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Simultaneous Measurements of Fluorescence and Beam Attenuation: Instrument Characterization and Interpretation of Signals from Stratified Coastal Waters

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Hydrographic (conductivity, temperature, depth), optical (fluorescence and beam attenuation) and chemical (suspended particulate material, chlorophyll, gelbstoff) observations were made in sea lochs off the west coast of Scotland. A strong link was observed between hydrographic and optical layering in these waters. In order to assist the interpretation of the optical data, relationships between fluorescence and extracted chlorophyll and dry weight and beam attenuation were determined in the laboratory for five species of cultured phytoplankton and three types of inorganic particles. The inherent variation in these relationships from one class of material to another precluded the development of generally applicable algorithms for retrieving mass concentrations from 2-parameter optical data. However the instrument calibrations were used to partition a bivariate plot of fluorescence against attenuation on which the *in situ* data formed well defined clusters. This made it possible to deduce distribution profiles of phytoplankton and suspended sediment in the water column. The results indicate that even in the absence of absolute calibrations, multiparameter optical measurements can provide valuable information on fine-scale variations in seawater composition, and enhance the identification and discrimination of water masses in fjords and other highly structured water bodies.

Keywords: fluorescence; beam attenuation; optical layering; sea lochs; Scotland coast

Introduction

Patterns of variation in standard hydrographic parameters can be rapidly mapped using modern CTD instrumentation, but it is difficult to measure concentrations of phytoplankton, gelbstoff and suspended sediments with equivalent spatial resolution. The difficulty stems from the amount of labour required to carry out a sufficient number of direct chemical and gravimetric analyses, and there is much interest in deriving the required information from proxy variables which lend themselves more easily to rapid data acquisition. Optical measurements are obvious candidates for this purpose, but they are subject to considerable calibration uncertainties. These arise from differences in absorption and scattering properties between phytoplankton species (Bricaud *et al*., 1988), strong dependence of scattering from inorganic particles on their size and shape (Bishop, 1986; Morel, 1991) and significant variability in the yield of *in vivo* fluorescence from phytoplankton per unit chlorophyll *a* (Cunningham 1996).

In order to assess the practical effect of calibration difficulties on the interpretation of optical signals, the authors have made a series of simultaneous optical, hydrographic and chemical observations in sea lochs off the south west coast of Scotland. Sea lochs provide excellent sites for work of this nature since their optical and hydrographic properties vary widely on a fine spatial scale. They are mainly fjordic in character, with deep glacially excavated basins partially isolated by rock sills, and most receive significant flows of fresh water from the surrounding hills. In some areas the input of tidal energy is sufficient to produce mixing of the water column, but in others marked stratification occurs (Simpson & Rippeth, 1993). Primary production in sea lochs is likely to be limited by irradiance rather than inorganic nutrients for most of the year, though nutrient limitation may occur at the peak of the spring diatom bloom (Ross *et al*., 1993). Since the exposure of phytoplankton cells to photosynthetically available irradiance depends on the degree of vertical mixing, the growth of phytoplankton is strongly influenced by the stratification of the water column (Tett

