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Chlorophyll variability in the Baltic Sea: a pitfall for monitoring

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Within the time and space scales of quasi-synoptic areal surveys (~1 day, ~50 km) the fluorescence, measured by an *in situ* profiler, is shown to be a reliable measure of the chlorophyll *a* concentration, although significant areal and temporal differences in the fluorescence yield were observed. Analysis of variance is used to partition the total chlorophyll variance into effects due to differences between large-scale areas in different basins, to time differences between surveys, and to synoptic-scale (~10 km) and fine-scale (~100 m) spatial variability. It is shown that the large-scale areal differences in chlorophyll concentration are inevitably missed, being over-shadowed by the other components of variance. The dominant sources of variance are due to the time and to the synoptic-scale space variability. With respect to the variance structure, the total water column chlorophyll is preferable for monitoring purposes. The results point up weaknesses in the conventional practice of monitoring a highly variable parameter via a sparse grid a few times a year.

Introduction

The need to improve our present knowledge of the state and dynamics of marine environments under heavy anthropogenic influence, such as the Baltic Sea, has been fully acknowledged (Melvasalo *et al.*, 1981). In recent years there has been much discussion in the Baltic oceanographic community on the methods and potential of monitoring studies for assessing the long-term changes in the Baltic environment. Specifically, the questions most often raised are: what parameters are to be measured, and what should be the time and space intervals, which are both cost effective and scientifically sound?

Agreement on these matters is urgently needed because the international Baltic Monitoring Programme and many national and bilateral monitoring programmes are either already initiated or to be initiated in the near future. Unfortunately, much of the discussion so far has been speculative without proper statistical argument. This is understandable because data sets allowing this kind of argument are hard to obtain and hard to analyse. A good exception is a paper by Wulff (1979) which shows, by simple omission of some of the data points from a time series of primary production data, that if a highly variable parameter is measured 4–6 times a year, as proposed in the Baltic Monitoring Programme, any long-term changes will inevitably be missed unless they are of extreme magnitude.

For many reasons the concentration of chlorophyll *a* is one of the most frequently used biological oceanographic parameters (Platt *et al.*, 1977). In recent years we have collected a data set, unequaled in the Baltic, of vertical chlorophyll and CTD profiles. The profiles were obtained using an *in situ* chlorophyll *a* fluorescence/CTD profiler and the sampling methodology of quasi-synoptic areal surveys (Kahru *et al.*, 1981). Additional evidence will be given here of the plausibility of using the *in situ* fluorescence for mapping chlorophyll concentrations in limited space and time scales. For the first time, we combine all the chlorophyll surveys from two areas to identify, by the analysis of variance, the different components of variance, i.e. those due to differences in the large-scale area (basin), survey (time), and synoptic-scale (~10 km) spatial variability, respectively. Here we follow the general approach developed by Platt *et al.* (1970).

Undoubtedly, there are more advanced and powerful methods to analyse variance, such as one-dimensional or two-dimensional spectral analysis (Platt, 1978; Gower *et al.*, 1980). However, the stringent requirements of spectral analysis on the data and on the mechanisms to be resolved limit its applicability, and may invalidate the results (Star and Cullen, 1981). In the Baltic we have already revealed the existence of chlorophyll patches (diameter ~1 km) by the use of spectral analysis (Kahru, 1981). Results of the analysis of variance point up weaknesses in the practice of monitoring a spatially and temporally "patchy" parameter by taking infrequent samples from a sparse network of monitoring stations. The dominant sources of variance – the temporal (from days to months) and the synoptic-scale (~10 km) spatial variability – over-shadow the obvious areal differences between basins and, most probably, the long-term changes. On the other hand, the vari-

ability of a conservative parameter (salinity in deeper layers) is mainly determined by the general hydrography of the basin.

