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Differences in *in vivo* Fluorescence Yield for Netplankton and Nanoplankton Size Classes

Chang-Ho MOON and Seung-Yong LEE
Department of Oceanography, National Fisheries University of Pusan, Pusan 608-737, Korea

In the South Sea of Korea, *in vivo* fluorescence intensity (IVF) and extractable chlorophyll *a* concentration were measured to determine whether there was significant defference in *in vivo* fluorescence per unit chlorophyll *a* (R) between netplankton and nanoplankton size classes (less than 22μm). IVF and chlorophyll *a* were linearly related for both size classes, but R's were significantly different between two size classes. The R of nanoplankton was about 7 times higher than that of netplankton. Therefore, the size dependency of R must be taken into consideration when size fraction of phytoplankton biomass is determined from the measurements of *in vivo* fluorescence intensity.

Introduction

Techniques currently usued for the measurements of chlorophyll involve the filtration of samples, extraction of pigment with 90% aceton followed by either measuring the absorption of light by the trichometric method (Richard and Thompson, 1952) or by the fluorescence (Yentsch and Menzel, 1963; Holm-Hansen *et al.*, 1965). These two methods, however, take time to extract chlorophyll and experiments are not convenient on the shipboard. Lorenzen (1966) described the use of *in vivo* fluorescence chlorophyll *a* technique to obtain the algal biomass quickly and conveniently. The basic assumption of the method is that there is a constant ratio (R) between the observed fluorescence intensity (arbitary units) of an *in vivo* sample (IVF) and the extractable chlorophyll *a* (μg/l).

Numerous works have shown large variations in R values of the natural phytoplankton population. Strickland (1968) found that the ratio (R) could vary 2-fold in the water off Peru and California. Studies in San Francisco Bay (Alpine *et al.*, 1979) and Chesapeake Bay (Loftus and Seliger, 1975) showed 10-fold variation in R's over relatively short time and distance scales. The variations in R were attributed to the intensity of irradiance to which the cells have been exposed (Kiefer, 1973a), the difference in photosynthetic capacity (Vincent, 1983), the physiological condition (Kiefer, 1973b) and the species composition or size classes (Strickland, 1968; Heaney, 1978; Vincent, 1983).

In Korean waters, there have been numerous measurements of chlorophyll by the *in vivo* fluorescence technique (Shim and Yeo, 1988; Shim and Shin, 1989; Shin *et al.*, 1990; Shim *et al.*, 1991), but there were no comments on the factors affecting the R values. Moreover, most of those studies showed the biomass of netplankton and nanoplankton size classes, separately. The purpose of this study is to determine whether there is significant difference in *in vivo* fluorescence per unit chlorophyll *a* (R) between netplankton and nanoplankton size classes. If there is significant variation in R, the size dependency of R must be taken into consideration when using IVF to estimate size fractionate

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biomasses.

Materials and Methods

Field observations (Fig. 1) were performed during the day time in the South Sea of Korea on August 16~17, 1993. Surface water samples were

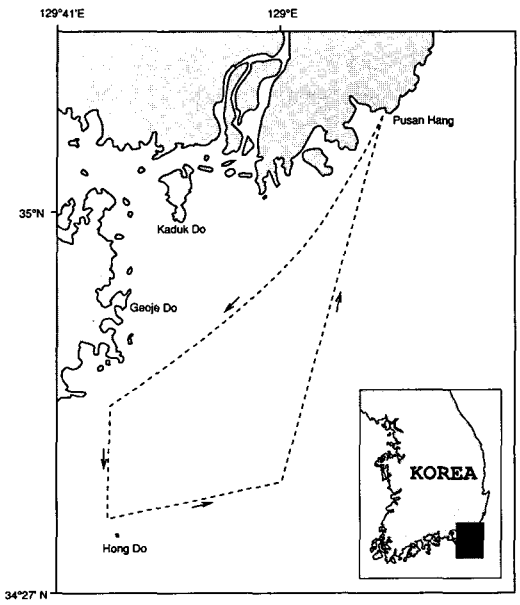

Fig. 1. Location of sampling area. Surface water samples were collected at every 30 minute during the cruise.

collected at every 30 minute during the cruise and held for about 2 hours in darkness before measuring *in vivo* fluorescence intensity (Heaney, 1978; Alpine and Cloern, 1985). The measurements of IVF and chlorophyll *a* were made on an "unfractionated" whole sample and "nanoplankton", defined as the fraction passing through a 22*μm* nitex screen. Values for netplankton were obtained by subtracting the values of nanoplankton from the values of whole samples. All measurements were made in duplicate and the averages were taken for the results. Calculations of the IVF and chlorophyll *a* were corrected by subtracting the values of the filtrate, the fraction passing through a 0.45*μm* membrane filter.

