sampling period, which were confirmed by the higher chlorophyll fluorescence values (Fig. 3 a,b,c). The values were slightly lower during the winter

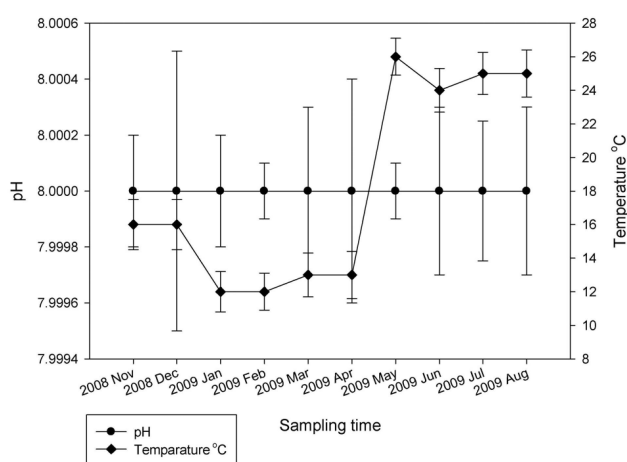


Fig. 2. Temperature and pH profile of the water samples at different sampling time.

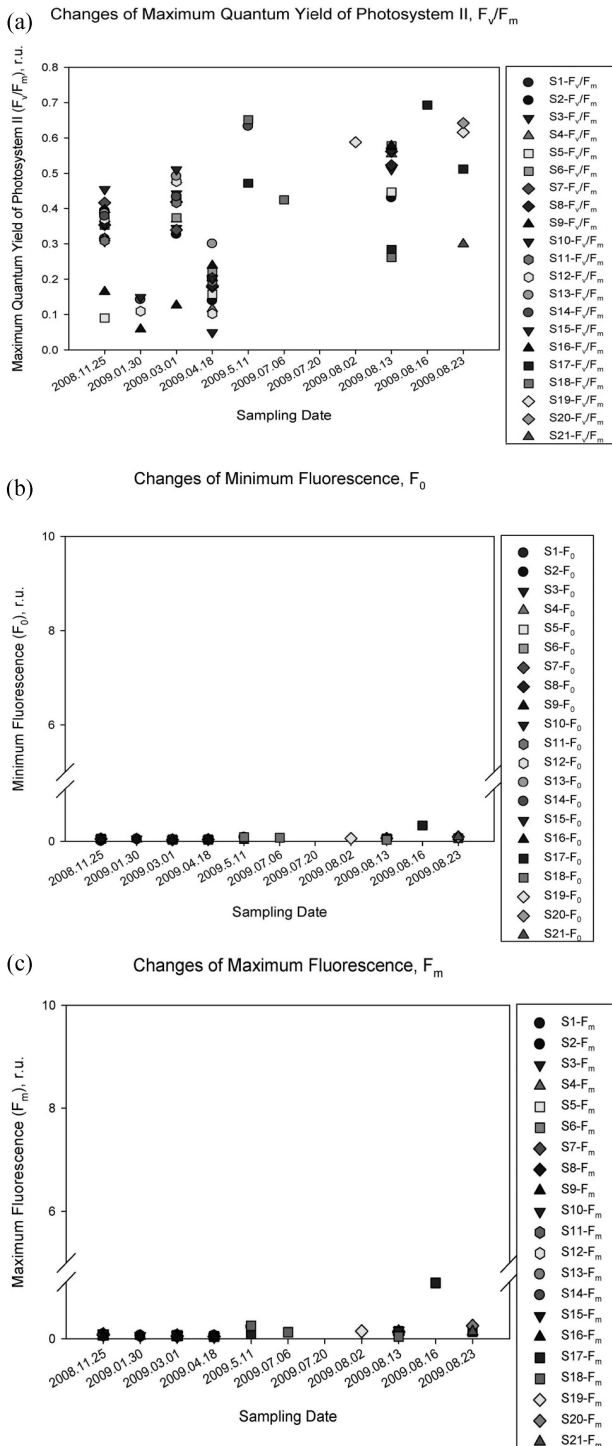


Fig. 3 a,b,c. Chlorophyll fluorescence Measurement

season. This is probably due to the low temperature profile during this period. However the values were relatively became higher with the onset of spring and summer. The species diversity index was measured only once in the month of May and was carried out for only five stations (Table 2). The diversity and abundance of

