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Differences in *in vivo* fluorescence yield between three phytoplankton size classes

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Abstract. The size-dependent relationship between *in vivo* fluorescence (IVF) and chlorophyll *a* was determined for monthly phytoplankton samples from the San Francisco Bay estuary. Chlorophyll *a* and IVF were both measured on netplankton (>22 μm), nanoplankton (5–22 μm), and ultraplankton (<5 μm) samples that were separated with screens. IVF and chlorophyll *a* were linearly related for each size class, but the IVF per unit chlorophyll *a* (R) was significantly different between these three size classes. The ultraplankton R was twice that of the nanoplankton which was in turn twice the netplankton R. Hence, accurate size fractionation of phytoplankton biomass from measures of IVF requires correction for size-dependent variations in R.

Introduction

In vivo fluorescence (IVF) is commonly used to estimate chlorophyll *a* (i.e., phytoplankton biomass) in natural waters. Many investigators have found that the IVF intensity per unit chlorophyll *a* (R) varies with species composition and physiological state of the phytoplankton community. The purpose of this study was to determine if R is constant between three size classes of phytoplankton from San Francisco Bay. If so, then phytoplankton biomass can be size-fractionated quickly and easily in the field by measuring the *in vivo* fluorescence of screened filtrates. The size classes investigated were those commonly used by aquatic researchers: netplankton (>22 μm); nanoplankton (5–22 μm); and ultraplankton (<5 μm).

Work in numerous environments has shown large (up to 10-fold) variations in R of natural phytoplankton populations. In waters off Peru and California, Strickland (1968) found that the factor relating IVF to chlorophyll *a* could vary 2-fold. Studies in San Francisco Bay (Alpine *et al.*, 1979) and Chesapeake Bay (Loftus and Seliger, 1975) have demonstrated 10-fold variations in R over relatively short time and distance scales. Variations in R are attributable to the intensity of irradiance to which the cells have been exposed (light history) (Kiefer, 1973a; Heaney, 1978; Harris, 1978; Vincent, 1979), differences in photosynthetic capacity (P_{max} per unit chlorophyll *a*) (Vincent, 1983), physiological condition (e.g., degree of nutrient stress) (Blasco, 1973; Kiefer, 1973b), and to interspecific differences (Strickland, 1968; Heaney, 1978; Vincent, 1983).

Photoinhibition of fluorescence occurs at high light intensities (0.15 ly/min) (Kiefer, 1973a), but this is a transient property that can be greatly reduced by holding phytoplankton samples in darkness for an hour (Heaney, 1978). Differences in photosynthetic capacity cause variations in IVF. In culture studies, Samuelsson and Oquist (1977) found an approximately inverse relationship between fluorescence and photosynthetic rate. Thus if differences in photosynthetic capacity between groups were significant, this could cause variation in R. Nutrient stress causes R to increase in phytoplankton (Blasco, 1973; Kiefer, 1973b). These variations in R are probably caused by structural and chemical changes of chloroplast lamellae rather than by changes in accessory

pigments (which have different fluorescence responses and could conceivably increase R) (Kiefer, 1973b). Morphological variations also influence R. For example, Kiefer (1973b) observed movement of chloroplasts to the valvar ends of diatoms and shrinkage of chloroplasts associated with high light intensities and an accompanying drop in R.

Differences in R between species and between classes of phytoplankton have been found (Strickland, 1968; Vincent, 1983; Heaney, 1978), but there is no universal hierarchy of R values among phytoplankton classes. For example, high values of R have been reported for diatoms (Heaney, 1978), coccolithophorids (Kiefer, 1973c) and chlorophytes (Vincent, 1983) relative to other phytoplankton taxa. Size-specific variations in R were reported by Loftus *et al.* (1972), who found that large cells are characterized by low R values.

These studies have shown that R can vary over a large range between species of phytoplankton. Since each size class in San Francisco Bay could contain different phytoplankton species, we measured R for each size class. This study was designed to test the null hypothesis that there is no difference in R among size classes of phytoplankton in natural populations. Samples taken from an estuarine environment during 1980 were separated into size classes, and then IVF and chlorophyll *a* were measured in each class so that R values could be compared.