IVF was measured with a Turner Designs Model 10 Fluorometer equipped with a 100*ml* cuvette holder and appropriate filters (Lorenzen, 1966). Extractable chlorophyll *a* was determined fluorometrically as described by Yentsch and Menzel (1963) and Holm-Hansen *et al.* (1965). The samples were collected onto 0.45*μm* membrane filter and then the filters were soaked in glass tubes with 10*ml* of 90 % aceton for 24 hours in the dark refrigerator. After extractions were completed, the fluorescence were measured 30 seconds before and after acidification with a drop of 5% HCl (to correct for phaeopigments) on the same fluorometer used for IVF measurements, which was calibrated with pure chlorophyll *a* (Sigma Chemical).

Results and Discussion

The IVF intensity of filtrate, the fraction passing through a 0.45*μm* membrane filter, ranged from 1.2 % to 19.9% of total IVF with an average 7.3%. Most of this filtrate IVF seem to be free from chlorophyll *a* because the filtrate chlorophyll *a* was only between 0.3% and 1.3% with an average 0.6% (Table 1). Total extractable chlorophyll *a* concentrations ranged from 0.50 to 13.59*μg/l*, while the concentrations of netplankton and nanoplankton

Table 1. Comparison of 0.45*μm* filtrate and nanoplankton percentage (%) of total chlorophyll *a* and total *in vivo* fluorescence (IVF)

	chlorophyll *a*		IVF	
	0.45*μm*/total	nano/total	0.45*μm*/total	nano/total
Average	0.6	42.7	7.3	67.7
STD	0.4	24.0	5.3	17.7
Range	0.3~1.3	3.6~92.0	1.2~19.9	31.1~97.2
n	6	32	32	32

were in the range of 0.06~11.67μg/l and 0.19~1.92 μg/l, respectively (Fig. 2). Percent nanoplankton chlorophyll *a* of total ranged from 3.6% to 92.0% with an average 42.7%, while percentage of nanoplankton IVF were between 31.2% and 97.2% with an average 67.7% (Table 1). This implies that the IVF of nanoplankton is larger than that of netplankton.

There was good linear relationship between IVF and chlorophyll *a* (R=slope) for each totalplankton, netplankton and nanoplankton. The coefficients of determination (r^2) were 0.55 for totalplankton, 0.64 for netplankton and 0.41 for nanoplankton (n =32, Fig. 2). However, there was significant difference ($p < 0.005$) in slopes (R) for net and nanoplankton size classes (Table 2). The R value of nanoplankton was about 7 times higher than that of netplankton. These results are consistent with Loftus *et al.* (1972) who found that small sized phytoplankton have higher R values than large species in a laboratory study of unialgal culture. Alpine and Cloern (1985) also reported in the field study of San Francisco Bay that ultraplankton R was twice that of the nanoplankton which was in turn twice the netplankton R.

It is generally believed that small cells have higher photosynthetic rates per unit chlorophyll *a* than large cells (Taguchi, 1976) and fluorescence yield might therefore be lower in smaller cells. However, we found that R was much higher in smaller cells. If there is no difference in photosynthetic rates per unit chlorophyll *a* between these two size classes in the study area, which have not been reported previously, our data suggest that the greatest influence on R in the study area is associated with factors affecting the rate of the absorption of light, such as cell size and species composition (i.e. accessory pigment) as noted by Alpine and Cloern (1985). According to Kirk (1975), small cells are more efficient at capturing photons than larger

