DETERMINING CHLOROPHYLL ON THE 1984 CALCOFI SURVEYS

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ABSTRACT

Two sources of error may be relevant to the 1984 CalCOFI chlorophyll data set. These are discussed and their magnitude estimated. The first error is due to the use of GF/C glass fiber filters, which do not completely retain the smallest phytoplankters. This error is important mainly in more oligotrophic waters, where its relative bias is of the order of −15%. The second error arises from the incomplete recovery of chlorophyll from the filter when the filter is not ground in acetone but is allowed to extract in acetone for 24 hours. The relative bias introduced is about −8%. Taking into account both of these errors, the most accurate estimate of the true chlorophyll concentration falls in the range 1.1 to 1.3 times the observed concentration.

RESUMEN

Existen dos posibles fuentes de error en los datos de clorofila de CalCOFI de 1984. Sus magnitudes son discutidas y estimadas. El primer error se debe al uso de filtros de fibra de vidrio tipo GF/C los cuales no retienen completamente los fitoplanctontes más pequeños. Este error es importante principalmente en aguas oligotróficas, donde el sesgo relativo es del orden de un −15%. El segundo error proviene de la recuperación incompleta de clorofila desde el filtro cuando este filtro no es macerado sino extraído en acetona por 24 horas. El sesgo relativo que se introduce es aproximadamente −8%. Considerando ambos errores, una estimación más exacta de la concentración real de clorofila estará comprendida en un rango de 1.1-1.3 veces la concentración observada.

INTRODUCTION

The 1984 CalCOFI program includes measurements of chlorophyll *a* and phaeopigments in the upper 200 meters. These are made at every hydrographic station and at the noontime productivity stations. Each two-ship survey includes approximately 2,000 individual chlorophyll determinations.

Approximately 140 ml of seawater is removed from each Nansen or Niskin bottle, and is filtered through a Whatman GF/C filter to remove the particulate material. Each filter is then placed in a scintillation vial with 90% acetone and stored in the dark, under refrigeration, for 24 hours. The acetone is brought to room temperature, decanted into a cuvette, and the fluorescence is determined with a Turner 111 fluorometer. All fluorometers used on the survey are calibrated against a spectrophotometer using the trichromatic equations of Parsons and Strickland (Strickland and Parsons 1968). Cross-calibration of the instruments is checked at intervals during the program.

The selection of this analytical procedure involved several compromises, which are expected to reduce the accuracy of the measurements to some extent. Evaluation of the 1984 chlorophyll data set necessitates understanding the probable errors introduced by these compromises.

RESULTS

Filter Pore Size

There exists an extensive body of chlorophyll *a* data from the California Current based upon the material retained by the GF/C filter. This filter has a specified retention of 1.2 μ, but experimental determinations give varied results. Work by Parker (1981) suggests the average retention may be closer to 3 μ, while Eppley finds that GF/C filters will retain material that has passed through 1-μ Nuclepore filters (pers. comm.). Recent work in a variety of oceanic environments has indicated the existence of an important photosynthetic component 0.5-3 μ in diameter (the picoplankton), which is not quantitatively retained by the traditional GF/C filter. Our decision to continue using the GF/C filters, in spite of a potential negative bias, was based on the importance of maintaining long-term continuity of the data set.

On CalCOFI cruise 8105-J, comparisons were made between the chlorophyll retained on GF/C filters and on GF/F filters, which have a specified minimum retention size of 0.7 μ. Replicate subsamples from each of 71 samples were filtered, one through a GF/C filter, the other through a GF/F filter. Samples were allowed to extract for 24 hours in the dark, under refrigeration, before we determined fluorescence. Samples were collected throughout the euphotic zone and from a variety of water types. The GF/C filters did tend to underestimate the chlorophyll concentration, especially at lower chlorophyll concentrations (Figure 1). Of the 49 samples with chlorophyll concentrations less than 0.5 μ g/1, the GF/F filters retained more chlo-