The study area

San Francisco Bay is a large estuary with diverse phytoplankton communities. Two embayments were sampled, South Bay and San Pablo Bay (Figure 1). Two sites were chosen in each embayment to represent populations from the deep channel (>10 m) and the lateral shoals (<2 m). These areas have different chemical and physical characteristics which influence phytoplankton populations (i.e., turbidity, circulation, nutrient concentrations). For example, the annual mean attenuation coefficient for the four sampling sites varies by a factor of four (Cole and Cloern, 1984) and the ratio of mixed depth (H) to photic depth (Z_p) is also different between sites. At the channel stations (27 and 13) the annual mean ratio $H:Z_p$ is 3.8 whereas for the shoal stations (162 and 318) it is 1.9 (Cole and Cloern, 1984). These differences in light availability among channel and shoal populations could have an effect on the physiological condition and, hence, the fluorescence yield of the phytoplankton. Thus an aspect of the experimental design used here was to compare R in shallow versus deep subenvironments, where light prehistories may differ, and among northern and southern San Francisco Bay where phytoplankton community compositions differ (Cloern *et al.*, 1985).

South Bay is a large shallow embayment with a phytoplankton community that is numerically dominated by nanoplankton and ultraplankton, mostly cryptomonads, throughout the year (Wong and Cloern, 1982). Biomass remained low for most of this sampling period (1980); chlorophyll *a* concentrations were <5 μg/l, except for a spring diatom bloom in which chlorophyll *a* concentrations increased to a maximum of 60 μg/l. San Pablo Bay is part of the northern reach of the San Francisco Bay which is a partially mixed estuary (Conomos, 1979). Large seasonal variations occur in species composition, but commonly there is a small population of microflagellates most of the year and a netplankton bloom of diatoms in spring (Cloern *et al.*, 1985). In the spring of 1980 a large unicellular diatom (*Coscinodiscus* sp., >100 μm) dominated the

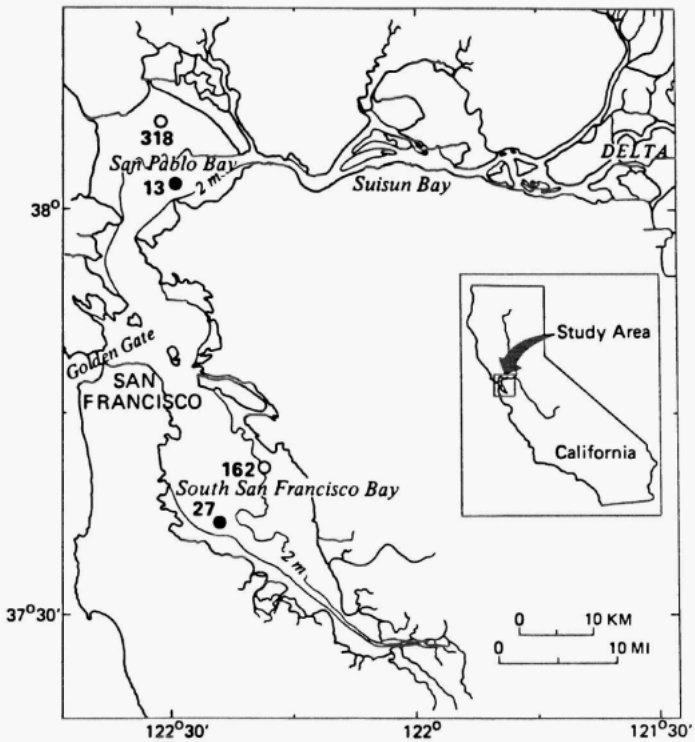

Fig. 1. Map of San Francisco Bay showing major embayments and sampling stations. (●) channel station locations, depth >10 m; (○) shoal sampling stations, depth <2 m.

phytoplankton biomass (Wong and Cloern, 1982).

From species enumeration and identification information (Wong and Cloern, 1982) the major species in each size class are shown in Table I. The netplankton is composed primarily of diatoms, the ultraplankton primarily of cryptomonads, and the nanoplankton is a combination of the two.

