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Integrated Samples Provide Accurate Means of Parameters Characterizing Aquatic Ecosystems

key words: field samples, integrated samples, normal distribution, phytoplankton counts

Abstract

The measurement of ecosystem parameters normally involves considerable effort in terms of sampling and analysis due to their variation in time and space. This contribution describes the advantages and properties of the integrated sample and demonstrates them by comparing the statistical properties of separate samples (number of *Filinia longiseta* per unit volume), and integrated sample (total chlorophyll *a* concentration) and phytoplankton samples analyzed by the Utermöhl method. A working hypothesis is presented to explain the causes of the overdispersion that hinders phytoplankton analysis and to suggest ways of eliminating it.

Contents

1. Key to Symbols

n	sample size
$\bar{x}$	arithmetic mean
s	standard deviation
χ^2	test statistic for the χ^2 test
α	general: level of significance (first kind risk); for the χ^2 test in the sense of probability of fit (Lienert)
β	probability of error (second kind risk)
$\varphi(u)$	relative frequency of the standardized normal frequency distribution
$\frac{s^2}{\bar{x}}$	dispersion (or overdispersion)
f	degrees of freedom
M	momentary value
M^*	measurement at interval of days
DM	dry mass

2. Introduction

The quantitative estimation of ecological parameters by means of routine measuring programmes is important for the analysis and modelling of aquatic ecosystems, but it involves four basic difficulties.

The first is the heterogeneity in the distribution of the biological and chemical components of the water body. This irregularity in spatial distribution is caused by hydrodynamic and other physical or chemical factors or by specific behavioural patterns of the organisms themselves (STEELE 1978, DUMONT 1977, ARNDT, SCHNESE & HEERKLOSS 1981 a, b, cf. also for further references). In this respect it is necessary to distinguish between variations in horizontal and vertical distribution and patchiness.

Fig. 1 illustrates the uneven distribution of one biological component.

The second difficulty is that the concentrations of some ecosystems components can vary by 100 % or more within only a few days. These are the components whose generation times or larval development times are measured in periods of only a few hours to a few days, such as phytoplankton, microorganisms, copepods and rotifers. Accurate quantitative estimations of such components are possible only if sampling is performed every three or four days.

