A photometer for the continuous measurement of Calcite-dependent light scatter in seawater

Katherine A. Kilpatrick, William M. Balch
University of Miami, Rosenstiel School for Marine and Atmospheric Science,
Miami, Florida 33149

Yuntao Ge, Kenneth J. Voss
University of Miami, Physics Department,
Coral Gables Florida 33124

ABSTRACT

Biogenically-produced calcite represents a significant source of light scatter in the ocean, poorly defined in space and time. One reason for this lack of observations is that standard shipboard techniques for measuring light scatter are slow and labor intensive. We describe an automated photometer for measuring 90° light scatter due to calcite on time scales as short as a minute. Raw seawater continuously flows through a 1 mL glass cuvette illuminated by a helium-neon laser. A photodiode is used to measure the 90° light scatter of particles in the incident beam. During each sampling interval, a calibrated addition of weak acid lowers the seawater pH to dissolve the calcite and another 90° light scatter measurement is made. Standard curves are prepared using calcium carbonate coccoliths from the coccolithophore *Emiliania huxleyi* . The relationships between acid-labile light scatter and coccolith abundance give an r^2 of 0.96-0.99. The light scatter photometer is coupled to a fluorometer, temperature and salinity probe to relate the suspended calcite concentration to algal distributions and hydrography. We show some examples of its performance at sea.

1.INTRODUCTION

In recent years, there has been much interest in the phenomenon of mesoscale coccolithophore blooms. These blooms increase the visible reflectance of seawater from 2-5% to values as high as 25%. The spectral effect of these blooms results in a color change of the water from blue to a turquoise. Sightings of this condition have been noted as "whitings" in ship's logs from around the world[1]. Many of these white water blooms have been reported to be predominantly composed of the coccolithophorid *Emiliania huxleyi* [2] but can also be caused by other coccolithophore species[3]. The coccolithophoridae order of phytoplankton are covered with calcium carbonate scales (coccoliths) which can dramatically affect the shape and magnitude of the volume scattering function. In blooms of coccolithophores, as much as 75% of the total backscatter is due to calcite coccoliths[4]. Our estimates suggest that even in non-bloom conditions calcite may represent 10-20% of the total backscatter. Backscatter due to these calcite particles dramatically changes ocean reflectance as seen by remote sensing ocean color satellites.

The impact of calcite on the backscattering coefficient has been assessed by measurement of the volume scattering function before and after dissolution of the calcite particles [4]. This measurement is extremely tedious to perform, which limits sample frequency. We report here the development of an automated flow thru 90° light scatter instrument which can be calibrated to the coccolith concentration.

2. OPTICAL INSTRUMENT DESIGN

The scattering meter uses a 5mW CW He-Ne laser at 632.8nm as the light source (Fig. 1). Light from the laser passes through a glass beam splitter which sends a portion of the light (4%) to a 10

 0-8194-1574-X/94/$6.00

mm^2 silicon photodiode(Melles Griot 13DSI007) which is used to monitor the laser power. The remaining light travels thru a lens which focuses the beam on a 1 cm glass cuvette. The light scattered to 90° is collected by a plano-convex 25.4 mm focal length lens which focuses the light onto another photodiode detector (Melles Griot 13DSI007). This geometry gives a full acceptance angle of 22 degrees. Each of these detectors have independent gain adjustments. Voltage from each diode is passed to a pre-amp and amplifier and the analog signal from each is converted to digital form by a 16 bit A/D board in the computer controller (286 PC). The signal detector voltage is divided by the laser monitor voltage to compensate for any changes in laser power during sampling.

3. DATA ACQUISITION

The instrument was fitted with a microvolume 0.5 ml rectangular flow thru cuvette. A small glass mixing coil was placed on the bottom entry port of the cuvette. Seawater was continuously pulled thru the system by a peristaltic pump at 11.6 ml per minute resulting in a 10 second turnover time of water in the cuvette. It is important that the sample be pulled rather than pushed thru the system because the delicate cells and particles can be damaged as they pass over the pump head, dramatically changing the shape and hence the optical characteristics. Every minute for a 30 second duration a second peristaltic pump was activated injecting 0.3 % glacial acetic acid at a rate of 0.23 mL per minute upstream of the mixing coil, into the stream line, dropping the pH to 5.8. As the acidified sample passed around the mixing coil, calcite in the sample dissolved. Previous lab results have demonstrated that a pH of 5.8 was not low enough to damage the cultured phytoplankton cells on this time scale (unpublished data.). The amount of light scatter before and after the addition of the acid was recorded. If calcite was present in the sample then the 90° light scatter was greater in the non-acidified (calcite present) than the acidified (calcite dissolved). The difference in scatter was proportional to the amount of calcite in the sample, henceforth this will be referred to as acid-labile light scatter. In the absence of calcite, no change in light scatter was detected.