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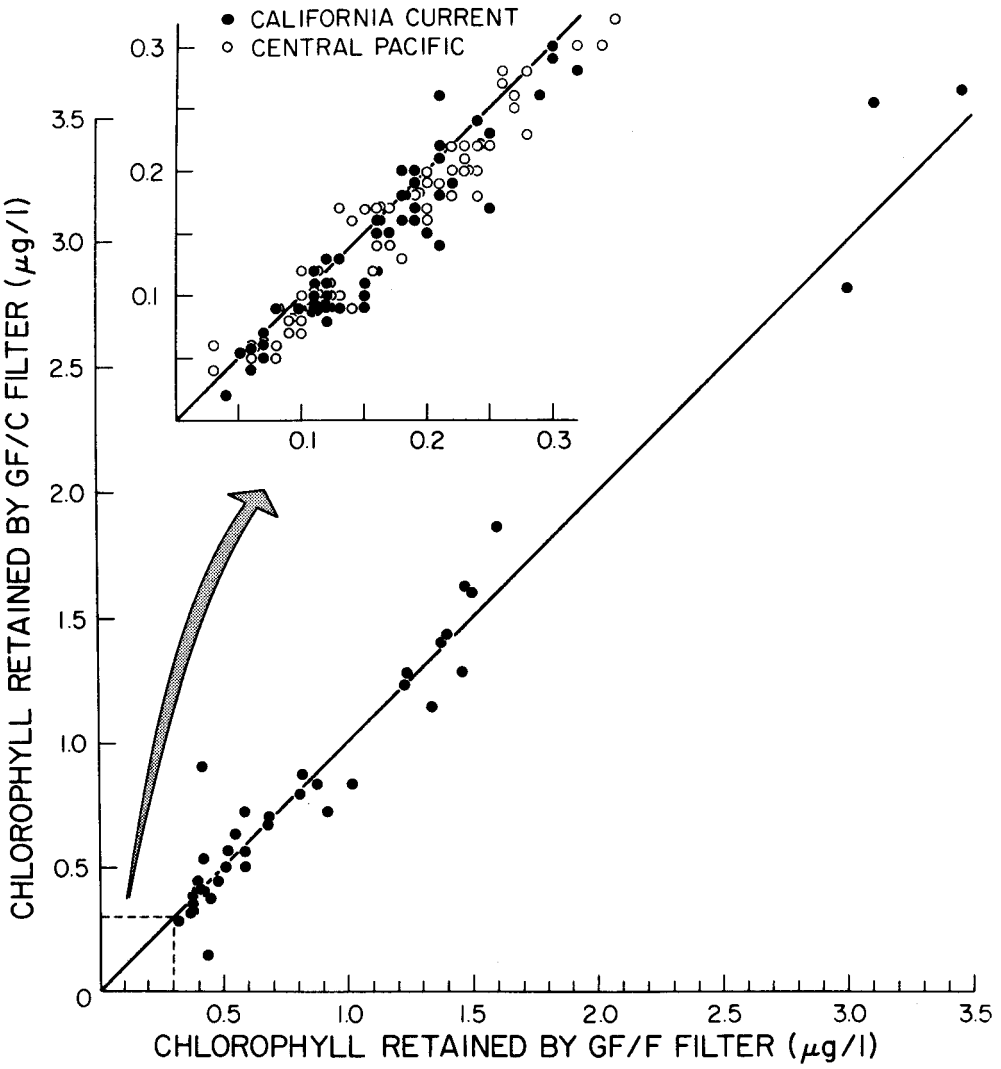

Figure 1. Comparison of the amount of chlorophyll retained by a GF/C glass fiber filter and that retained by a GF/F glass fiber filter for samples from the California Current and the Central Pacific environment. Solid line represents equal retention.

rophyll 41 times (Figure 2; $p < .01$). This pattern was similar to that observed in the Central Pacific (Figure 1, insert). However, at higher chlorophyll concentrations, this bias was not apparent. Over the entire data set the mean relative bias [(GF/C - GF/F)/GF/F] was −13%. When the 22 samples with chlorophyll concentrations in excess of 0.5 μ g/l were excluded, this bias increased to −15%. This relationship between bias and chlorophyll concentration is compatible with previous work which indicates a higher proportion of smaller phytoplankton in oligotrophic environments (e.g., Malone 1971 a,b, 1980; Li et al. 1983).