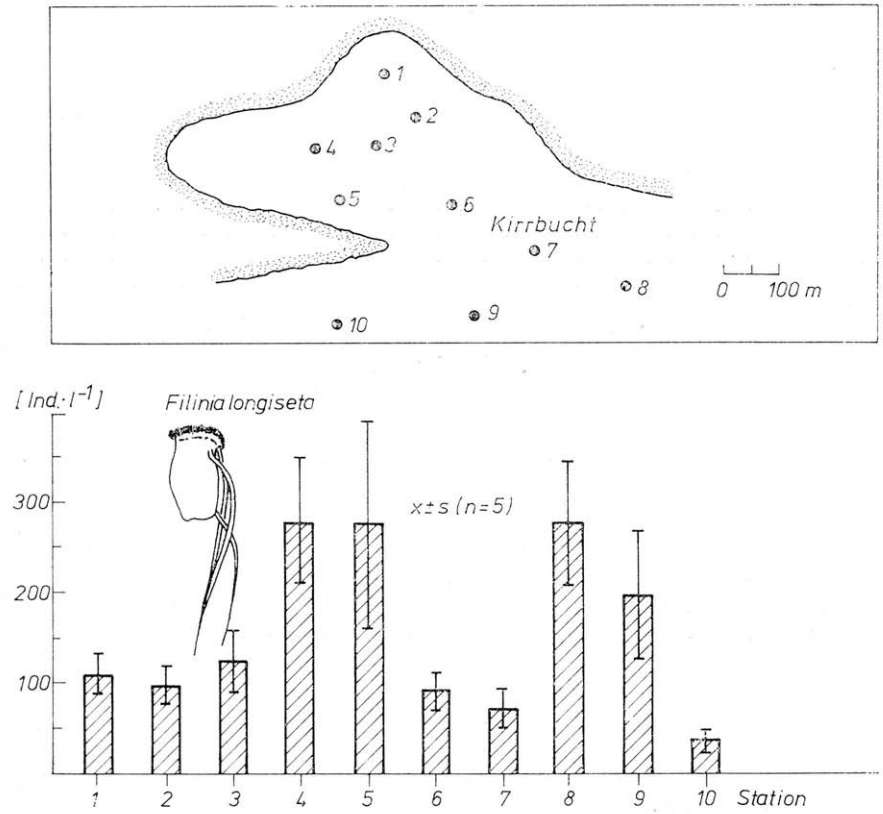

Figure 1. Horizontal distribution of *Filinia longiseta* (Rotatoria) in Kirr-Bucht and Zingster Strom (Barther Bodden) (3 Aug. 1980; taken at depth 0.25 m; water temperature 13.3 °C; salinity 4.2 ‰)

It is this that gives rise to the third difficulty. As a rule biological measurements are made manually or at best semiautomatically. Automatic measurements are the rare exception. This means that analysis of the samples is very time consuming (cf. RHEINHEIMER 1977), and the number of samples a laboratory can cope with is therefore limited.

The fourth difficulty concerns the statistical analysis of the results. Statistical analysis of the measured concentrations or rates of biotic and abiotic parameters of aquatic ecosystems should not be restricted to point estimations (means and standard deviation) but should permit the calculation of confidence intervals at least; this, however, is possible only if the type of statistical frequency distribution of the measured values is known. The theoretical distributions used hitherto to describe the statistical distribution of the measured values have varied considerably (VENRICK 1978, p. 8 and p. 244), and their selection and application requires a detailed knowledge of statistics. If ignored, this is bound to lead to simplification and errors in the results yielded by routine programmes.

The difficult task facing us is, therefore, to obtain more representative primary data of greater statistical reliability in order to draw more correct conclusions from our analyses without increasing the manual and personnel effort in proportion. In our opinion during a routine measuring programme serving as a basis for system analysis it is necessary to obtain one representative measured value a week for the chemical and biological parameters, and this sample should take into account the differences in horizontal and vertical distribution, patchiness, diurnal rhythms etc revealed by appropriate pilot studies. It must be stressed that we are not talking here about special investigation programmes serving, for instance, the detailed analysis of variations in space, sociological studies and so on; these require a special approach that cannot be considered here.

There seem to be two ways out of this difficult situation:

1. Automatic or semi-automatic techniques should be introduced wherever possible, even if this involves a certain loss of accuracy. Table 1 gives an overview of the currently available ways of measuring ecological parameters automatically and semiautomatically (cf. WUHRMANN 1973, VIETINGHOFF 1981, cf. also for further references).

2. Where the data cannot be measured automatically or semiautomatically, integrated samples should be taken at appropriate intervals (WUHRMANN 1973, BOTTRELL et al. 1976, NOWAK 1975, HOLMES & WIDRIG 1956, JANSSON & WULFF 1977, VENRICK 1978, VIETINGHOFF 1980, 1981, ARNDT, SCHNESE & HEERKLOSS 1981).

The methods used to obtain integrated samples involve the taking of samples from various depths at various localities and then, instead of studying them separately, mixing them and performing the quantitative estimations with the mixed, or integrated, samples.

Compared with separate samples, integrated samples have several useful properties which must be exploited. For instance,

- the mean value yielded by a mixed sample is the same as the mean obtained from all single samples, so that in this respect there is no difference;
- the variance will be considerably lower than that calculated from single samples, and low variances imply high accuracy or relatively small samples sizes for a given accuracy;
- reliable means obtained from mixed samples are completely adequate for ecosystem analysis and modelling purposes and should be given preference over the relatively inaccurate means calculated from individual samples.