Data collection and fluorescence calibration

The vertical profiler consisted of a Variosens *in situ* fluorimeter (Impulsphysik GmbH, Hamburg) and an NBIS Mark III CTD (conductivity-temperature-depth) probe. Both signals were interfaced to an HP 9825A desk-top calculator. The fluorescence profile, with a vertical resolution of ~18 cm, was interpolated to a set of equi-spaced (50 cm) data points from 0·5 m to 60 m depths. More details may be found in Kahru *et al.* (1981). The influence of micro-scale patchiness (see Astheimer and Haardt, 1984) was minimized by numerical averaging.

It is well known that the *in vivo* chlorophyll *a* fluorescence yield is variable (Kiefer, 1973; Brand, 1982), in contrast to the constant yield *in vitro*. Unfortunately, to our knowledge, no studies have been conducted in the Baltic of the natural variability of the chlorophyll *a* fluorescence yield. These data are needed, however, to employ the more productive *in situ* method for chlorophyll monitoring.

The calibration equation used for the Variosens signal was $C = a_0 + a_1 \exp(a_2 S)$, where C is the chlorophyll *a* concentration and S is the output signal. The coefficient a_2 is dependent only on the receiver electronics, and is determined with high precision using successive dilutions of a phytoplankton culture. The coefficients a_0 and a_1 are to be determined by regression analysis between the fluorescence ($F = \exp(a_2 S)$) and the extracted chlorophyll concentration. Preparation of the samples for extraction and the photometric analysis (Jeffrey and Humphrey, 1975) were carried out according to Edler (1979). The water samples were obtained after the *in situ* vertical profiling (down- and up-trace). Owing to the ship's drift in between profiling and water sampling, and to the low vertical accuracy (~1 m) of the bottle sampling, there is no one-to-one correspondence between these measurements. The extent of the discrepancy depends on the intensity of the small-scale (horizontal and vertical) chlorophyll variability. Bearing in mind also the inherent analytical errors associated with the filtration, extraction, etc., it is evident that both fluorescence and extracted measurements are subject to error. This invalidates the application of the conventional (Model I) regression analysis which assumes that the independent variable is known without error. In the field of marine biology this frequently overlooked aspect has been addressed by Ricker (1973) and Laws and Archie (1981); the latter advocate the use of the geometric mean Model II technique. The method minimizes the absolute value of the sum of the products of the deviations of the observations from the regression line in both directions.

In July/August 1982 about 250 extracted chlorophyll measurements were made from different marine environments to determine the natural variability of the regression line $C = a_0 + a_1 F$. As maximal variability in the fluorescence characteristics is expected in coastal as opposed to offshore areas, most of the measurements were made in Estonian coastal waters. The data were grouped in six surveys: S (in the central Gulf of Finland), D (hourly series in the southern Gulf of Finland), K (hourly series in the southeastern Gulf of Finland), P (in the Moonsund, Estonian west coast), R (in the Gulf of Riga), and G (in the offshore southeastern Gotland Basin). Model II regression lines were fitted to the data of individual surveys as well as to the whole data set.

The general conclusion supported the experience gathered during the calibration of our earlier surveys in the offshore Baltic: at least within the limited space and time intervals of our areal surveys, of the order of 50 km and a few days, the fluorescence profiles can be reliably converted to chlorophyll concentrations using a reasonable number of calibrations. The correlation coefficients on individual surveys are almost always above 0·90. Bearing in mind the inherent errors associated with both variables, a better relationship cannot be expected even if a perfect relationship exists between the fluorescence and the chlorophyll *a* concentration. To test the differences between the slopes on individual surveys i and j with the slopes b_1 and b_2, correlation coefficients r_1 and r_2, and sample sizes n_1 and n_2, the following test statistic (Clarke, 1980) was calculated.

$$T_{ij} = \frac{|\ln b_1 - \ln b_2|}{[(1 - r_1^2)/n_1 + (1 - r_2^2)/n]^{\frac{1}{2}}}.$$