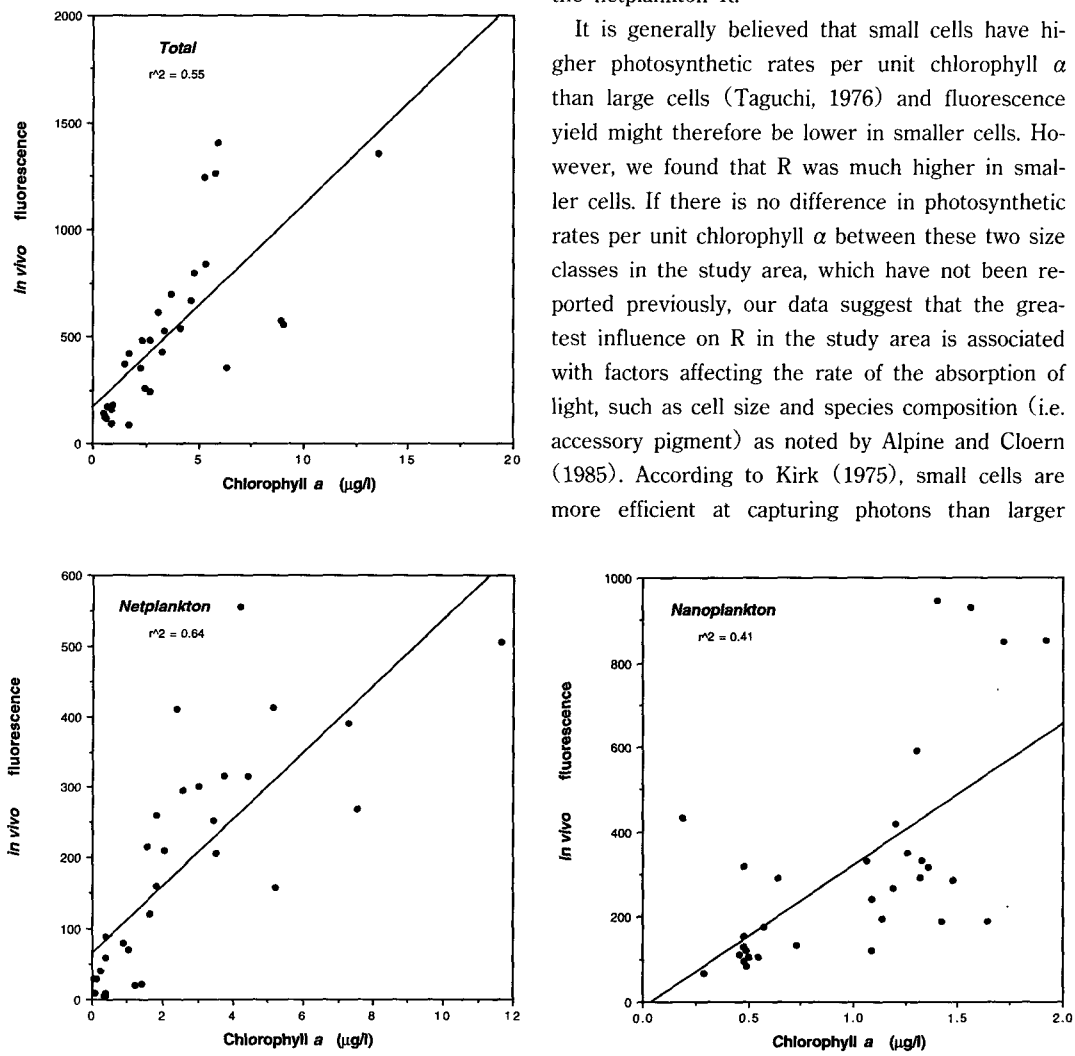

Fig. 2. *In vivo* fluorescence plotted against chlorophyll *a* for total (a), net (b), and nanoplankton (c) size classes.

Table 2. Regression and coefficient of determination for the linear regression of *in vivo* fluorescence (IVF) against chlorophyll *a* for netplankton and nanoplankton size classes

size classes	intercept (IVF units)	slope (R) (IVF/chl. *a*)	95% C.L. for R	r^2	n
netplankton	65.0	47.0	32.9~ 61.1	0.64	32
nanoplankton	−15.6	335.4	184.0~486.8	0.41	32

cells and this lead to higher R values.

Nutrient stress and photoinhibition, which may cause the variation in R (Kiefer, 1973b; Heaney, 1978), are unlikely to affect the R values reported in this study. Nutrients in the study area are generally exceed rate-limiting levels (Cho, 1985) and all samples were dark adapted before fluorescence measurements. Most of light-induced variations in fluorescence yield can be eliminated with this pretreatment (Loftus and Seliger, 1975; Alpine and Cloern, 1985).

References

Alpine, A.E. and J.E. Cloern. 1985. Differences in *in vivo* fluorescence yield between three phytoplankton size classes. J. Plankton Res. 7, 381~390.