Methods

During 1980, monthly samples were collected for simultaneous measurement of IVF and chlorophyll *a* at the four sites (Figure 1). Water was collected from 2 m in the channel and 1 m in the shoals in opaque containers and held for at least 2 h in darkness before measuring IVF (Heaney, 1978). Aliquots of ~125 ml from each sample were gravity filtered through three different screens: a 22-μm Nitex screen; a 5-μm Nuclepore filter; and a 0.45-μm Gelman A/E glass fiber filter. An unscreened aliquot (total) was also analyzed. Chlorophyll *a* and IVF measurements were made in triplicate for the total and screened aliquots. The average coefficient of variation (CV) for the unscreened samples was 5% for chlorophyll *a* and 2% for IVF measurements.

IVF was measured with a Turner Designs Model 10 fluorometer equipped with a small volume (10 ml) cuvette holder, red-sensitive photomultiplier (R446) and a blue lamp (10-045). The filters used were Corning 5-60 for excitation, Corning 3-66 for reference

Table I. Major species by size class found in South and San Pablo Bay embayments of San Francisco Bay. These species comprised >50% of phytoplankton biomass consistently in 1980 (Cloern *et al.*, 1985).

Netplankton		Nanoplankton		Ultraplankton	
Diatoms					
Coscinodiscus sp.	31 × 68[a]	*Cyclotella caspia*	6 × 8	*(Cyclotella caspia)*	6 × 8
Thalassiosira rotula	2 × 45	*Cyclotella* sp.	7 × 15	*(Cyclotella* sp.)	7 × 15
Coscinodiscus sp.	107 × 162	*(Pleurosigma fasciola)*	117 × 16		
Amphora sp.	74 × 37				
Coscinodiscus radiatus	34 × 101				
Pleurosigma fasciola	117 × 16				
Thalassiosira sp.	28 × 83				
Non-diatoms					
(Mesodinium rubrum)[b]	25 × 17	*Chroomonas amphioxeia*	11 × 6	*Chroomonas minuta*	6 × 3
		Mesodinium rubrum	25 × 17		
		(Chroomonas minuta)	6 × 3		

[a]Dimensions are given in μm (length × width or depth) for each species (Wong and Cloern, 1982).
[b]Parentheses indicate that the species may sometimes occur in this size class because its dimensions are close to the borderline between size classes.

and Corning 2-64 for emission. After IVF measurements were made, the screened and unscreened aliquots were filtered for chlorophyll *a* analysis. The analytical procedure basically followed the fluorometric method of Strickland and Parsons (1972) (for details see Alpine, 1983). The samples were collected onto glass fiber filters (Gelman AE) and ground with a Teflon tissue homogenizer in 90% acetone buffered with $MgCO_3$. Fluorescence was measured before and after acidification (to correct for phaeopigments) with HCl to a concentration of 3×10^{-3} M (Riemann, 1978) on the same fluorometer used for IVF measurements. The fluorometer was calibrated with pure chlorophyll *a* (Sigma Chemical).

The method for calculating the amount of *in vivo* fluorescence and chlorophyll *a* in each size class began with the averaging of replicate values from each screened fraction or unscreened sample. The 0.45-μm filtrate, which we interpret as the 'soluble' fraction, ranged from 7% to 76% of total IVF with an average of 33%. This 'soluble' IVF is not primarily chlorophyll *a* because the percentage of 'soluble' chlorophyll *a* was much lower, ranging from 0% to 6% with an average of 2%. Values for netplankton, nanoplankton, and ultraplankton biomass were calculated by differences between the 'soluble-corrected' values. Thus:

$$\text{Netplankton} = T - 22 \quad (1)$$

$$\text{Nanoplankton} = 22 - 5 \quad (2)$$

$$\text{Ultraplankton} = 5 \quad (3)$$

where T = unfractionated (total) sample − S; S = 0.45 μm filtrate (soluble); 22 = the 22 μm screened filtrate − S; 5 = the 5 μm screened filtrate − S.