The recent quantitative work on picoplankton has been based on filters with pore sizes on the order of 0.2 to 0.4 μ (Waterbury et al. 1979; Platt et al. 1983), considerably smaller than the minimum cell diameter of 0.5 (Johnson and Seiburth 1979) to 0.9 μ (Waterbury et al. 1979) reported for picoplankton. The GF/F filters used in the present study have a reported minimum retention of 0.7 μ, and the smallest phytoplankton components may be incompletely removed. However, Li et al. (1983) found that GF/F filters retained 94% of the radioactivity retained by 0.2 μ Nuclepore filters.

Extraction Procedure

In the early 1960s, glass fiber filters, ground in acetone to extract chlorophyll, largely replaced Millepore filters, which were extracted in acetone for some period of time without grinding. Use of glass fiber filters and the grinding procedure offers the advantage of rapid sample analysis (one or two hours). In addition, extraction of chlorophyll may be more complete with ground glass fiber filters, especially when benthic or estuarine species are prevalent (Strickland and Parsons 1968). On the other hand, the grinding procedure is more time-consuming and requires more equipment on board ship. Preliminary work in the

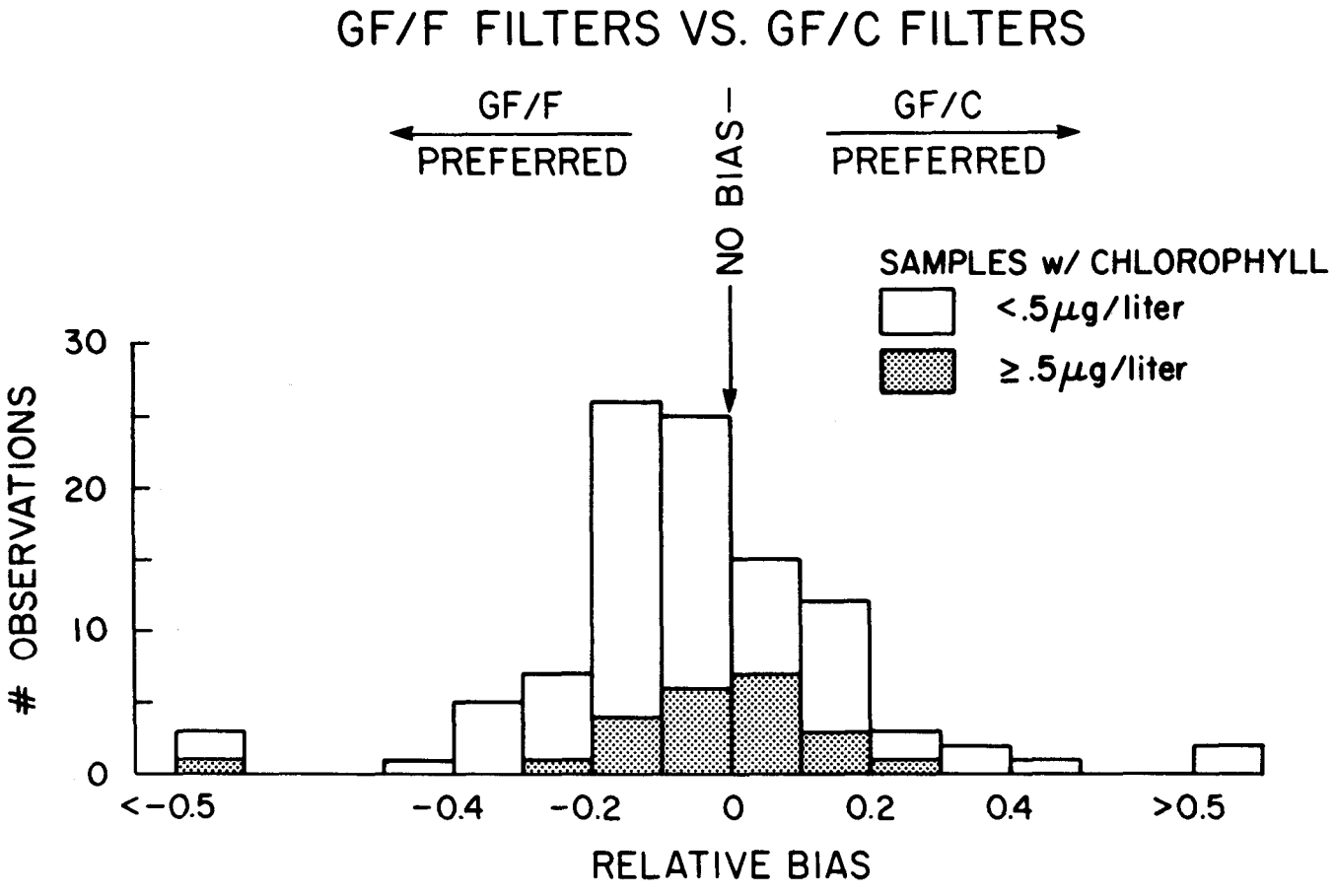

Figure 2. Data from Figure 1 (California Current only) expressed as a frequency distribution of relative biases. Relative Bias = (GF/F-GF/C)/(GF/F); GF/F = chlorophyll retained by a GF/F filter; GF/C = chlorophyll retained by a GF/C filter.

California Current suggested that in some situations grinding may recover even less chlorophyll (8%) than 24-hour extraction, apparently because some of the chlorophyll is degraded to phaeopigments during the grinding process (Table 1). Chlorophyll on past CalCOFI surveys has been determined by the grinding procedure. Our decision to return to the 24-hour extraction procedure was based on the savings in time and effort, which appeared to justify the risk of a slight bias.