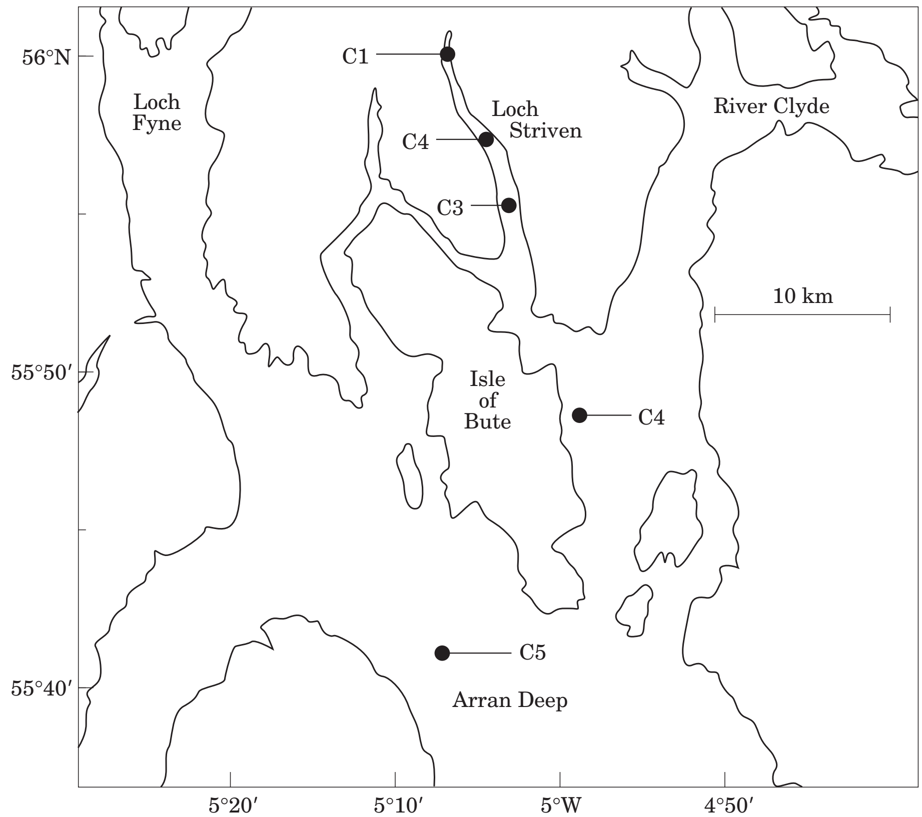

FIGURE 1. Map of the Clyde Sea with the positions of stations C1 to C5 indicated.

et al., 1986). For illustrative purposes this paper presents data obtained in the northern section of the Firth of Clyde in the autumn of 1996, but many of the features described have been found in other sea loch locations and at different times of the year.

Materials and methods

Stations and sampling

Measurements were carried out on a single day (20 October 1996) at a series of five stations extending from the head of Loch Striven to the deep water west of the island of Arran (Figure 1). A useful summary of the hydrography of this area may be found in Edwards *et al.* (1986). The transect passes down a small fjord with significant freshwater input, through a channel to the east of the island of Bute, and into a deep basin east of the island of Arran. Water samples were taken from two or three depths at each station and filtered immediately using Whatman GF glass fibre filters. The filters were stored frozen and analysed within 2 weeks for chlorophyll *a* by acetone extraction (Parsons *et al.*, 1984) and for suspended particulate material (SPM) by drying and weighing. In addition small quantities of water (25 ml) were passed through 0·2 μm membrane filters and their gelbstoff concentration assessed by measuring absorption at 440 nm using a 100 mm pathlength cuvette (Bricaud, 1981).

Hydrographic and optical profiles were obtained using a Neil Brown CTD system with a Seatech fluorometer and Seatech transmissometer attached. The fluorometer provided broad-band blue excitation (approximately 400 nm to 600 nm) and collected red fluorescence from 650 nm to 720 nm (Bartz *et al.*, 1988). The transmissometer employed a collimated red beam with a peak wavelength of 660 nm (Bartz *et al.*, 1978). The instruments were deployed from the hydrographic winch on RV *Calanus*, the Dunstaffnage Marine Laboratory research vessel.

Laboratory characterization

Before the cruise, the response of the optical instruments was investigated by adding known concentrations of algal cultures and inorganic particles separately to filtered seawater in a 200 l black plastic tank. Monospecific algal cultures were grown in 10 l bottles containing Provasoli's ASP6 medium (Sigma, Poole, U.K.) at a temperature of 15 °C. Kaolin powder