Results and Discussion

To determine whether there was a significant difference in the *in vivo* fluorescence per unit chlorophyll *a* between phytoplankton size classes, R was determined separately for each size class by regressing [Model II linear regression, Laws and Archie (1981)]

Table II. Regression and correlation coefficients for the linear regression (Model II) of *in vivo* fluorescence against chlorophyll *a* for netplankton, nanoplankton, and ultraplankton size classes.

Size class	Intercept (IVF units)	Slope (R) (IVF units/ chl *a* μg/l)	95% C.L. for R	r	n
Netplankton	−0.1	5.6	5.2 − 6.2	0.96	40
Nanoplankton	5.2	11.6	10.0 − 13.3	0.87	51
Ultraplankton	4.4	24.6	20.1 − 30.2	0.73	48

IVF against chlorophyll *a* (R = slope). Among all size classes, IVF and chlorophyll *a* were linearly related ($p < 0.001$) and a large percentage (73 − 96%) of the variation in IVF for each size class was correlated with variations in chlorophyll *a* (Table II, Figure 2). In this analysis data from all sites were pooled. Individual analyses for each site demonstrated that this was a justified grouping; no significant differences were found in slopes (R) between channel and shoal populations or between embayments (Alpine, 1983). However, there were significant differences ($p < 0.001$) in slopes among phytoplankton size classes (Table II, Figure 2). The R value (i.e., the slope) for the ultraplankton was the highest followed by the nanoplankton. The lowest R value was found for the netplankton size class. The R value of ultraplankton was four times that of the netplankton and twice that of the nanoplankton.

Also of significance is the strong correlation between IVF and chlorophyll *a* within each size class through a wide range of environmental conditions. Samples were collected from four different sites, over a year long period, so these samples were collected over a range of salinity, temperature, turbidity and nutrient concentrations. These environmental parameters can influence R but results from this study suggest that R is primarily a function of cell size and/or species composition of the phytoplankton community.

Note that the screen size chosen for a particular area must truly separate the size classes to discern differences in R. In an embayment where two diatoms, *Skeletonema costatum* and *Thalassiosira eccentrica*, dominated the netplankton a difference in R between size classes could not be discerned because these forms were not quantitatively separated by the 22-μm screen (Alpine, 1983).

The ratio, R, between IVF intensity and chlorophyll *a* can be thought of as the product of two terms (Kiefer, 1973b):

$$R = ab \tag{4}$$

where

$$a = \frac{\text{quanta absorption rate}}{\text{chlorophyll } a} \qquad b = \frac{\text{quanta emission rate}}{\text{quanta absorption rate}}$$

The first term (a) is influenced by factors that affect quanta absorption efficiency. These include accessory pigments which increase the quanta absorbed per unit chlorophyll *a* and thus increase R. Cell size is also a factor in the absorption of light. Small cells are more efficient at capturing photons than large cells (Kirk, 1975), and this would lead to higher R values. The intracellular position and orientation of chloroplasts also affect the rate of quanta absorption and emission (Kiefer, 1973b). Kiefer did not look at large versus small cells but it is possible that the structure of the large cells found