With regard to the statistical distribution type of the subsamples of mixed samples used for studying the components of aquatic ecosystems, in view of the central limit theorem of statistics it can be assumed to be normal (cf. BENDAT &

Table 1. Possibilities for the use of automatic and semi-automatic measuring systems for ecosystem analysis

M Momentary value M* Measured at intervals of days

Parameter	Measuring device or sensor available	Remarks
a) Physical		
Current field	+	M → hourly mean
Water level	+	M → hourly mean
Wind field	+	M → hourly mean
Irradiance	+	M → hourly mean → daily sum
Water temperature field	+	M → hourly mean
b) Chemical		
Salinity by conductivity	+	M → hourly mean
pH	+	M* and daily variation
O_2	+	M* and daily variation
PO_4^{---}, dissolved organic P, particulate bonded P, NH_4^+, NO_2^-, NO_3^-, dissolved organic N, particulate bonded N, inorganic dissolved C, dissolved organic C, particulate bonded C	Semi-automatic laboratory analysis (integrated sample)	M*
c) Biological		
Biomass [mg DM · m^{-3}]	(+)	Chlorophyll in vivo fluorimetrically in the case of phytoplankton
	(+)	Electronic particle counts; particle size taken into account in the case of phyto- and zooplankton
	(+)	Aerial photography in the case of Macrophytobenthos
Routine: Still manual by microscope. Counts performed every 2–4 weeks; generation times sometimes only about a day.		
Productivity [mg DM · m^{-3} · d^{-1}]	(+)	by electronic O_2 measurement
	((+))	by electronic particle counts befor and after time interval (still in infancy)
Routinely still manual by ^{14}C marker experiments		

PIERSOL 1971, WARD *et al.* 1979). We have studied the distribution type of the total chlorophyll concentration, a parameter characterizing the phytoplankton biomass, in 100 subsamples of a mixed sample by means of the χ^2 test. This parameter was chosen because the conventional method of analyzing plankton samples after Utermöhl yields results that differ (overdispersion) considerably from the theory. Another parameter (individual numbers of *Filinia longiseta* EHRENBERG) was used to study statistical distributions in samples taken at a single locality (spot samples). And finally

we wish to present a few remarks regarding the methodological aspects of quantitative phytoplankton analysis by the Utermöhl method regarding in particular the choice of the dispersion medium and the elimination of overdispersion.

3. Material and Methods

The individual numbers of *Filinia longiseta* were calculated from 69 full samples of 750 ml each. The samples were taken on 6 June 1980 from the Zingster Strom (part of the Barther Bodden, a shallow, eutrophic, brackish landlocked water on the South Baltic coast–cf. JOST & NAUSCH 1980), filtered through a 56 µm mesh plankton net, transferred to a plankton counting chamber and counted completely with a microscope (ERGAVAL, VEB Carl Zeiss Jena, GDR) at a magnification of ×45. For the total chlorophyll concentration studies over 60 surface and deep samples (100 ml each) were taken on 8 October 1980 along a north-south transect through the Barther Bodden and mixed to form an integrated sample. One hundred subsamples of 3.5 ml each were then taken from the integrated sample and the chlorophyll concentration in each subsample was measured fluorometrically with the Unicam SP 800 (Unicam Instruments Ltd., Cambridge). The samples used for the phytoplankton counts were taken in the Arkona Sea (station in the central part of the Baltic Sea–cf. KELL 1973) in June 1980, fixated with Lugol's solution and counted in 50 ml chambers in an inverted microscope (WILD 40, Heerbrugg, Switzerland). The values for the total chlorophyll concentration and the individual numbers of *Filinia longiseta* were divided into classes, a reasonable class interval and reduction level respectively for the classes being found by trial and error. The types of distribution were checked by means of the χ^2 test (SOKAL & ROHLF 1969, CLAUSS & EBNER 1971) using the decision criteria of LIENERT (in CLAUSS & EBNER 1971). The tables used for $\varphi(u)$ and χ^2 are given in the annex in WEBER (1972).