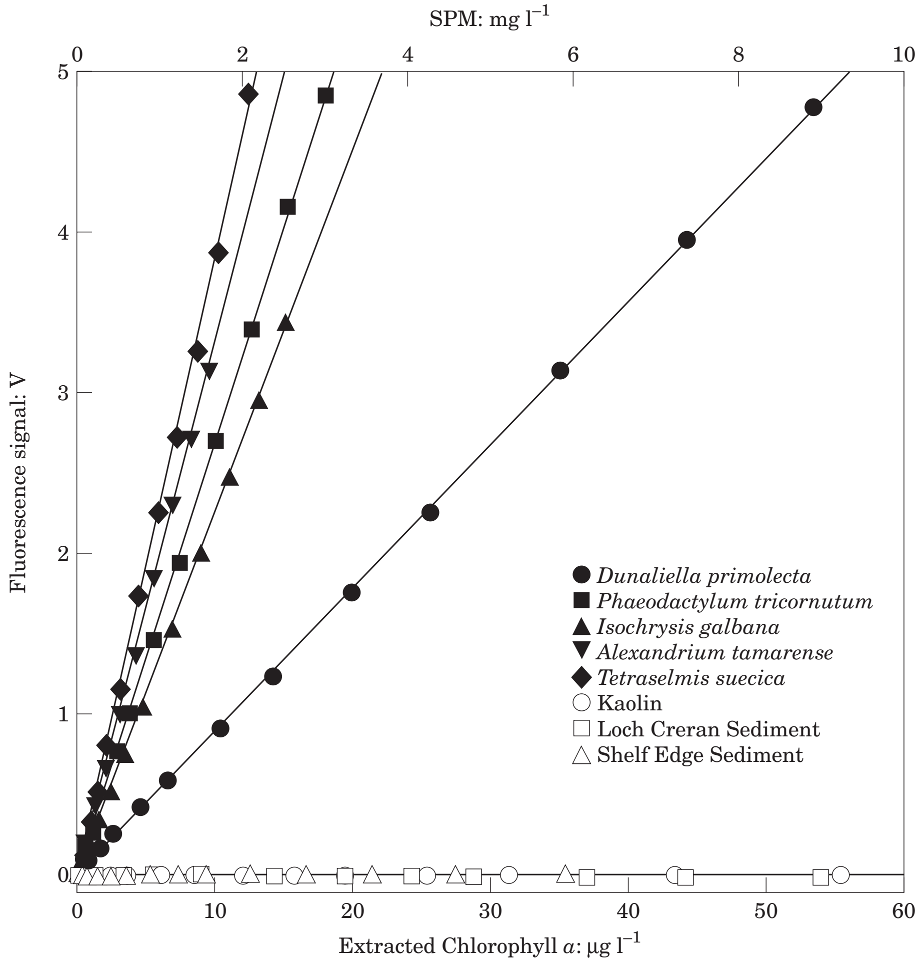

FIGURE 2. Response of the Seatech fluorometer to varying concentrations (expressed in terms of extractable chlorophyll) of five species of cultured phytoplankton. The negligible signals produced by scattering from inorganic particles are shown on a second x-axis.

was obtained from a laboratory supply company. Sediment samples were collected in Loch Creran and from a depth of 2000 m on the shelf edge west of Scotland: their negligible organic content was verified by baking in a furnace at 500 °C and measuring the resultant loss of weight. The inorganic materials were resuspended by vigorous agitation in distilled water and passed through a 60 µm nylon mesh to remove large particles before adding to the calibration tank.

Results

Laboratory calibrations

The fluorometer output varied linearly with chlorophyll concentration for all five species of phytoplankton, and degradation of the fluorescence signal by scattered light was negligible for inorganic particle concentrations up to at least 10 mg l^{-1} (Figure 2). However the gradient of the fluorescence/chlorophyll relationship varied significantly (by a factor of 4) according to species (Table 1). The transmissometer data is presented as the beam attenuation coefficient measured relative to a 0·2 µm filtered seawater blank: it can be converted to absolute attenuation coefficients

TABLE 1. Fluorometer sensitivity for monospecific phytoplankton samples

Species	Sensitivity (V µg Chl^{-1} l)
Dunaliella	0·09
Isochrysis	0·23
Phaeodactylum	0·27
Alexandrium	0·34
Tetraselmis	0·39