Two procedures were investigated from February to November 1983, using water collected from the end of the pier at Scripps Institution of Oceanography (SIO). Two replicate samples were collected with a Nansen bottle from just below the sea surface. From each bottle, four subsamples were drawn. Two were filtered through GF/C filters, ground in acetone, and the fluorescence determined within one hour; the other two subsamples were filtered through GF/C filters and allowed to extract, without grinding, for 20-24 hours. To determine bias, we compared the two means of the duplicate samples within the same Nansen bottle. There were 72 such comparisons. Most of these, however, are paired (duplicate Nansen bottle samples on the same day) and, as discussed below, the results are not independent. Thus, the effective sample size is 38. In 80% of the observations, the recovery of chlorophyll was greater when the filter was ground ($p < .05$; Figure 3). The mean relative bias [(unground − ground/ground] was −7.6% (Figure 4). Examination of the fluorescence ratios, (before acidification:after acidification) did not indicate any differential degradation of chlorophyll, as was observed in the preliminary experiment. However, the chlorophyll concentrations during the pier experiment rarely dropped below 0.3 μ g/l, so that experiment did not include material from a truly oligotrophic environment where benthic and estuarine forms are rare.

There was no evidence for a difference in precision between the two techniques. Indeed, there was a tendency (sign test, $p \sim .18$) for the 24-hour extraction procedure to yield more precise replicates.

The two experiments run on the same day (one experiment from each of two Nansen bottles) tended to