Table 2.

2.1. Total chlorophyll estimation by fluorimetry (rel. units; integrated sample from Barther Bodden)

116.0	128.0	126.5	123.0	122.2	128.0	134.2
128.0	129.6	128.8	126.0	121.0	139.3	131.9
131.0	148.0	130.5	131.0	131.4	130.6	128.0
140.4	133.8	135.5	128.4	139.0	124.4	128.6
132.5	136.3	130.5	133.6	126.3	127.3	127.5
132.8	130.3	133.9	134.8	134.3	135.1	130.5
133.3	133.4	130.5	126.1	137.3	134.3	125.1
125.0	128.8	120.7	114.5	131.3	132.8	124.9
127.9	135.8	133.1	137.4	129.7	130.3	132.8
129.3	131.1	126.2	131.2	132.3	131.2	127.4
132.8	127.5	123.0	131.6	126.6	132.0	130.0
129.9	130.1	130.7	129.0	132.6	133.0	134.0
132.4	133.8	135.9	132.0	131.0	133.1	126.5
132.9	122.0	135.8	131.0	134.5	131.5	119.4
132.0	128.0					

2.2 *Filinia longiseta* (Ind. per 750 ml, spot samples, Zingster Strom)

318	280	256	223	201	200	196
192	189	189	179	176	163	162
161	151	151	147	147	146	145
144	140	133	132	129	129	128
127	126	123	118	118	117	103
103	103	102	101	98	97	94
92	90	89	87	87	84	80
75	75	74	74	72	70	65
64	62	60	59	55	54	53
41	39	39	25	19	10	

Table 3. Values from phytoplankton

3.1. Undiluted sample

Species	Repeats								
	1	2	3	4	5	6	7	8	9
Aphanizomenon flos-aquae	1019	967.5	1113.8	848	1515	1268	932.3	724.62	843.8
Chaetoceros spec.	227	159	236	226	210	134	192	169	252
Nodularia spumigena	43.56	57.15	55.92	42.42	37.65	35	100.7	39.54	51.39
Cerataulina pelagica	23	16	46	25	13.5	23.5	10.5	15.5	15
Anabaena flos-aquae	11.28	15.48	31.38	20.61	17.46	20.67	3	14.55	36.06
Achnanthes taeniata	10	27	19	31	11	9	8	–	15
Cylindrotheca closterium	6	8	6	15	22	6	11	18	26
Anabaena inaequalis	5.13	2.49	6.18	8.34	4.38	6.18	4.2	0.6	13.89

3.2. Sample diluted 1:5

Species	Repeats								
	1	2	3	4	5	6	7	8	9
Aphanizomenon flos-aquae	204	227	194	276	244	209	231	302	202
Chaetoceros spec.	44	51	48	35	48	44	56	51	41
Nodularia spumigena	11	9	14	12	11	17	6	5	12
Cerataulina pelagica	5	4	2	6	4	3	4	5	6
Anabaena flos-aquae	3	1	5	3	4	4	2	3	3
Achnanthes taeniata	7	6	5	5	8	4	3	6	6
Cylindrotheca closterium	2	3	3	2	5	1	–	6	5
Anabaena inaequalis	1	1	–	–	2	2	1	–	–

3.3 Sample diluted 1:15

Species	Repeats								
	1	2	3	4	5	6	7	8	9
Aphanizomenon flos-aquae	71	69	80	82	49	84	68	76	71
Chaetoceros spec.	12	16	14	10	17	15	19	21	23
Nodularia spumigena	4	3	6	4	5	4	3	4	7
Cerataulina pelagica	–	2	1	4	3	1	2	1	–
Anabaena flos-aquae	2	–	1	1	–	4	1	–	2
Achnanthes taeniata	4	3	2	2	–	2	4	3	3
Cylindrotheca closterium	–	–	2	1	–	1	3	–	–
Anabaena inaequalis	–	–	–	2	–	1	–	1	1