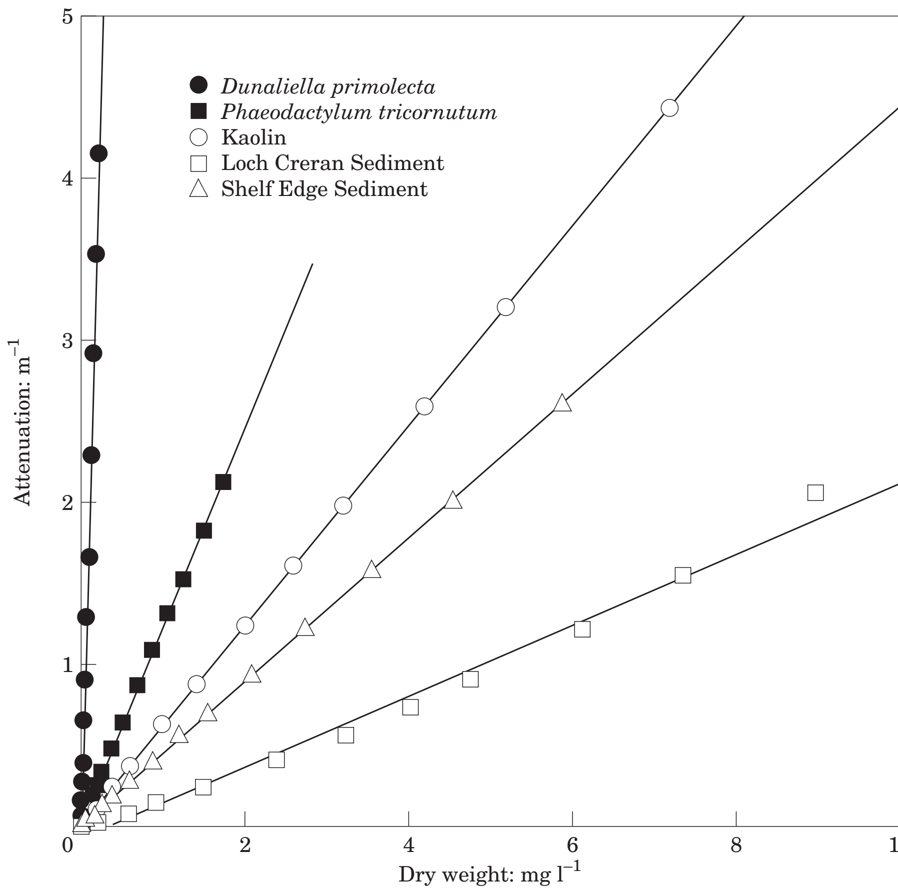

FIGURE 3. Response of the Seatech transmissometer to varying concentrations (expressed in terms of dry weight) of two species of cultured phytoplankton and three types of inorganic particles.

by adding the attenuation coefficient of seawater at 660 nm (0·41 m^{-1}). The relationship between the beam attenuation signal and dry weight was linear for each type of suspended particulate material (Figure 3), but the gradient varied from one particle type to another and was highest for the organic particles (Table 2). These data indicate that it is not possible to calculate mass concentrations from the beam attenuation signal unless the type of material is known. A broad indicator of material type (algal or non-algal) can be derived from the measurement of fluorescence, but the variation in mass-specific and chlorophyll-specific fluorescence from one species to another means that fluorescence intensity alone is not sufficient to adequately constrain the mass determination problem. The general inverse problem of obtaining quantitative information from 2-parameter optical measurements on samples containing an arbitrary mix of organic and non-organic particles would therefore appear to be intractable. Nevertheless, the field results discussed below indicate that useful information can be retrieved from specific *in situ* optical measurements when they are accompanied by regular chemical analyses and when they are made over restricted ranges in time and space.

Field calibrations

Aggregated results from the analysis of water samples from all stations are summarized in Table 3. The first interesting observation was that gelbstoff-related attenuation coefficients derived from laboratory spectrophotometry showed a high inverse correlation with salinity, which suggested that the low-salinity surface water at the head of sea lochs was likely to be distinctly coloured. The attenuation coefficient of gelbstoff is relatively low at the red wavelengths employed by the transmissometer (Bricaud *et al.*, 1981) and so this feature was not detected by our optical instrumentation: a blue transmissometer would clearly be a useful addition to our instrument array. There was a good linear relationship between extracted chlorophyll and fluorescence, implying that the fluorescence characteristics of the phytoplankton population were

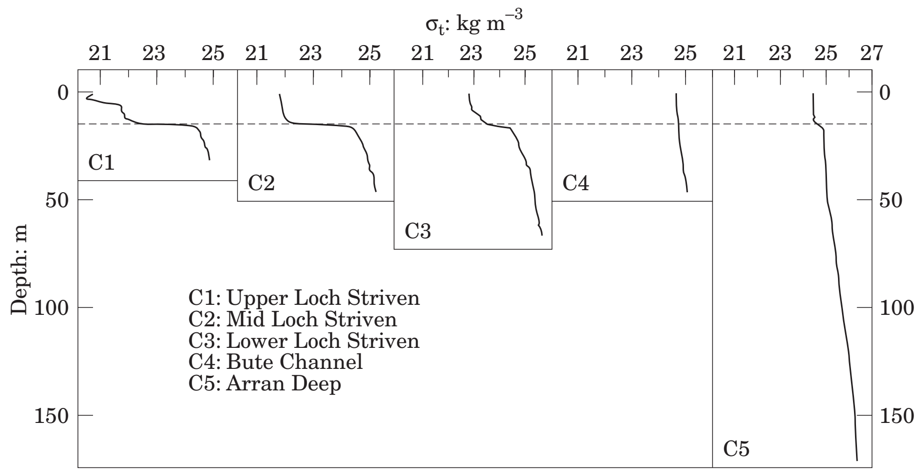

FIGURE 4. Density profiles for stations C1 (head of Loch Striven) to C5 (Arran Deep). The dotted line at around 15 m indicates the major pycnocline.