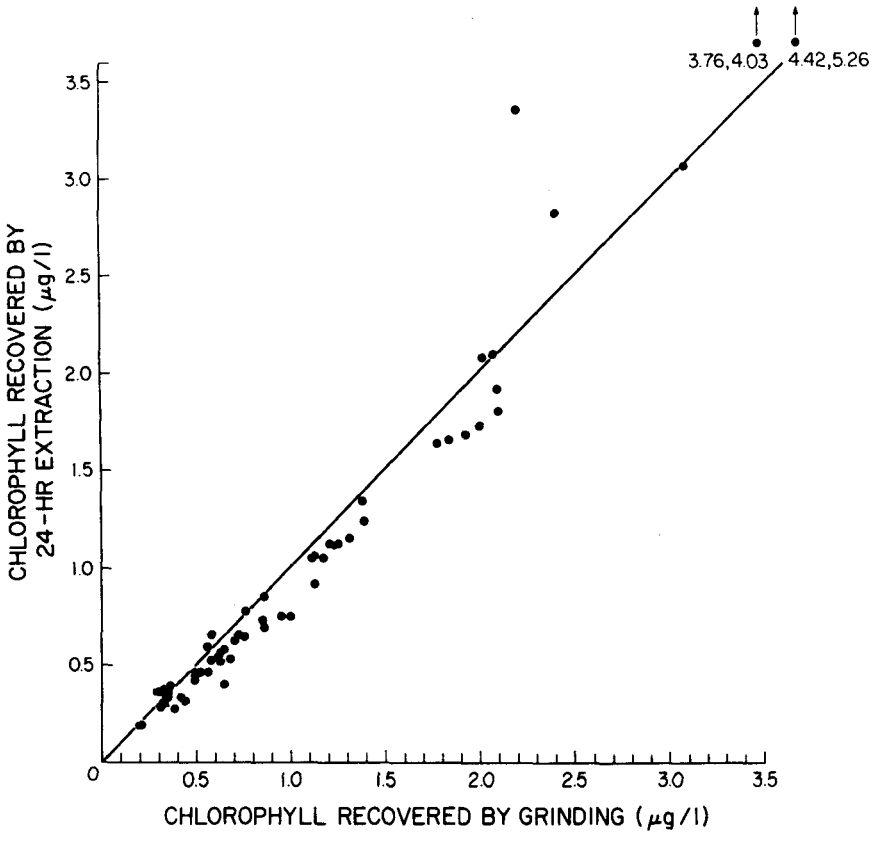

Figure 3. Comparison of the amount of chlorophyll recovered after 24-hour extraction to that recovered after grinding the filter. Solid line represents equal recovery.

TABLE 1

Preliminary Experiment on the Effects of Filter Size and Extraction Procedure on the Determination of Chlorophyll (Chl.) and Phaeopigments (Ph.) in the California Current*

	GF/C filters				GF-F filters			
	Extracted		Ground		Extracted		Ground	
Depth (m)	Chl.	Ph.	Chl.	Ph.	Chl.	Ph.	Chl.	Ph.
0	0.22	0.04	0.23	0.04	0.24	0.04	0.24	0.05
10	0.27	0.02	0.24	0.06	0.26	0.04	0.24	0.06
20	0.30	0.05	0.25	0.08	0.32	0.08	0.30	0.09
30	0.28	0.07	0.25	0.10	0.28	0.10	—	—
40	0.56	0.18	—	—	0.59	0.17	0.54	0.19
50	0.51	0.22	0.46	0.24	0.47	0.25	—	—
60	0.28	0.20	0.27	0.23	0.26	0.25	—	—
70	0.22	0.20	0.20	0.20	0.23	0.23	—	—
80	0.16	0.15	—	—	0.14	0.19	—	—
90	0.12	0.15	—	—	0.11	0.20	—	—

Summary of Chlorophyll Recovery

Extraction procedure: filters ground (G) vs extracted only (Ex)

	G > Ex	G = Ex	G < Ex
GF/C filters	1	0	6
GF/F filters	0	1	3

Filter type: GF/C filters vs GF/F filters

	GF/F > GF/C	GF/F = GF/C	GF/F < GF/C
G	4	1	6
Ex	2	1	0

*CalCOFI station 90.28, August 1982.

Filters (GF/C or GF/F) were extracted in acetone for 24 hrs, and the fluorescence of half of the extract was determined (Extracted). The filter was then ground in the remaining extract and a second determination was made (Ground). Results are µg/l.

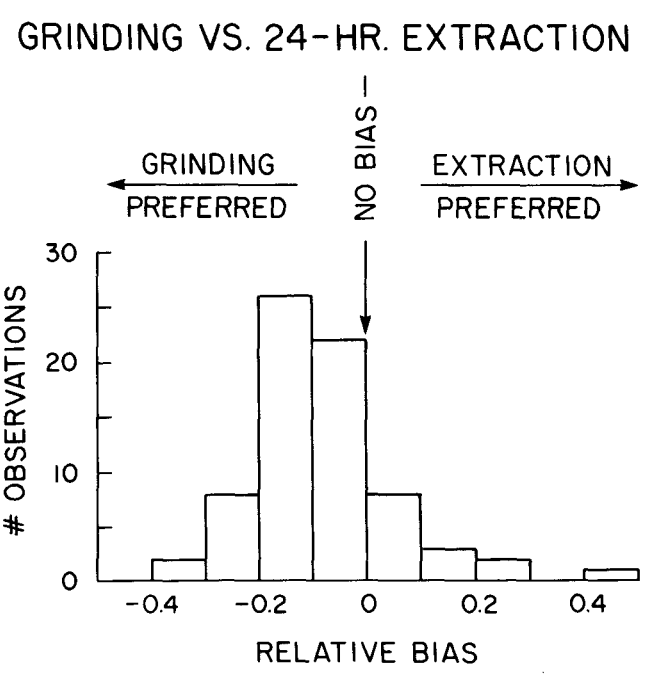

Figure 4. Data from Figure 3 expressed as a frequency distribution of relative biases. Relative Bias = (G - Ex)/G; G = chlorophyll recovered when filter is ground; Ex = chlorophyll recovered when filter is extracted for 24 hours.