A Turner 1-11 fluorometer configured for in- vivo chlorophyll fluorescence and an Inter-Oceans temperature and salinity probe were also in the flow stream. All data were collected on an IDS Turbo 286 computer with a National Instruments 16 channel A/D board and Labtech Notebook software. During along-track measurements seawater came from the ship's seawater chest (2m depth) , then through stainless steel pipe to the ship's lab. Data was collected at 3 minute intervals. Hourly discrete samples were analyzed for extracted chlorophyll and particulate calcite.

Vertical profiles were obtained with a pumping system, the hose of which was attached to a CTD rosette. Thehose was raised at 2 meters per minute with a maximum possible depth of 120 meters. The seawater was brought into a large tub on deck for debubbling, where a Little Giant pump in the bottom of the tub moved the water into the main lab.

4. ANALYTICAL METHODS

Particulate calcite was determined by flame photometeric atomic absorption spectroscopy (Perkin Elmer model 2380). One liter of seawater was filtered onto a pre-combusted Whatman GFF 25mm filter. The filter was rinsed with 20mM borate buffer, pH 8.0 to remove interstitial seawater. Filters were then frozen for later analysis. Samples were processed by digesting filters in 2 mL of 50% trace-metal clean hydrochloric acid and maintained in a water bath at 40°C overnight. Eight mL of 1% lanthanium chloride were added to the sample, then centrifuged to remove the filter. Dissolved calcium in solution was analyzed by absorption at 422.7nm. using a 10cm flame The instrument was calibrated using a commercial calcium standard from Fisher Scientific. The *in vivo* chlorophyll fluorescence was calibrated by linear regression with discrete

samples. The extracted chlorophyll and phaeopigment concentrations were determined by the method of Yentsch and Menzel [5] as modified by Holm-Hansen[6]. Particles and cells were enumerated in a Palmer Maloney counting chamber using an epi-fluorescence microscope with a polarization attachment.

5. CALIBRATION OF THE GE-METER

The linearity of the detector was checked using a series of neutral density filters. A culture of the coccolithophore *Emiliania huxleyi* at a concentration of 9.62×10^2 cells ml^{-1} and 8.75×10^3 coccoliths ml^{-1} was pumped thru the cuvette at 3.5 ml per minute. Combinations of neutral density filters were placed in front of the signal detector to attenuate the 90° light scatter of the sample and the voltage of each detector was recorded. The ratio of the signal to laser monitor was found to have a linear response over 3 orders of magnitude (Fig.2). The detector was able to discriminate between 0.2 um filtered distilled water (Milli-Q) and filtered seawater with a signals of 0.639 and 0.785, respectively. These values are within the mid-range of the dynamic response of the detectors set at the lowest gain. A dilution series was measured of seawater containing only free coccoliths from *E. huxleyi* (Fig. 3). The data in this figure represents the total 90° light scatter for the sample which includes both seawater and coccoliths. The curvilinear response is due to the fact that below 10,000 coccoliths ml^{-1} most of the total 90° light scatter is due to water. The instrument was found to be sensitive to 1,000 coccolith ml^{-1} and remained linear past 90,000 coccoliths ml^{-1} which represents bloom conditions (Fig. 4). This range of concentrations of coccoliths represents 0.2-20 ug of inorganic Carbon per liter as calcite, assuming 0.2pg of carbon per coccolith[7].

6. DATA ANALYSIS

The data from the scattering meter was continually acquired at a rate of one sample every 0.2 seconds. As the acid pump was triggered on and off, the resulting data stream over a 1 minute period had a square wave appearance when calcite was present. The minimum duration of the plateaus in the wave form was about 20 seconds with a 10 second transition interval between the peak and trough as the calcite dissolved. A 40 point average was applied to the data during the 10 second interval before the acid pump was triggered on or off. The non-acidified and acidified data were independently smoothed using a 4 point running average. Since determination of the acidified and non-acidified value were not synchronous, rapidly changing light scatter caused an artifactual value for the acid labile light scatter. To correct for this, data from the non-acidified samples were interpolated to the time of the acidified sample. The acidified value subtracted from the interpolated, non-acidified data point represented the acid labile light scatter.