TABLE 2. Transmissometer sensitivity for single-component suspension

Material	Sensitivity (m^{-1} mg^{-1} l)
Dunaliella	16·28
Phaeodactylum	1·22
Kaolin	0·61
Shelf edge sediment	0·44
Loch Creran sediment	0·22

rather constant and that fluorescence could be used as a semi-quantitative indicator of phytoplankton abundance throughout the survey area. The relationship between attenuation and SPM is complicated by the known differences in mass-specific attenuation coefficients for algal and non-algal particulates, and this is reflected in the low regression coefficient in Table 3.

Water column profiles

Figure 4 shows density profiles for each station. There was a clear pycnocline at around 15 m in Loch Striven, with an additional surface layer of less dense water at the head of the Loch where a small river enters. The density stratification was lost in the channel to the east of Bute, but it reappeared at about the same depth east of Arran and appeared to be a general feature of the stratified sections of the upper Firth. The accompanying fluorescence profiles are shown in Figure 5. In spite of the late season, there was a marked development of the surface phytoplankton population in those stations where stratification occurs, with a peak chlorophyll concentration of around 2·5 μg l^{-1}. In the unstratified Bute channel, however, fluorescence levels were low throughout the water column. The transmissometer profiles (Figure 6) showed the influence of phytoplankton near the surface at all the stratified stations. Beam attenuation fell to a minimum around the pycnocline, and rose again towards the bottom where sediment resuspension was the most likely cause.

Fluorescence/attenuation relationships in Loch Striven

Fluorescence and transmissometer data from stations C1 to C4 are plotted as x,y pairs in Figure 7. Different symbols are used on the scatterplot to distinguish between readings obtained from above and below the pycnocline in Loch Striven and from the mixed station off Bute. In principle, for seawater containing

TABLE 3. Linear regression coefficients derived from the analysis of water samples

x-variable	y-variable	slope	r^2 coefficient
Salinity	Gelbstoff (a_{440})	− 0·08	0·95
Chlorophyll (μg l^{-1})	*In-situ* fluorescence (V)	0·12	0·99
SPM (mg l^{-1})	Attenuation coefficient. (m^{-1})	0·54	0·61

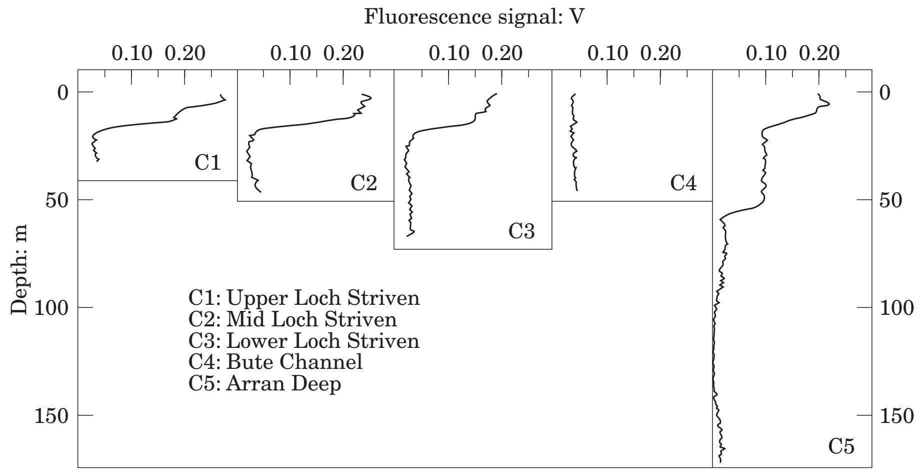

FIGURE 5. Fluorescence profiles for all stations.