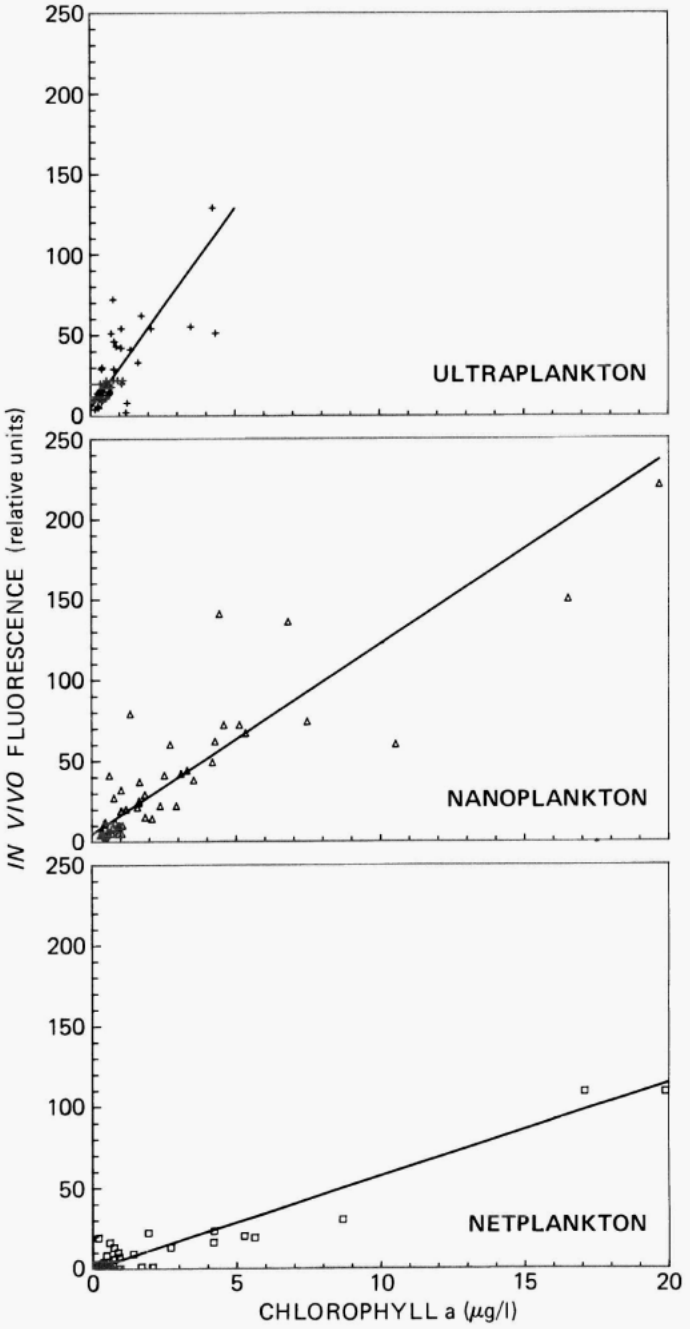

Fig. 2. *In vivo* fluorescence plotted against chlorophyll *a* for each size class. Data are pooled from four sites in San Francisco Bay over a year long period. Regression lines are shown for each size class.

here (i.e., diatoms) causes a lower R. Malone *et al.* (1979) found that nanoplankton (<22 μm) have twice the chlorophyll *a* per unit particle volume of netplankton. This makes for a more dense 'packaging' of the chlorophyll which could also increase the quanta absorption rate.

Another influence on (a) relates to the pigmentation of each species in the population. Differences in pigment composition between taxa produce different emission and excitation spectra which can lead to differences in R because the fluorometer has fixed wavelengths for excitation and emission (Loftus and Seliger, 1975). Thus differences we have found in R between size classes could be related to differences in species composition within each size class. In this study the smallest size class was commonly dominated by cryptomonads and had a higher R than the netplankton which was dominated by diatoms.

The second term (b) of equation (4) is the fluorescence yield (Kiefer, 1973b) which is affected by a wide range of physiological variables. Nutrient stress and photoinhibition are two conditions which cause large variations in (b), but these are unlikely to affect the R values reported here. All samples were dark adapted before fluorescence measurements were made. Light-induced variations in fluorescence yield can nearly be eliminated with this 'pretreatment' (Loftus and Seliger, 1975; Heaney, 1978). Plant nutrients generally exceed rate-limiting levels in San Francisco Bay (Peterson, 1979). Thus increases in fluorescence associated with nutrient-stressed populations are probably negligible here.

Differences in photosynthetic rates between size classes could influence fluorescence yield and thus 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 have found that R is higher in smaller cells. Productivity studies during 1980 in San Francisco Bay demonstrated no difference in photosynthetic rates per unit chlorophyll *a* between size classes (B.E. Cole, personal communication). Thus our data suggest that the greatest influences on R in this environment are associated with factors affecting the term (a) in equation (4); namely cell size and species composition (i.e., accessory pigments).