7. FIELD DATA

Surface data collected from 2°N-1°N latitude along 140°W longitude during the JGOFS equatorial pacific cruise in August-September 1992 are shown in figure 5. A hydrographic front and an increase in chlorophyll fluorescence occurred between 1.6°-1.5°N. This front was associated with a decrease in temperature (Fig.5A). The total 90° light scatter (non-acidified) began increasing at 1.6°N but remained high until 1.3°N even though chlorophyll had begun to decrease (Fig.5B). The non-acidified and acidified 90° light scatter were the same from 1.9°N to 1.65°N and again between 1.34°N to 1°N. The acid labile 90° light scatter had a small peak at 1.65°N with three major peaks between 1.44 °to 1.32°N.(Fig. 5C). These acid labile peaks were not located in the region of highest chlorophyll concentrations. Calcite as carbon ranged from 2.2-5.0 ug per liter along the transect. Differences in sample size and frequency between the light

scatter and calcite measurements precluded a direct comparison, however the two values generally co-varied over the transect.

A depth profile from the vertical pumping system at 12°N 140°W in the equatorial Pacific is shown in Figure 6. Values for acid labile 90° light scatter were close to zero for most of the profile with a well defined peak at about 50m (Fig.6A). Broad peaks in both chlorophyll and total 90° light scatter occurred at 75m (Fig.6B), 25 meters below the peak in acid labile 90° light scatter, suggesting that coccolithophores were living above the chlorophyll maximum. Samples of particulate calcite taken from a rosette cast 12 hours prior to the pump profile showed a peak in calcite carbon at 45m.

8. CONCLUSIONS

Acid labile light scatter calibrated well to coccolith concentration in the lab cultures (r^2=0.99) and varied rapidly over short spatial scales both in the vertical and horizontal. Future work will involve absolute calibration of the detector at various gains and improved sampling design such that a direct comparison between acid labile light scatter and particulate calcite can be made at sea. This instrument will be a useful shipboard tool for high resolution mapping of calcite particles in the sea.

9. ACKNOWLEDGEMENTS

W.M.B. and K.A.K. were supported by the Ocean Optics program of the Office of Naval Research (N0014-91-J-1048), National Science Foundation (OCE-9022227) ,and NASA(NAGW2426). K.J.V. and Y.G. were supported by ONR (N0014-90-J-1505).

10. REFERENCES

1. Brongersma-Sanders,M. (1957) "Mass mortality in the sea". Mem. Geol. Soc. Am., 67,941-1010.

2. Holligan,P.M., Viollier,M., Harbour,D.S., Camus,P. and Champagne-Phillipe,M. (1983) "Satellite and ship studies of coccolithophore production along a continental shelf edge". Nature, 304,339-342.

3. Balch,W.M., Eppley,R.W., Abbott,M.R., Reid,F.M. (1989) " Bias in satellite-derived pigment measurements due to coccolithophorees and dinoflagellates". J. Plank. Res.,11,575-581.

4. Balch, W.M, Holligan,P.M, Ackelson,S.G, Voss,K.J. (1991) "Biological and optical properties of mesoscale coccolithophore blooms in the Gulf of Maine". Limnol. Oceanogr,, 36,629-643.

5. Yentsch,C.S. and Menszel,D.W. (1963)" A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence". Deep-Sea Res, 10,221-231.

6. Holm-Hansen,O., Lorenzen,C.J.,Holmes,R.W.,Strickland,J.D.H. (1965) "Fluorometric determination of chlorophyll". J. Cons. perm. int. Explor. Mer., 30,3-15.

7. Balch, W.M., Holligan,P.M., Kilpatrick,K.A., (1992) "Calcification, photosynthesis and growth of the bloom-forming coccolithophore, *Emiliania huxleyi*.". Cont. Shelf Res.,12,1353-1372.