4. Results and Discussion

.4.1 The probability distributions of spot samples and subsamples from integrated samples

The results of our investigations are given in Tables 2 and 3 and Fig. 2 and 3. The figures show the "observed" and "expected" frequencies to permit comparison. As can be seen, it was assumed on the basis of LIENERT's decision criteria that in both cases distribution was normal.

counts (500 ml samples from Arkona Sea

10	11	12	13	14	15	16	17	18	$\bar{x}$	s	$\frac{s^2}{\bar{x}}$
839	1113.55	766.4	996	996.29	1157.87	1018.56	1100.3	1115.4	1018.6	190.2	35.5
253	263	230	204	193	221	225	180	160	207.4	36.3	6.3
38.1	30.45	26.46	62.49	64.8	52.29	62.79	40.8	51.75	49.6	17.1	5.9
22	27	20	5	20	49	22.5	12	17	21.3	11.1	5.8
18	9.27	14.07	16.5	36.87	22.41	9.87	12.6	6.9	17.6	9.4	5.0
74	41	33	46	24	11	15	17	24	24.4	17.1	11.9
12	16.5	9.5	23	13	19	34	16	19	15.6	7.6	3.7
3.72	1.14	7.47	9.66	13.68	8.25	7.08	5.67	12.75	6.7	3.9	2.3

10	11	12	13	14	15	16	17	18	$\bar{x}$	s	$\frac{s^2}{\bar{x}}$
184	235	245	216	199	201	301	264	212	230.3	35.6	5.5
36	47	50	42	43	47	49	34	53	45.5	6.2	0.84
12	13	19	21	11	9	10	21	12	12.5	4.5	1.6
4	5	8	3	2	4	7	3	8	4.6	1.8	0.72
4	5	7	7	4	4	2	8	5	4.1	1.8	0.83
5	4	7	4	6	5	5	8	4	5.4	1.4	0.37
3	3	1	4	4	2	3	3	5	3.0	1.6	0.83
–	–	2	–	3	2	1	–	1	1.0	1.0	1.0

10	11	12	13	14	15	16	17	18	$\bar{x}$	s	$\frac{s^2}{\bar{x}}$
59	77	71	79	51	64	78	82	76	71.5	10.2	1.46
18	21	11	24	20	16	27	12	13	17.2	4.2	1.36
3	4	5	2	5	1	4	4	2	3.9	1.45	0.54
–	2	1	2	4	–	–	2	2	1.5	1.34	1.20
1	–	1	3	2	4	–	–	2	1.2	1.15	1.13
3	–	–	1	1	2	–	2	1	1.8	1.42	1.11
4	1	1	2	1	1	–	–	–	0.9	1.16	1.43
–	1	2	–	–	–	2	–	1	0.6	0.78	0.99

Calculation of the confidence interval for the chlorophyll estimation yielded a very promising result: the sample size tables in RASCH *et al.* (1978), page 480, showed that on account of our a priori information (standard deviation $s = 4.94$ relative units) and the specified accuracy $\alpha = 0.05$; $\beta = 0.1$ a sample size of $n = 3$ is sufficient. With the help of random number tables (WEBER 1972) we selected $n = 3$ values [130.0; 133.8; 135.8] from among the 100 chlorophyll concentration values and calculated the 95% confidence interval [$125.8 < \bar{x} = 133.2 < 142.5$]. In terms of both effort ($n = 3$) and accuracy (width of the confidence interval $< \pm 10$ % of the mean) this result is very