The distribution of T_{ij} is approximated by the Student's *t* distribution with the degrees of freedom given in Clarke (1980). The results outlined in Table 1 show that some of the differences are highly significant, and there seems to be a cluster of the first three surveys from the Gulf of Finland with higher slopes, and a cluster of the last three surveys with lower slopes. (Higher slopes infer lower fluorescence yields and *vice versa*.) However, when all the surveys are summed, the correlation coefficient is still 0·88. It might be expected that owing to the strong vertical stratification in the Baltic, considerable variance around the regression line would stem from the inaccuracy in the depth of the water bottle sampling. To compensate for that, the computer was allowed to choose the closest fluorescence value to the fitted line in the depth interval ±1 m from the nominal water sample depth. This procedure produced a considerable increase in the correlation values of the total data set (from 0·88 to 0·93) as well as of the individual surveys, e.g. to 0·98, 0·97 and 0·96 for surveys P, K, and D, respectively. Contrary to some observations (e.g. Aiken, 1981), no significant differences between the day and night fluorescence yields could be found. The

Table 1. Results of Model II regression analysis between the *in situ* fluorescence and the extracted chlorophyll *a* concentration. The upper right corner of the matrix contains values of the test statistic, T_{ij}, with the corresponding degrees of freedom in the lower left corner. The significance of the difference between slopes i and j is indicated at the probability levels 5 % (+), 1 % (++), 0·1 % (+++).

Survey	S	D	K	P	R	G
Slope	0·20	0·18	0·23	0·13	0·17	0·15
Correlation coefficient	0·81	0·94	0·92	0·96	0·80	0·84
Sample size	*50*	*16*	*43*	*13*	*62*	*52*
Degrees of freedom/T_{ij}						
S	–	0·76	1·25	4·00+++	1·60	2·81++
D	26	–	2·13+	3·22++	0·81	2·02
K	49	20	–	5·97+++	3·18++	4·63+++
P	24	16	18	–	2·15+	1·29
R	57	26	56	23	–	1·25
G	52	25	50	22	59	–

surveys with lower correlations comprise either observations from a larger area (Gulfs of Finland and Riga) over a longer period of about a week (surveys S and R), or from a mass occurrence of the blue-green algae (survey G).

The principal sampling schemes with the profiler were quasi-synoptic areal surveys, which typically comprised grids of 5 × 6 or 6 × 6 stations, with the covered areas respectively 37·0 × 46·3 km or 46·3 × 46·3 km. The spacing between neighbouring stations (grid points) was 5 nautical miles (9·3 km). Details of the surveys are summarized in Table 2. Occasionally, some stations were missed on account of instrument failure or unexpected weather conditions. Since the time interval between successive stations was ~1 hr, the duration of each survey was approximately the number of stations in hours. Hence, within these time and space scales, mapping of chlorophyll distribution by means of fluorescence is plausible, if a reasonable number of calibrations is made. On one occasion we detected a significant increase in the fluorescence yield between two consecutive surveys (18/2 and 18/3) within two days. This was associated with a sudden stabilization of the water column and an increase in the irradiance and the water temperature to which the phytoplankton was subjected.

Table 2. Summary of the chlorophyll surveys used in the analysis of variance. Profiles of chlorophyll fluorescence and CTD were obtained at stations on rectangular grids with a 5-mile step (surveys 29/G and 29/H comprise profiles along a section).

Area	Survey	Date	Station no.
G	14/1	5 Jul 79	21
	14/2	15 Jul 79	21
	17/1	8 May 80	29
	18/1	30 May 80	30
	18/2	8 Jun 80	30
	18/3	10 Jun 80	25
	19/1	1 Jul 80	30
	23/1	3 Jun 81	42
	23/2	15 Jun 81	25
	29/G	6 Aug 82	17
	29/H	7 Aug 82	9
B	22/1	25 Apr 81	36
	22/2	29 Apr 81	36
	28/2	13 Jun 82	36
	28/3	19 Jun 82	26
	28/4	21 Jun 82	24

Total: 16 surveys and 437 stations.