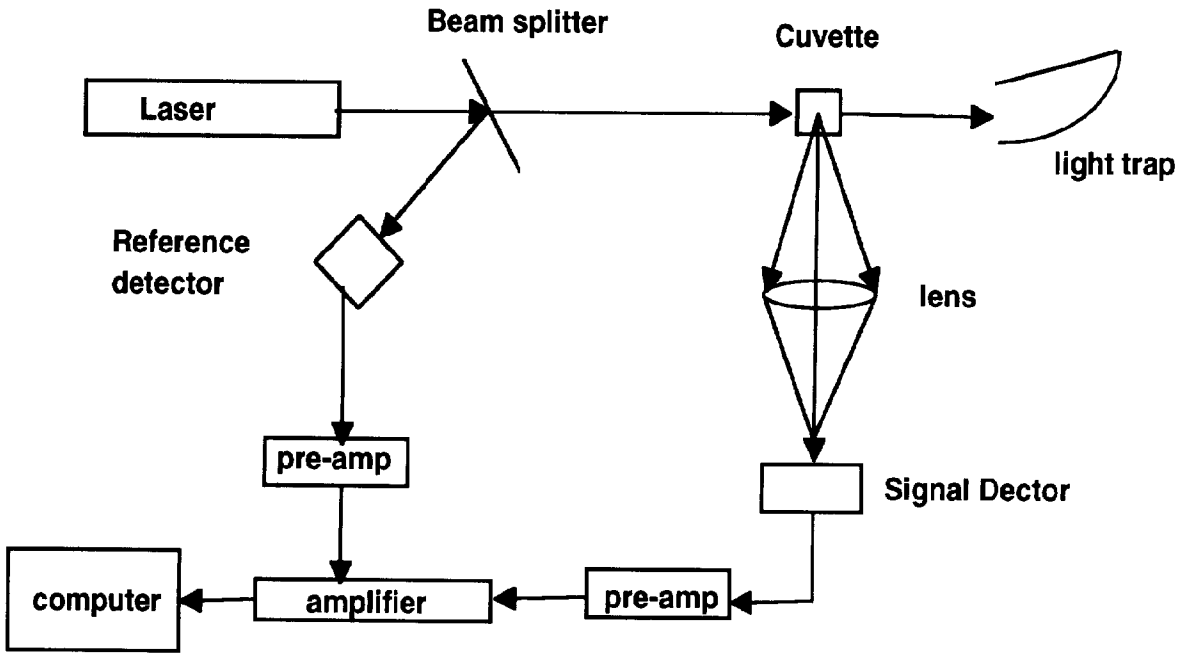

Figure 1 Scattering meter optical layout

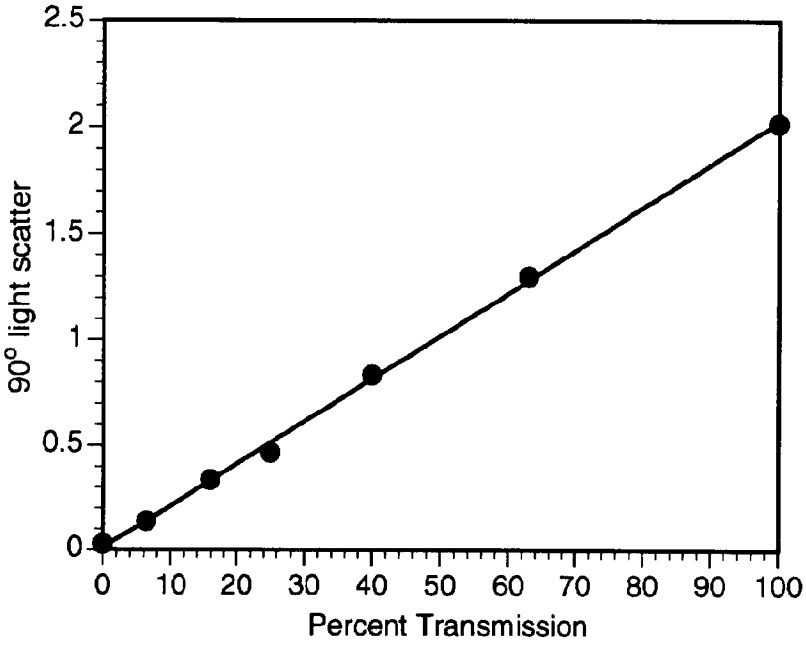

Figure 2 Linearity response of the Signal detector: The scattered light was attenuated with neutral density filters.