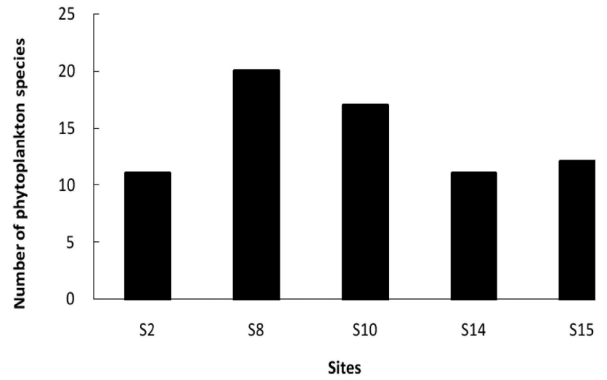


Fig. 4. Diversity index of microalgae of selected sites.

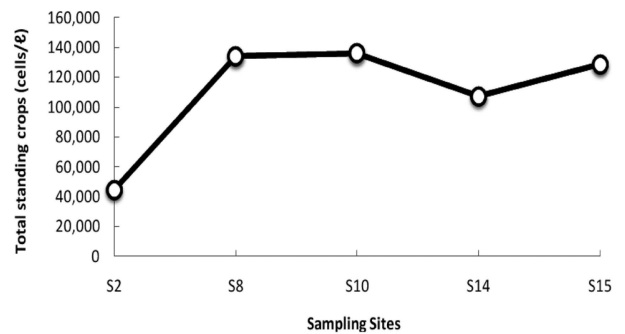


Fig. 5. Total number of microalgae of selected sampling sites.

Table 1. Location of sampling sites

Sample Sites	Latitude	Longitude
1	35.177977	129.216313
2	35.175217	129.215835
3	35.171418	129.213078
4	35.166865	129.207963
5	35.158783	129.205895
6	35.155718	129.20144
7	35.153808	129.197555
8	35.152822	129.191763
9	35.152838	129.181127
10	35.155308	129.171297
11	35.156153	129.166598
12	35.153815	129.156732
13	35.140225	129.140883
14	35.143287	129.121335
15	35.147393	129.124473
16	35.158918	129.140688
17		Sea port 30 m
18		Sea port 50 m
19		Sea port 50 m
20		Sea port 150 m
21		Yachts anchorage

**Table 2.** Taxonomic diversity of microalgae at selected sites along the coastal area of Sooyoung Bay.

Species	S2	S8	S10	S14	S15
<b>BACILLARIOPHYCEAE</b>					
<i>Asterionellopsis glacialis</i> CASTRACANE	5,728	15,833	10,393	3,723	5,266
<i>Biddulphia longicruris</i>	0	556	0	0	0
<i>Chaetoceros pseudocrinitus</i>	0	4,722	9,270	12,766	4,681
<i>Chaetoceros didymus</i> var protuberans	4,637	3,611	14,045	3,723	0
<i>Chaetoceros</i> sp.	17,728	65,834	67,978	59,574	87,181
<i>Cylindrotheca closterium</i>	3,273	12,500	7,023	1,064	1,170
<i>Ditylum brightwellii</i>	273	556	0	0	0
<i>Eucampia zodiacus</i>	0	5,000	3,652	7,447	5,851
<i>Licmophora dalmatica</i>	0	278	281	532	0
<i>Melosira</i> sp.	0	3,611	7,023	7,447	13,458
<i>Navicula</i> sp.	1,909	3,333	2,809	2,660	1,170
<i>Nitzschia</i> sp.	3,000	3,056	2,528	532	1,170
<i>Pleurosigma angulatum</i>	0	556	562	0	0
<i>Pleurosigma directum</i>	273	0	0	0	0
<i>Pseudonitzschia</i> sp.	0	0	843	0	0
<i>Rhizosolenia</i> sp.	0	278	281	0	585
<i>Rhizosolenia pungens</i>	0	278	0	0	0
<i>Skeletonema costatum</i>	0	3,889	1,124	0	1,170
<i>Striatella uipunctata</i>	273	278	0	0	0
<i>Thalassiosira</i> sp.	2,727	556	3,371	3,191	585
<i>Thalassionema natzschioides</i> HUSTEDT	273	1,111	843	0	5,851
<i>Thalassiosira hyalina</i>	0	1,111	0	0	0
<i>Thalassiothrix</i> sp.	0	0	562	0	0
<b>DINOPHYCEAE</b>					
<i>Prorocentrum triestinum</i>	0	0	0	0	0
<i>Protoperdinium breve</i>	0	0	0	0	0
<i>Protoperdinium</i> sp.	0	0	0	0	0
<b>SILICOFLAGELLATE</b>					
<i>Dictyocha fibula</i>	0	0	0	0	0
<b>CRYPTOPHYCEAE</b>					
<i>Dictyocha fspeculum</i>	0	0	0	0	0
<i>Cryptomonas</i> sp.	0	0	0	0	0
<b>UNKNOWN</b>	4,637	7,222	3,652	4,787	585
Total standing crops (cell/l)	44,729	134,168	136,237	107,446	128,724
Number of species	11	20	17	11	12