Alpine, A.E., J.E. Cloern and B.E. Cole. 1979. Variations in the reltionship between *in vivo* fluorescence and chlorophyll *a* in the San Francisco Bay estuary. Trans. Am. Geophys. Union, 60, 851.

Cho, C.H. 1985. Chlorophyll *a* concentrations of microplankton and nanoplankton in the coastal seas of Korea in fall. Bull. Korean Fish. Soc. 18, 63~66.

Heaney, S.I. 1978. Some observations on the use of the *in vivo* fluorescence technique to determine chlorophyll *a* in natural populations of freshwater phytoplankton. Freshwater Biol. 8, 115~126.

Holm-Hansen, A.E., C.J. Lorenzen, R.W. Holms and J.D.H. Strickland. 1965. Fluorometric determination of chlorophyll. J. Cons. Perm. Int. Explor. Mer. 30, 3~15.

Kiefer, D.A. 1973a. Fluorescence properties of natural phytoplankton populations. Mar. Biol. 22, 263~269.

Kiefer, D.A. 1973b. Chloropyll *a* fluorescence in marine centric diatoms: Response of chloroplast to light and nutrient stress. Mar. Biol. 23, 39~46.

Kirk, J.T.O. 1975. A theoretical analysis of the contribution of algal cells to the attenuation of light within natural waters. New Phycol. 75, 11~20.

Loftus, M.E. and H.H. Seliger. 1975. Some limitation of the *in vivo* fluorescence technique. Chesapeake Sci. 16, 79~92.

Loftus, M.E., D.V. Subba-Rao and H.H. Seliger. 1972. Growth and dissipation of phytoplankton in Chesapeake Bay. I. Response to a large pulse of rainfall. Chesapeake Sci. 13, 282~299.

Lorezen, L.J. 1966. A method for the continuous measurements of *in vivo* chlorophyll concentration. Deep-Sea Res. 13, 223~227.

Richard, F.A. and T.G. Thompson. 1952. The estimation and characterization of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigments. J. Mar. Res. 11, 156~172.

Shim, J.H. and Y.K. Shin. 1989. Biomass of primary producer in the Chonsu Bay: Relationship between phytoplankton carbon, cell number and chlorophyll. J. Oceanol. Soc. Kor. 24, 194~205.

Shim, J.H. and H.G. Yeo. 1988. Spatial and temporal variations of phytoplankton in Chonsu Bay. J. Oceanol. Soc. Kor. 23, 130~145.

Shim, J.H., H.G. Yeo and Y.K. Shin. 1991. Ecological effect of thermal effluent in the Korean coastal waters. I. Significance of autotrophic nano and picoplankton in the adjacent waters of Kori Nuclear Power Plant. J. Oceanol. Soc. Kor. 26, 77~82.

Shin, Y.K., J.H. Shim, J.S. Jo and Y.C. Park. 1990. Relative significance of nanoplnkton in Chonsu Bay: Species composition, abundance, chlorophyll and primary productivity. J. Oceanol. Soc. Kor. 25, 217~228.

Strickland, J.1968. Continuous measurement of *in vivo* chlorophyll α: Precautionary note. Deep-Sea Res. 15, 225~227.

Taguchi, S. 1976. Relationship between photosynthesis and cell size of marine diatom. J. Physiol. 12, 185~189.

Vincent, W. 1983. Fluorescence properties of the freshwater phytoplankton: Three algal classes compared. Br. Phycol. J. 18, 5~21.

Yentsch, C.S. and D.W. Menzel. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res. 10, 221~231.

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Netplankton과 Nanoplankton 크기별 *in vivo* Fluorescence의 차이

문창호 · 이승용
부산수산대학교 해양학과

식물플랑크톤의 엽록소 *a* 당 *in vivo* fluorescence 세기 (R)가 netplankton 과 nanoplankton 사이에 차이가 있는지 남해에서 조사하였다. *In vivo* fluorescence (IVF)와 엽록소 *a*는 서로 다른 두 크기의 플랑크톤에서 각각 좋은 상관관계를 보였으나 비율 R은 netplankton 과 nanoplankton 사이에 유의적으로 차이가 있었으며 nanoplankton의 R 값이 netplankton 보다 약 7배 높았다. 그러므로 IVF로 식물플랑크톤의 크기별 생물량을 측정할 때 R에 대한 식물플랑크톤 크기의 영향을 고려하여야한다.