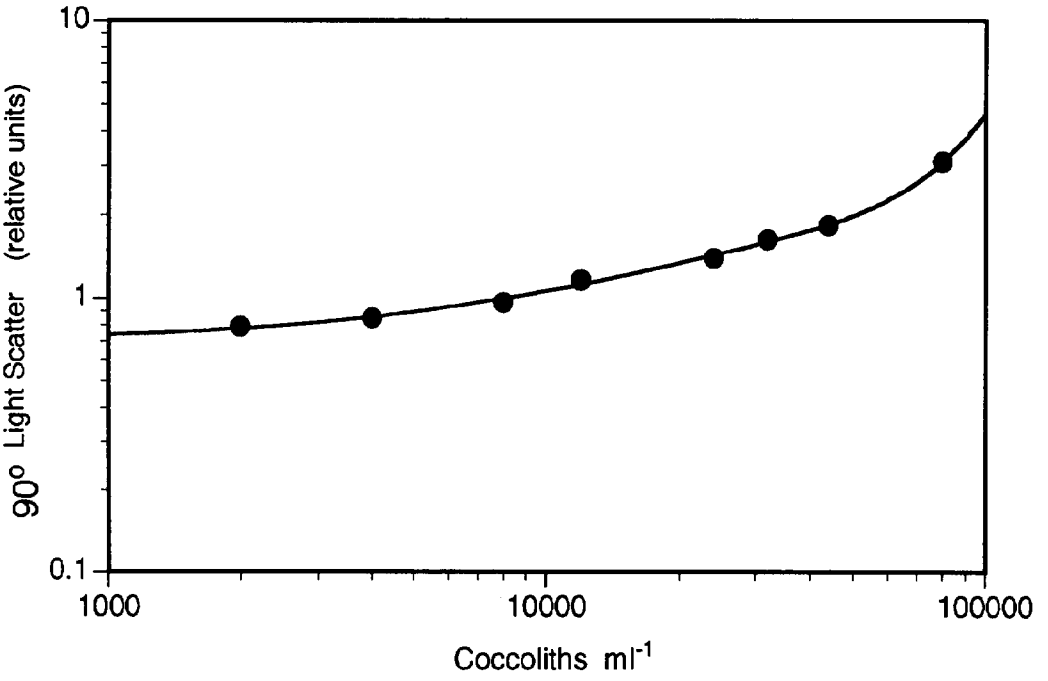

Figure 3 Dector response to increasing coccolith concentration: The data represent the contribution of both seawater and coccoliths to the total 90° light scatter signal.

$f(x) = 2.875192E\text{-}5*x + 7.279006E\text{-}1 \qquad R^2 = 9.912294E\text{-}1$

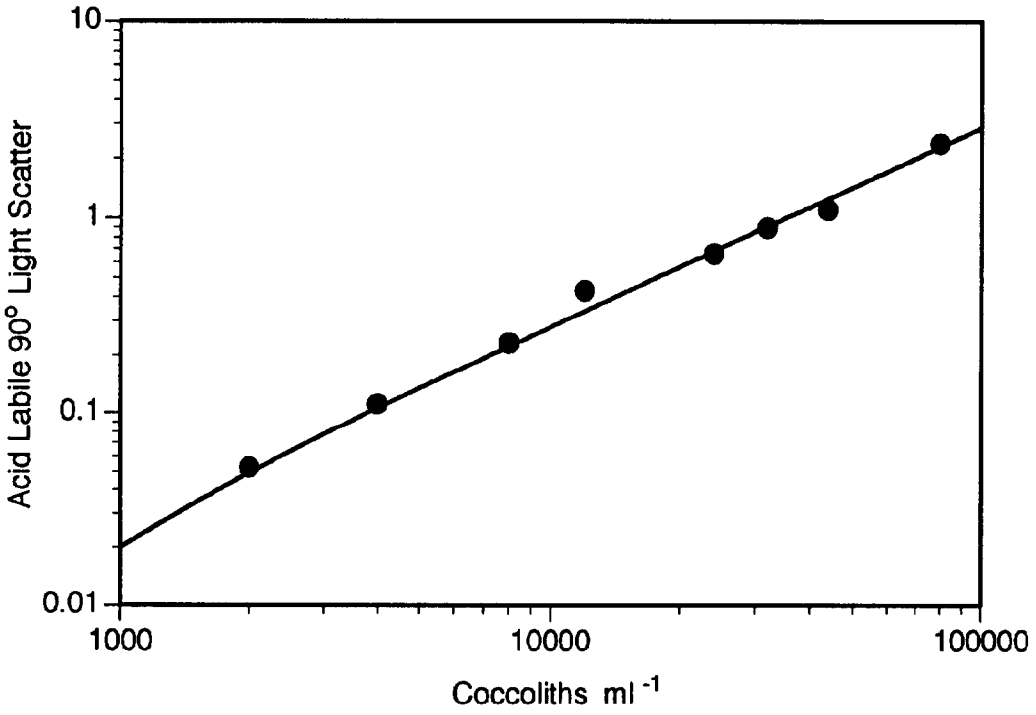

Figure 4 Acid-labile 90^0 light scatter versus coccolith concentration. The acid-labile value is calculated as the difference between the total and acidified light scatter.