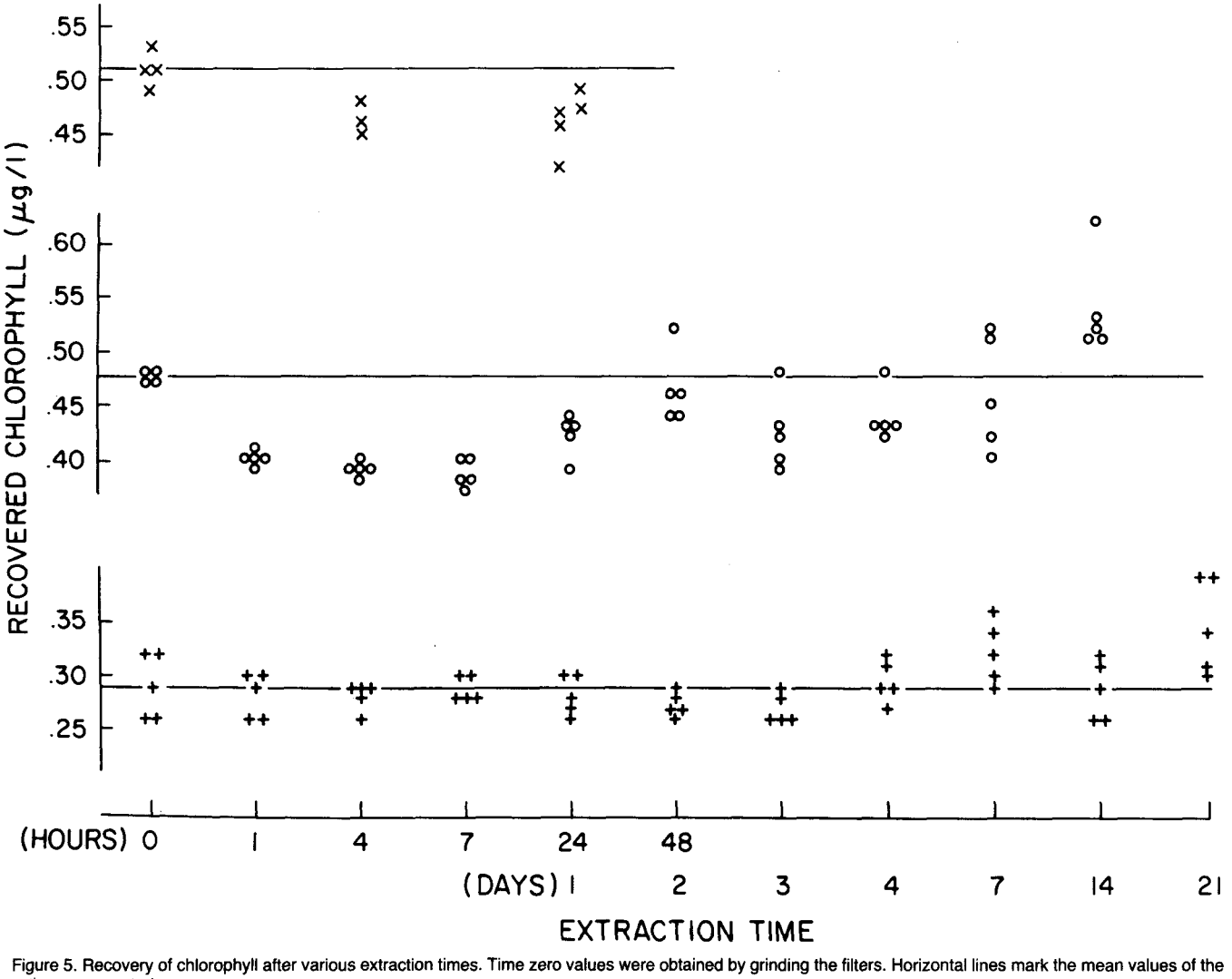

Figure 5. Recovery of chlorophyll after various extraction times. Time zero values were obtained by grinding the filters. Horizontal lines mark the mean values of the time zero controls.