Analysis of variance in quasi-synoptic areal surveys

Our earlier publications (Kahru *et al.*, 1982; Kahru, 1982), based on recurrent quasi-synoptic areal surveys, describe the interaction between chlorophyll distribution and hydrography. Here we combine the data from all the surveys obtained so far from two areas: area G in the Gotland Basin, ~50–100 km SSE from the island of Gotland and area B in the Bornholm Basin, east to the island of Bornholm. The two areas, which belong hydrographically to different basins, are separated by a distance of about 270 km. Their differing hydrography is manifested, for example, in the time of the commencement of the spring phytoplankton bloom which starts in the middle of April in the Bornholm Basin and in the middle of May in the Gotland Basin (Kaiser and Schulz, 1978). The salinity stratification is stronger and the halocline is shallower (50–60 m *vs* 60–80 m) in the Bornholm Basin, which is closer to the inflow of the more saline North Sea water.

Following the general approach of Platt *et al.* (1970) we have attempted to resolve the different sources of variance. It was assumed that the total variance of chlorophyll observations at a given depth or in a depth interval has three components, i.e. $\sigma_T^2 = \sigma_A^2 + \sigma_S^2 + \sigma_B^2$, where σ_T^2 is the total variance, σ_A^2 is the variance due to differences between areas, σ_S^2 is the variance between different surveys, and σ_B^2 is the between-station variance during individual surveys. σ_B^2 also includes the errors due to non-synopticity, that is, the changes during the

Table 3. Grand mean and coefficient of variation (C.V., %) and area G and B means of chlorophyll and salinity, together with the analysis of variance results: estimates of the total variance (s_T^2) and the components of the variance of a single measurement, i.e. due to differences between areas (s_A^2), surveys (s_S^2), and stations (s_B^2). For convenience, the variances are multiplied by 1000. If not indicated by NS (not significant), all the values different from zero are significant at the probability level 1 %. +0 stands for a small positive number not specified due to rounding errors; if significantly different from zero, it is marked with S. The analysis of variance was made on the data transformed by log(x + 1).

	Depth (m)	Grand mean	C.V.	G mean	B mean	s_T^2	s_A^2	s_S^2	s_B^2
Chlorophyll	0·5	1·64	72	1·54	1·80	31	0	11	20
mg m^{-3}	1	1·68	70	1·58	1·83	30	0	12	18
	2	1·73	66	1·67	1·85	28	0	12	16
	5	1·99	54	1·97	2·03	21	0	11	10
	10	2·22	52	2·24	2·18	21	0	11	10
	15	2·01	57	1·90	2·22	27	0	13	14
	20	1·41	79	1·21	1·79	31	0	20	11
	30	0·81	101	0·60	1·20	22	1 NS	16	6
	50	0·45	62	0·52	0·33	5	1 NS	2	3
mg m^{-2}	0–10	18·2	51	17·2	20·1	40	0	21	19
	10–20	18·7	52	17·4	21·2	54	0	31	23
	0–30	46·9	55	42·1	56·1	47	0	29	18
	30–60	15·6	63	14·1	17·9	48	0	30	18
	0–60	62·6	56	55·3	74·2	46	0	30	16
Salinity	10	7·870	2	7·884	7·845	0·04	0	+0 S	+0
‰	30	7·931	1	7·962	7·877	0·03	0	+0 S	+0
	60	9·638	19	8·333	11·841	5	5	+0 S	+0
	70	10·801	21	9·296	13·969	7	7	+0 S	+0

time taken to complete the survey. We may further assume that $\sigma_B^2 = \sigma_B'^2 + \sigma_W^2$, where $\sigma_B'^2$ is the true between-station variance and σ_W^2 is the within-station variance. σ_W^2 is determined mainly by the intensity of the fine-scale spatial variability. It has been shown earlier (Kahru *et al.*, 1981) that the between-station variance, σ_B^2, is dominated by the synoptic-scale spatial variance which exceeds, by an order of magnitude, the sum of the fine-scale (~100 m) and the error variance. The synoptic scale is a natural scale of the horizontal variability, and is defined by the internal Rossby radius of deformation (~10 km in the open Baltic). σ_S^2 comprises the time variance with a range of time scales from seasonal to a few days.