Our results are consistent with those of Loftus *et al.* (1972), who also found small sized phytoplankton have higher R values than large species in a laboratory study of unialgal cultures. They used seven different organisms: two large dinoflagellates (60 μm and 70 μm); three diatoms (6 μm, 10 μm and 15 μm); a green flagellate (*Dunaliella* sp., 6 μm); and an unidentified nanoplankter species (2 μm). The 2 μm flagellate had an R value five to ten times that of the dinoflagellates and two to three times that of the intermediate sized diatoms and *Dunaliella* sp.

Thus the size dependency of R must be taken into account when using IVF to size fractionate biomass. Table III demonstrates the improvement in estimating chlorophyll *a* in each size class by taking into account the size-dependent variation in R. Chlorophyll *a* was predicted from IVF values for each size class in two ways. The first method is to use a simple percent IVF to calculate the percent chlorophyll *a* in each size class. The other method is to predict chlorophyll *a* from the regression equations in Table II which take into account size-dependent variations in R. To determine if our estimate of chlorophyll *a* is improved by using separate regression equations we calculated the mean deviation (MD)

Table III. The mean deviation (MD) of predicted chlorophyll *a* from actual chlorophyll *a* for each size class. The percent mean deviation from the mean chlorophyll *a* concentration is also shown.

	Netplankton		Nanoplankton		Ultraplankton	
	MD (μg/l)	MD/$\bar{x}$ (%)	MD (μg/l)	MD/$\bar{x}$ (%)	MD (μg/l)	MD/$\bar{x}$ (%)
Chlorophyll *a* predicted from % IVF	0.35	16	0.26	9.5	0.19	21
Chlorophyll *a* predicted from R for each size class	0.19	8.7	0.26	9.5	0.10	11

Table IV. Comparison of linear regression slopes (R) of total IVF against total chlorophyll *a* for two areas of San Francisco Bay.

Date	R1-South Bay	R2-North Bay	R1/R2
4–5 August 1980	3.3	1.6	2.1
18–19 August 1980	3.3	1.4	2.3
3–4 September 1980	1.9	1.2	1.6

$$MD = \sum_{i=1}^{n} \sqrt{(\text{actual value} - \text{predicted value})^2}/n \qquad (5)$$

for both methods of estimating chlorophyll for all three size classes. As can be seen from this the estimate of chlorophyll *a* from IVF can be improved by a factor of two by taking into account the size-dependency of R.

Another significant implication relates to using IVF profiles to estimate chlorophyll *a* in natural environments. If there are large horizontal or vertical variations in phytoplankton cell size, then profiles of IVF may not be correlated with profiles of chlorophyll *a*. San Francisco Bay, like other estuaries, is an environment where there are potentially large horizontal variations in phytoplankton cell size. As an example we can compare the relationship between IVF and total chlorophyll *a* between the northern and southern reaches of San Francisco Bay. During late summer and early fall the southern reach is a nanoplankton-ultraplankton dominated system and the northern reach is a netplankton dominated system (Cloern *et al.*, 1985). During this time of the year the slopes of regressions of total IVF against total chlorophyll *a* are significantly higher for the southern reach (i.e., dominated by the smaller size classes) than that of the northern reach (Alpine *et al.*, 1979). In our example (Table IV) the slopes were approximately twice as great in the southern reach compared to the northern reach. Another example of this phenomenon can be seen in Heaney's (1978) work where an increase in R of a natural population occurred when a small single celled genus (*Chlorella*) bloomed. Hence, as others have found (Loftus *et al.*, 1972), care must be taken when using profiles of IVF to infer spatial variability of phytoplankton biomass, especially in systems having spatial variations in species composition.

In summary, we have found that *in vivo* fluorescence per unit chlorophyll *a* varies among three phytoplankton size classes and that the yield is highest for the smallest size class and lowest for the largest size class. Within a size class, R is fairly constant over a range of environmental conditions in this estuarine environment. Thus the most probable control of *in vivo* fluorescence per unit chlorophyll *a* for each size class is cell size and/or species composition. Furthermore we have demonstrated that this size-dependency in R must be taken into account to obtain accurate estimates of size class chlorophyll *a* from IVF.

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