give the same experimental result. On 31 of the 34 days, both experiments yielded either more chlorophyll from the ground filters or more chlorophyll from the 24-hour-extracted filters; on only 3 days were the results from the two experiments dissimilar. There were significantly more similar pairs than one would expect if the experimental results of the two replicate samples were independent (Chi-square = 16.8; $p <$ .01). This result does not appear to be an operator bias, but rather an interaction between the techniques and the composition of the flora being sampled.

Extraction Time

To examine the minimum and maximum acceptable extraction times, three experiments were run, using water from the end of the SIO pier. For each experiment a series of replicate subsamples was drawn from a 5-liter Niskin bottle. These were filtered onto GF/C filters and randomly assigned to the various treatments. The controls were ground in acetone, and the fluorescence was determined immediately. Other samples were placed in acetone and stored in the dark, under refrigeration, for various periods of time, before we determined the fluorescence.

The results of the three experiments (Figure 5) were inconsistent. Experiment 2 showed a significant increase in recovered chlorophyll with time (Kendall τ, $p <.05$). Experiment 3 showed no trend whatever, and the recovery of chlorophyll after extraction for one hour was not significantly different from the control. On the other hand, all experiments showed a tendency to lose precision with increasing extraction times (Kendall τ: experiment 2, $p < .01$; experiment 3, $p <$ .20). The choice of 24 hours as the target extraction time seems to offer acceptable accuracy without risking reduction of precision. However, under emer-

gency situations (such as the failure of a fluorometer), samples can be stored for at least 3 weeks without serious loss of chlorophyll. However, this conclusion is not supported by earlier observations of serious degradation of chlorophyll with time (Yentsch and Menzel 1963).

CONCLUSIONS

There are two sources of error in the CalCOFI 1984 chlorophyll data, arising from our choice of analytical procedures. The first is the loss of the smallest phytoplankton through the filter. The second is the failure to extract into acetone all of the chlorophyll retained on the filter. If these sources of bias are independent, their effects are additive. However, it is possible that the magnitudes of both biases are related to the composition of the flora being sampled, and that these two relationships are inverse. The loss of chlorophyll through the GF/C filter is proportionally greater in more oligotrophic systems. Conversely, the loss of chlorophyll in the absence of grinding is expected to be greater in nearshore systems where the tough-walled benthic and estuarine forms are more numerous. The information presently available does not allow us to determine whether these effects are independent or to directly evaluate the magnitude of the cumulative effects. We can only estimate that the average negative bias from the true chlorophyll concentration will be somewhere between 8%-15% (if the two effects operate in an inverse fashion) and 23% (if the two effects are additive). Expressing these biases in terms of the observed chlorophyll concentrations, we estimate the expected true value to be between 1.1 and 1.3 times larger than the observed value.

This discussion has primarily considered biases introduced by the adopted procedures. Such biases, if constant, will not distort the basic patterns of chlorophyll in the ocean, or the basic relationships between chlorophyll and other parameters. To the extent that these biases are a function of the phytoplankton composition, and thus not constant, some distortion may occur. However, the spatial variation of chlorophyll over the CalCOFI survey area is often at least a factor of 20 (Owen 1974; Hayward and Venrick 1982), so these biases are unlikely to obscure large-scale patterns. In any case, care must be taken in comparing chlorophyll values from the 1984 CalCOFI program with chlorophyll values derived with other analytical procedures. Since earlier CalCOFI cruises have used GF/C filters, the only bias in the 1984 data relative to past CalCOFI data will be due to the use of the 24-hour extraction procedure, and will be on the order of 8%. The available evidence does not indicate any loss of precision with the selected procedures, except perhaps in the case of prolonged extraction times. More exact determination of precision should be made during the 1984 CalCOFI program.

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