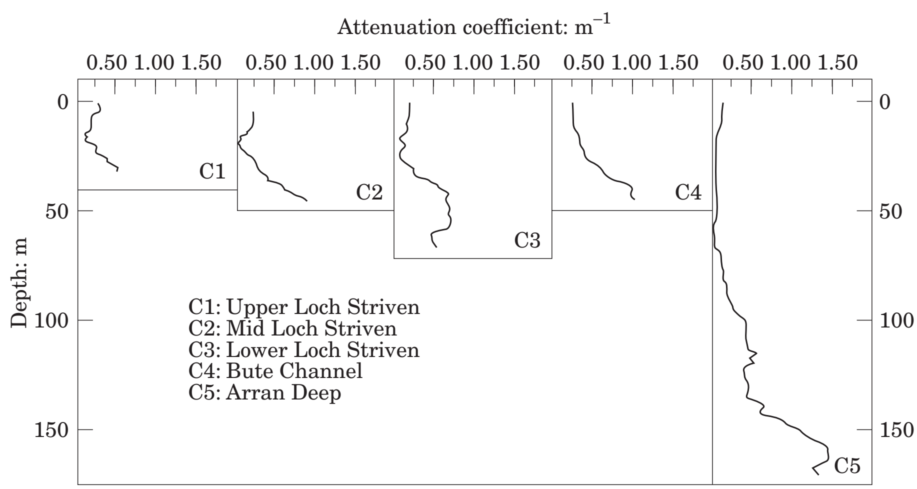

FIGURE 6. Attenuation profiles for all stations.

arbitrary mixtures of phytoplankton and inorganic particles, the data points could appear anywhere on this graph. In practice the data fall into two clear groupings, representing samples whose optical properties were dominated either by phytoplankton or by inorganic particles. Significant fluorescence values only occurred above the pycnocline. The general picture seems to be that there were two sources of attenuating material: phytoplankton near the surface, which did not penetrate the pycnocline, and sediment from the bottom which was resuspended either by tides or density-driven currents at all stations. This conforms to the typical situation in the Clyde Sea described by Balls (1990).

Hydrographic and optical stratification in the Arran Deep

The Arran Deep station provided an interesting example of a multi-layered system, and is shown in more detail in Figure 8. From the surface to a depth of 15 m (the major pycnocline) there was a layer which exhibited low density and high fluorescence: both characteristics were probably the result of stratification induced by the presence of water of lowered salinity. Below the surface layer was more dense but slightly warmer water with a fluorescence, which was lower by a factor of approximately two. This second layer extended down to roughly 50 m, which is close to the depth of the great shelf which separates the inner Clyde Sea from the well mixed waters of the North Channel (Simpson & Rippeth, 1993). The third layer (from 50 to 140 m) had steadily rising density, falling temperature and a further marked reduction in fluorescence. Finally at 140 m small but distinct changes in density and temperature were accompanied by a very pronounced rise in attenuation. There was also a very small increase in the signal registered by the fluorometer in this bottom layer,