The analysis of variance model is essentially a two-level nested anova with unequal sample sizes; i.e., the variability from differences between stations within a survey is nested within the areal and temporal variability. Sokal and Rohlf (1969, pp. 274–281) provide the formulas and the computational scheme. To compensate for the increase of variance at higher fluorescence values (see Platt *et al.*, 1970) the transformation $\log_{10}(x + 1)$ was applied to the data before the analysis of variance was made. For comparison, the same procedures were applied to salinities at selected depth levels.

The statistics and the analysis of variance results are presented in Table 3. In no case was the estimate of the areal variance s_A^2 significantly different from zero for the chlorophyll data. Even for the surface layer (down to 30 m) salinity was not a significant component of variance spatially, although a mean salinity gradient exists along the axis of the Baltic. In fact, the seasonal salinity trends influenced the results so that the areal sample means were against the long-term mean salinity gradient, i.e., the difference between the areal means was not significant.

In the topmost two metres, the dominant source of chlorophyll variance was due to the synoptic-scale spatial variability, whereas the temporal (between surveys) component dominated for the deeper levels and for the vertically integrated concentrations. It is important to note that the variance components are hierarchically ordered, i.e., from upper to lower levels: σ_A^2, σ_S^2, σ_B^2, σ_W^2. For monitoring purposes, the variables with higher shares of the higher levels of variance are obviously preferable to others. It may be inferred from the maximum ratio of the estimates of σ_S^2 and σ_B^2 (s_S^2/s_B^2) that, in such cases, the total water column chlorophyll (the concentration between 0 and 60 m), is in fact preferable to others. However, a high-resolution vertical profiler is needed to estimate the total concentration under conditions with highly stratified waters and sharp chlorophyll maxima. Chlorophyll concentrations at fixed depths, although most commonly used, are the worst for estimating both the temporal dynamics (lower s_S^2/s_B^2) and the synoptic-scale spatial variability (lower s_B^2/s_W^2, shown in Kahru *et al.*, 1981). The fine-scale horizontal and vertical variability interacts with the determination of the synoptic-scale structure, and they together interact with the determination of the temporal dynamics.

Therriault and Platt (1978) in a study of the spatial

heterogeneity in the surface layer of an exposed marine embayment concluded that the time variance contributed 91 % of the total chlorophyll variance (somewhat less for other parameters), while the contribution of the spatial component was rather low (6 %, together with the interaction term). This is in contrast to our results, which show a remarkable share of the space variance. A possible explanation could be that the bigger spatial scales of our study (10–50 km *vs* 1–5 km) introduced a drastic increase in the spatial variability. Also, the time variance component in our data was probably artificially reduced by the time of the surveys covering the period from April to August. Hence, only with these reservations in mind, can we agree with the conclusion of Therriault and Platt (1978) that for the study of seasonal and longer trends, it would be more economical to sample at higher frequency on fewer stations than at lower frequency on more stations. Nevertheless, the synoptic-scale variance component contributes to about 50 % of the total chlorophyll variance at fixed depths of the photic zone during the summer season. It remains to be shown – by increasing the frequency of surveys – how much of the synoptic-scale structure results from any permanent characteristics of stations. We may expect that, due to the strong topographic influence on the current pattern in the Baltic, even the synoptic-scale chlorophyll pattern may not be totally ephemeral, since higher chlorophyll levels are preferably associated with the favourable sites for upwelling, vertical mixing, etc.

Ironically, the impressive number of chlorophyll observations – several hundred vertical profiles, each containing 120 data points – was not sufficient to assign any difference to the mean concentrations in the Gotland and Bornholm basins. This must give pause to those workers who hope to reveal long-term trends or areal differences on the basis of four unsystematic monitoring point-samples per year, but we appreciate that a routine sampling programme of this kind will, at the least, back up more fundamental scientific investigations of such problems.

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