$f(x) = 2.878954E\text{-}5*x + -8.955551E\text{-}3 \quad R^2 = 9.902170E\text{-}1$

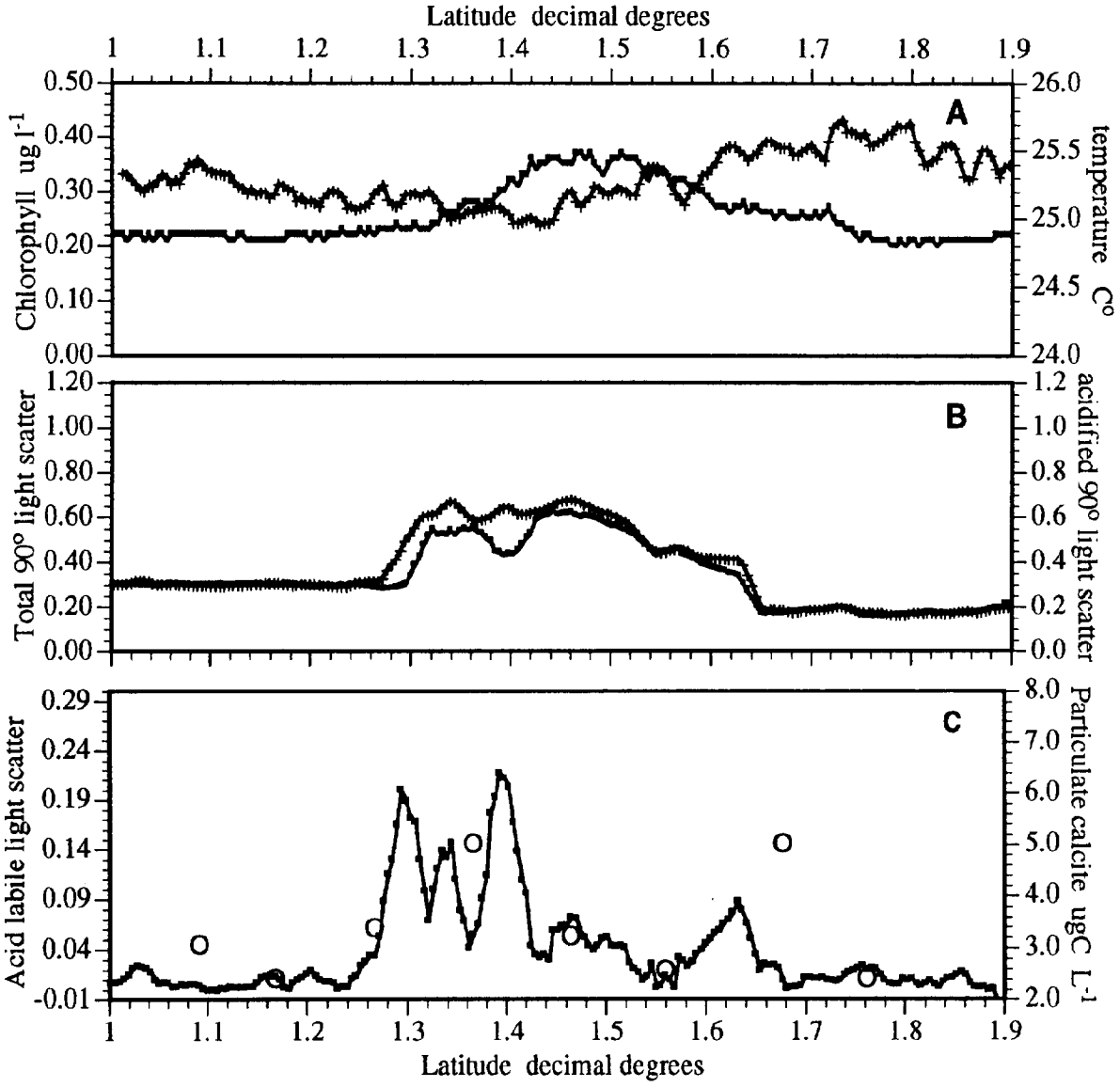

Figure 5 Portion of Transect from Equatorial Pacific along 140W Longitude.
A. + Temperature, ▪ Salinity; B. + Total 90° light scatter, ▪ acidified light scatter;
C. ▪ Acid labile light scatter, o Particulate Calcite.