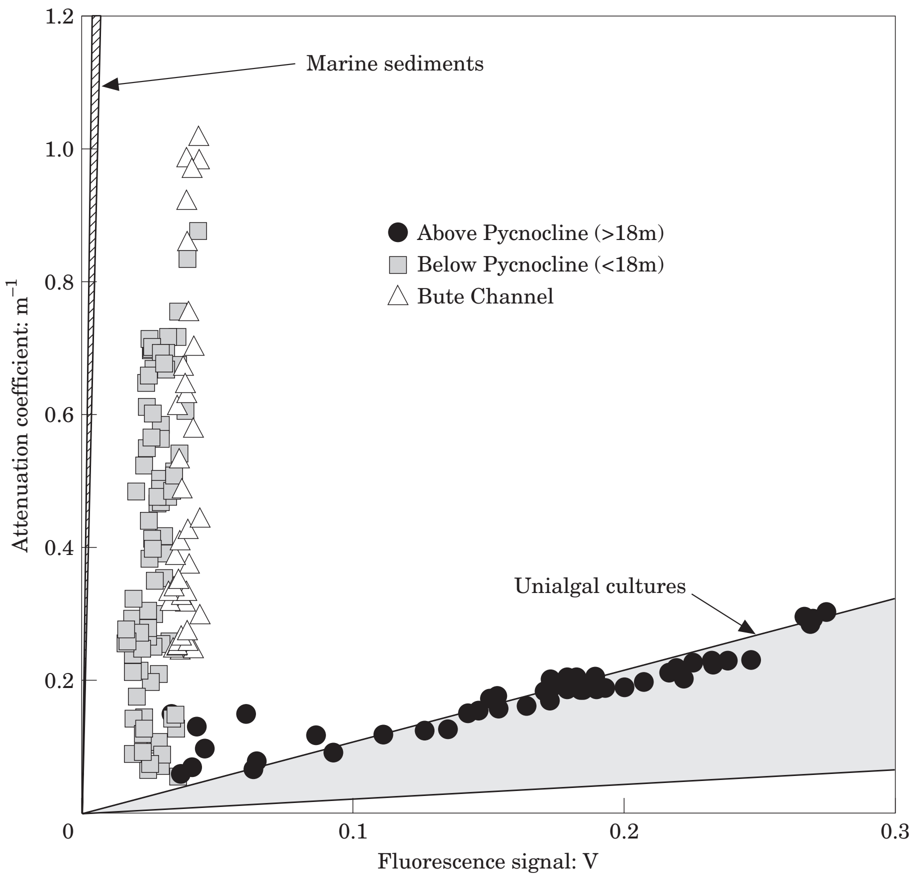

FIGURE 7. Scatterplot of fluorescence and attenuation signals from stations C1 to C4. The shaded areas indicate the limits of the signals generated by pure algal cultures and marine sediments. The phytoplankton-dominated data from above the pycnocline forms a cohesive group which is well separated from data from below the pycnocline and at the mixed station.

which might indicate the presence of phytodetrital material. The alignment of the major discontinuities in hydrographic characteristics with those in the optical properties is well illustrated at this station, but the effect appears to be a more general feature of the region.

Discussion

The results indicate that coastal water masses with different hydrographic characteristics frequently have distinct optical signatures. It appears that qualitative and semi-quantitative information can be extracted from these signals even in the absence of absolute instrument calibrations, but the value of the optical data would be greatly enhanced by better calibration methodologies. Here, therefore, the use of additional optical parameters to decrease the ambiguity inherent in fluorescence and attenuation measurements is investigated (McKee *et al.*, 1997). At the simplest level, multiparameter optical measurements can add clarity to the discrimination of water body types separated by only small differences in density or temperature. More interestingly, however, they allow deductions to be made about physical and biological processes. For example the strong link between fluorescence and density in Loch Striven in October shows that the part played by reduced-salinity layering in promoting early season phytoplankton growth in sea lochs (Tett & Wallis, 1978) also applies late in the year. Turbidity profiles (deduced from transmissometry and fluorometry, or measured directly by nephelometry) can indicate the resuspension of bed material and the injection of particles from land drainage and industrial sources. The variation in optical signatures which accompanies hydrographic stratification is likely to be associated with vertical layering in absorption, beam attenuation and scattering coefficients. Radiative transfer in such layered systems differs significantly from homogeneous water bodies with the same depth-averaged composition (Stramski & Mobley, 1997; Gordon & Boynton, 1998) and so the occurrence of