microalgae varied significantly at different sites. The microalgae diversity and number was highest at site S10, followed by sites S10, S15, S14 and S2 (Fig. 4, Fig. 5). The abundance of microalgae was relatively higher near the sea port areas whereas the diversity was lower. This might be due to the nutrient inflow and leading to bloom of a particular algal species. The most prominent class of microalgae was Bacillariophyceae. Among the total number of standing phytoplankton, nearly half of these algae were unidentified. This opens the gate for screening of exclusive and promising

Korean microalgae as potential production strains for high-value products.

In conclusion, the dynamics of the water temperature and pH of the water samples were similar at all the sites at any time of sampling. Microalgae were dominant in spring and summer. This diversity study may create an essential background to assess the marine biodiversity and its role in marine ecosystem. Further studies will be continued on the establishment of single cell culture (small scale to mass culture), biochemical and toxicity analysis of the cultured strains, taxonomic com-

parison of species level by using morphometric and molecular phylogenetic analysis including finding out of new genus or species, seasonal abundance and distribution of toxic genus/species of marine microalgae in South Korea.

## References

1. Pulz O, Gross W. 2004. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*. **65**, 625-648.
2. Falkowski P G, Katz M E, et al. 2004. The evolution of modern eukaryotic phytoplankton. *Science*, **305**, 354-360.
3. Litchman, E. and C.A. Klausmeyer. 2001. Competition of phytoplankton under fluctuating light. *Am. Nat.*, **157**, 170-187.
4. Boaventura, D., Re' , P., Cancela da Fonseca, L., Hawkins, S.J., 2002. Intertidal rocky shore communities of the continental Portuguese coast: analysis of distribution patterns. *Marine Ecology* **23**, 69-90.
5. Cecere, E. & Perrone, C. 2002. Morphology of *Acanthophora nayadiformis* (Ceramiales, Rhodophyta). *Phycologia* **41**: 523-532
7. Lund, J.W.C. 1949. Studies on *Asterionella formosa*. I. The origin and nature of the cells producing seasonal maxima. *J. Ecol.*, **37**, 389-419.
8. Talling, J.F. 1955. The relative growth rates of three planktonic diatoms in relation to underwater radiation and temperature. *Ann. Bot. N. S.*, **19**, 329-341.
9. Reynolds, C.S., M. Dokulil, and J. Padisak. 2000. Understanding the assembly of phytoplankton in relation to the trophic spectrum: Where are we now? p. 147-152. In: The trophic spectrum revised: the influence of trophic state on the assembly of phytoplankton communities, ed. by C.S. Reynolds, M. Dokulil and J. Padisak. Development in Hydrobiology 150. Kluwer Academic Publishers, London.
10. Hoshiai, G., T. Suzuki, T. Kamiyama, M. Yamasaki, and K. Ichimi. 2003. Water temperature and salinity during the occurrence of *Dinophysis fortii* and *D. acuminata* in Kesenuma Bay, northern Japan. *Fish. Sci.*, **69**, 1303-1315.
11. Reolke, D.L., P.M. Eldridge, and L.A. Cifuentes. 1999. A model of phytoplankton competition for limiting and nonlimiting nutrients: Implication for development of estuarine and near shore management schemes. *Estuaries*, **22**, 92-104.
12. Grover, J.P. 1989. Phosphorus-dependent growth kinetics of 11 species of freshwater algae. *Limnol. Oceanogr.*, **34**, 341-348.
13. Yamaguchi, K. 1997. Recent advances in microalgal bio-science in Japan, with special reference to utilization of biomass and metabolites: a review. *J Appl Phycol*, **8**, 487-502.
14. Olaizola, M. 2003. Commercial development of microalgal biotechnology: from the test tube to the marketplace. *J. Biomol. Eng.* **20**, 459-466.