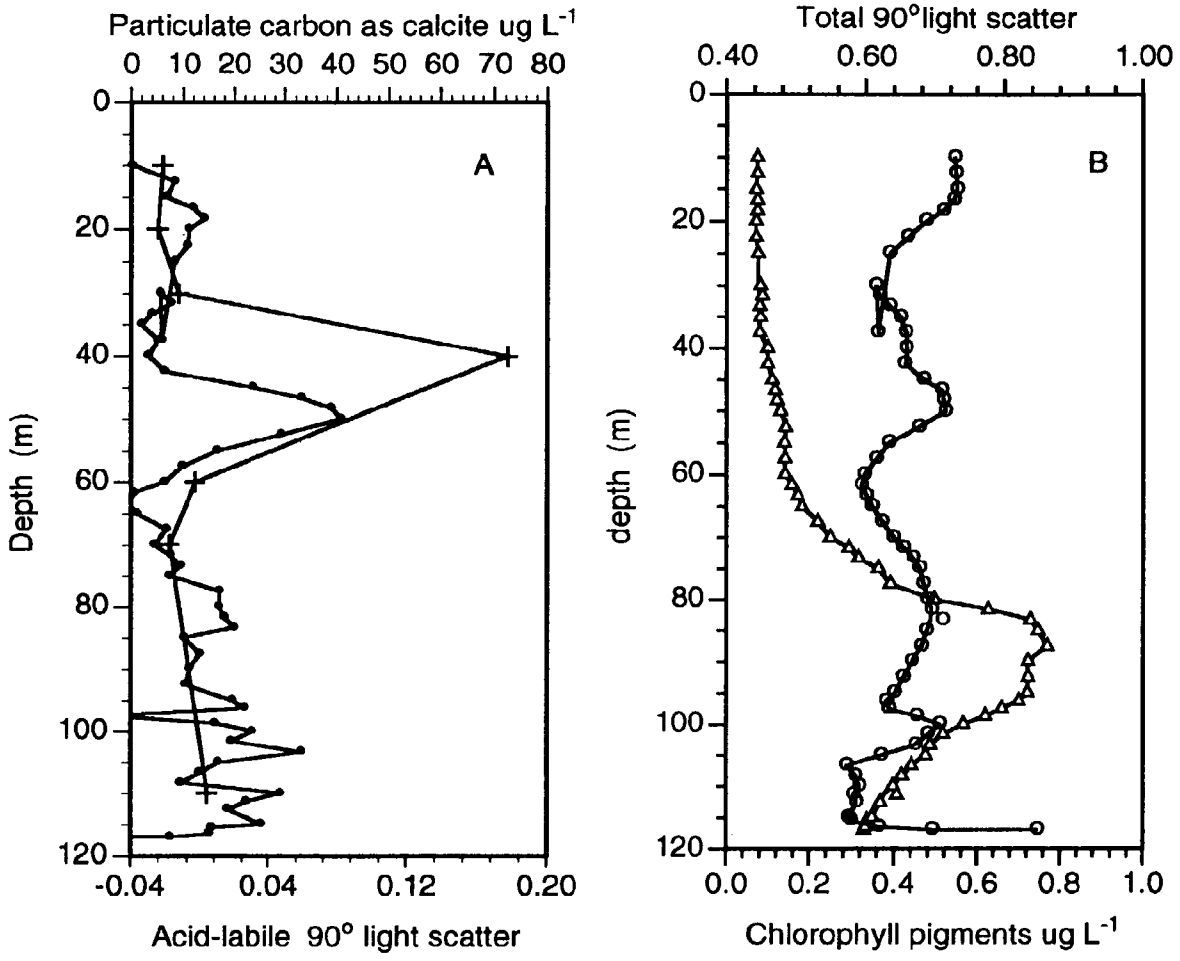

Figure 6, Verticle profile 12N 140W in the Equatorial Pacific
A; + Paticulate inorganic Carbon as calcite, • acid-labile 90° light scatter; B: Δ chlorophyll ug l-1,
O Total 90° light scatter