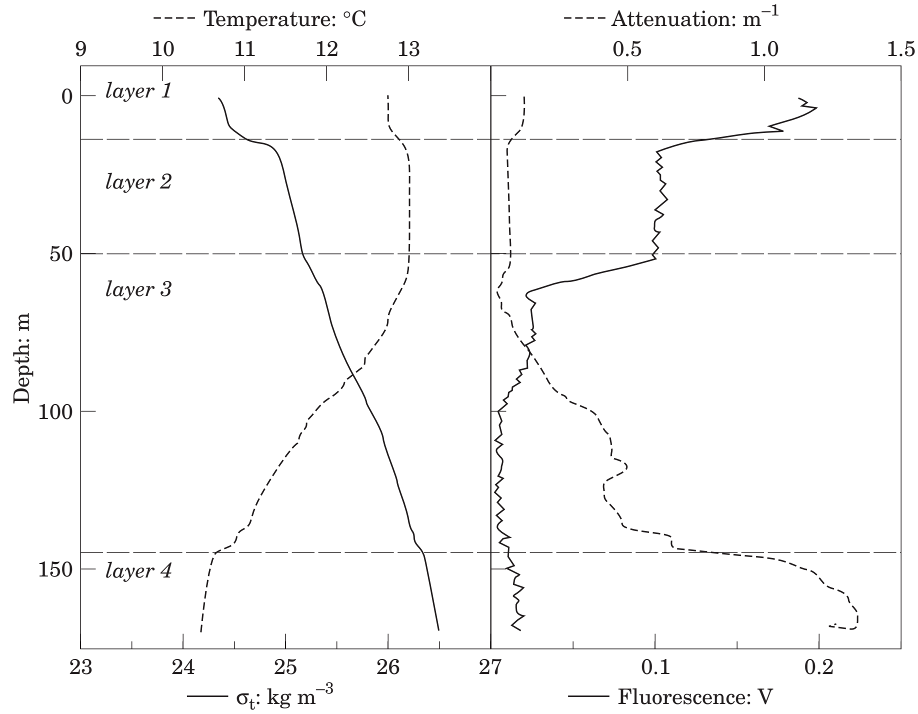

FIGURE 8. Temperature, density, attenuation and fluorescence profiles for the Arran Deep station, showing the alignment of hydrographic layers and optical properties.

marked optical layering has significant implications for the interpretation of remotely-sensed reflectance data from the Clyde Sea area, and presumably from other fjordic systems.

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References

Balls, P. W. 1990 Distribution and composition of suspended particulate material in the Clyde estuary and associated sea lochs. *Estuarine, Coastal and Shelf Science* **30,** 475–487.

Bartz, R., Zaneveld, J. R. V. & Pak, H. 1978 A transmissometer for profiling and moored observations in water. SPIE Vol. 160, *Ocean Optics* V. pp. 102–108.

Bartz, R., Spinrad, R. W. & Kitchen, J. C. 1988 A low power high resolution in situ fluorometer for profiling and moored applications in water. SPIE Vol. 925, *Ocean Optics* IX. pp. 157–170.

Bishop, J. K. B. 1986 The correction and suspended particulate matter calibration of Sea Tech transmissometer data. *Deep Sea Research* **33,** 121–134.

Bricaud, A., Morel, A. & Prieur, L. 1981 Absorption by dissolved organic matter of the sea (yellow substance) in the UV and visible domains. *Limnology and Oceanography* **26,** 43–53.

Bricaud, A., Bedhomme, A.-L. & Morel, A. 1988 Optical properties of diverse phytoplanktonic species: experimental results and theoretical interpretation. *Journal of Plankton Research* **10,** 851–873.

Cunningham, A. 1996 Variability of in-vivo chlorophyll fluorescence and its implication for instrument development in bio-optical oceanography. *Scientia Marina* **60,** 309–315.

Edwards, A., Baxter, M. S., Ellet, D. J., Martin, J. H. A., Meldrum, D. T. & Griffiths, C. R. 1986 Clyde sea hydrography. *Proceedings of the Royal Society of Edinburgh* **90B,** 67–83.

Gordon, H. R. & Boynton, G. C. 1998 Radiance-irradiance inversion algorithm for estimating the absorption and backscattering coefficients of natural waters: vertically stratified water bodies. *Applied Optics* **37,** 3886–3784.

McKee, D., Cunningham, A. & Jones, K. 1997 An integrated submersible fluorometer/nephelometer/transmissometer: design and testing at sea. *Optics and Laser Technology* **29,** 35–39.

Morel, A. 1991 Optics of marine particles and marine optics. In *Particle Analysis in Oceanography* (Demers, S. ed.). NATO ASI Series, Vol G 27, Springer-Verlag, Berlin, pp. 141–188.

Parsons, T. R., Maita, Y. & Lalli, C. M. 1984 *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, Oxford.

Ross, A., Gurney, W. S. C. G., Heath, M. R., Hay, S. J. & Henderson, E. W. 1993 A strategic simulation model of a fjord ecosystem. *Limnology and Oceanography* **38,** 128–153.

Simpson, J. H. & Rippeth, T. P. 1993 The Clyde Sea: a model of the seasonal cycle of stratification and mixing. *Estuarine, Coastal and Shelf Science* **37,** 129–144.

Stramski, D. & Mobley, C. D. 1997 Effects of microbial particles in ocean optics: methodology for radiative transfer modelling and example simulations. *Limnology and Oceanography* **42,** 550–560.

Tett, P. & Wallis, A. 1978 The general annual cycle of chlorophyll standing crop in Loch Creran. *Journal of Ecology* **66,** 227–239.

Tett, P., Gowen, R., Grantham, B. & Jones, K. 1986 The phytoplankton ecology of the Firth of Clyde sea-lochs Striven and Fyne. *Proceedings of the Royal Society of Edinburgh* **90B,** 223–238.