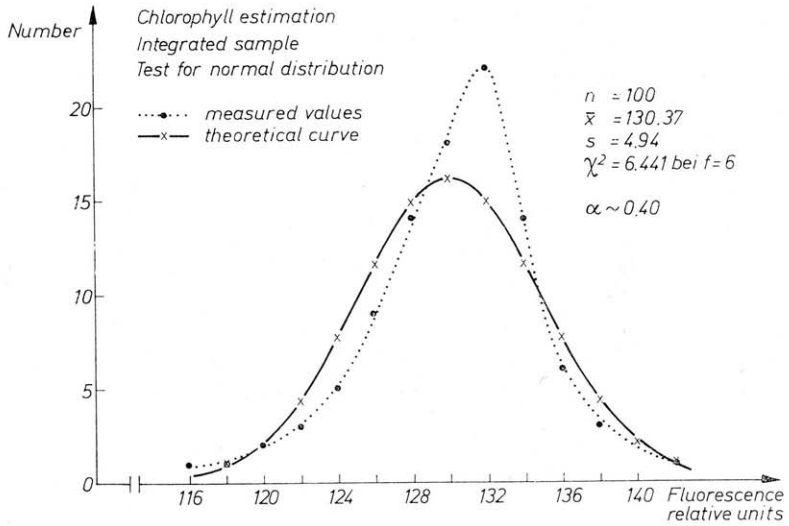

Figure 2. Chlorophyll estimation using an integrated sample and test for normal distribution; the null hypothesis (distribution is normal) is accepted. (Instead "bei" read "with".)

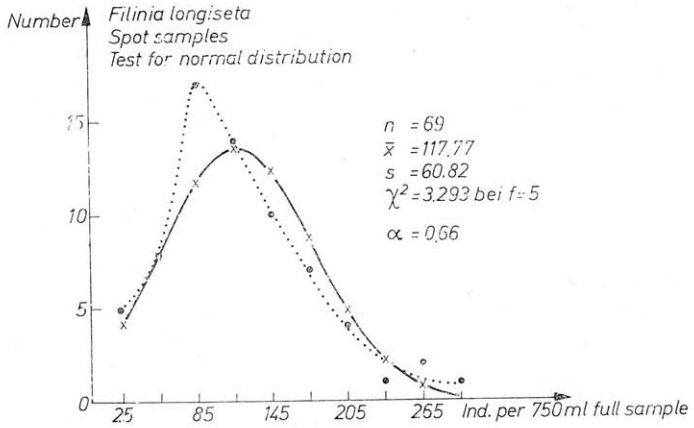

Figure 3. Abundance of *Filinia longiseta* as calculated from single spot samples and test for normal distribution; the null hypothesis (distribution is normal) is accepted. (Instead "bei" read "with".)

good. The reader is advised to compare it with the result given by NASEV, NASEV & GUIARD (1978)!

Attention must be drawn to one point, however. The taking of samples from an inhomogeneously distributed universe is an extremely critical step in making up integrated samples. Our samples were taken at different localities and from different depths along a transect leading straight across the water being investigated (cf. Part 2). Certain rules (BOTTRELL *et al.* 1976, VENRICK 1978) must be observed during this operation, and in particular it must be remembered that the sampling frequency must increase as the variability of the parameter being investigated increases.

Not unexpectedly, the data yielded by the *Filinia* samples had a high standard deviation ($s = 60.82$). The values for five samples [179; 101; 200; 103; 53) were selected with the help of random number tables und used to calculate the 95% confidence

interval [51.67 < $\bar{x}$ = 127.20 < 202.73]. This result must be considered unsatisfactory (values of the 95 % confidence interval = ca. ±60 % of the mean value) despite the larger number of samples.

4.2. Methodological problems involved in phytoplankton counts

In contrast to the phytoplankton biomass results yielded by fluorimetry (good agreement between parallel samples) the phytoplankton counts performed with the inverted microscope (Utermöhl method) sometimes differed considerably from the mean. This does not, however, throw doubt on the usefulness of the method as a whole: although it is possible to convert the values obtained by biomass counts directly into biomass equivalents, such conversions based on fluorimetrically measured chlorophyll concentrations can involve considerable errors. In the latter case the conversion of chlorophyll concentration into biomass equivalents may even be completely impossible because the chlorophyll concentration depends on phytoplankton group or species, light and nutrient conditions and the state of development of the algal cells.

Careful scrutiny of the phytoplankton values obtained by counts and shown in Table 3 reveals that the property of overdispersion is exhibited particularly by the relatively common species that have a major effect on production. This indicates the algal cells, if present in large numbers do not sink randomly and independently of each other to the bottom of the chamber but mutually affect each other; the distribution is clumped or contagious. It is this property that prevents the Poisson distribution from being applied to such material, and this must be considered a major drawback as far as analysis of the results is concerned.

The dispersion agent (Lugol's solution) obviously does not neutralize the charges of the algal cells sufficiently to prevent mutual attraction in the case of species exhibiting overdispersion such as *Aphanizomenon flos-aquae* (Sokal & Rohlf 1969). Red blood corpuscles, unless counted in Haym's solution, also mutually affect each other (bill-roll or rouleau effect) and the samples exhibit high variances and overdispersion. And the following comparison shows that phytoplankton counts have by no means reached their possible limits:

Baltic Sea, mean phytoplankton density $6 \cdot 10^6$ cells/ml (Poisson, sometimes overdispersion)
Boddens, mean phytoplankton density $120 \cdot 10^6$ cells/ml (overdispersion)
Blood, red corpuscles $5{,}000 \cdot 10^6$ cells/ml (Poisson)
(size of blood cells: $8 \times 8 \times 2$ µm)

In view of these figures we would suggest that improvement of the Utermöhl method for analyzing phytoplankton samples is more a question of biomethodology than a statistical problem.

5. Conclusions

The mixing and analysis of integrated samples seem to be the best of the currently possible procedures for taking and analyzing samples for the investigation of biotic and abiotic components by routine measuring programmes performed either to monitor the condition of the water or for the ecosystem analysis.

Integrated samples yield mean values with relatively small confidence intervals for samples of a relatively small size. They are easy to analyze statistically because the parameters have normal distributions, and their accuracy is completely adequate

for ecosystem analysis. The sampling of water at a single location at intervals of about four weeks, as is still quite common in connection with water management, must lead to means that are statistically very unreliable.

The analysis of phytoplankton samples by the Utermöhl method is a problem that has yet to be solved. It would appear advisable in this respect to seek fixatives which, like Haym's solution in the case of red corpuscles, will eliminate the phenomenon of overdispersion so that statistical analysis can be performed on the basis of a single distribution type (Poisson distribution) in this case, too. Under current conditions it seems advisable to measure the chlorophyll concentration (often) and count the phytoplankton by the Utermöhl method (less often) (cf. HALLEGRAEFF 1977, VENRICK 1978, p. 12, WILLEN & WILLEN, 1978, p. 300) in parallel.

6. Summary

The variability in time and space of ecosystem parameters and the considerable effort that is often involved in their quantitative estimation frequently make it difficult to obtain statistically reliable means for many ecosystem parameters. The application of automatic and semi-automatic measuring techniques and the analysis of integrated samples appear to be a suitable solution that is currently available. Integrated samples have a smaller variance than single samples for the same mean value, so that the size of the sample to be analyzed statistically can be reduced. The means yielded by integrated samples are completely adequate for many of the purposes involved in ecosystem analysis and the monitoring of waters. An integrated sample is used as an example (total chlorophyll concentration measured fluorimetrically) to show that the mean value with a small confidence interval can be obtained with only a small number of samples ($n=3$); in the case of single spot samples (numbers of *Filinia* per unit volume) the confidence interval was much broader despite the larger number of samples.

The considerations presented here do not hold for special investigation programmes (for instance sociological studies). Such experiments must be designed differently.

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