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Growth and Dissipation of Phytoplankton in Chesapeake Bay. II. A Statistical Analysis of Phytoplankton Standing Crops in the Rhode and West Rivers and an Adjacent Section of the Chesapeake Bay[1]

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ABSTRACT: It is possible to make statistically significant comparative measurements of similar sections of subestuaries under conditions where the large natural variations would mask all but drastic changes in the systems if they were studied individually. The comparative study is proposed as a modification to the baseline study of a single system for the assessment of the effects of man's activities in an estuary. We have made temporally coincident measurements of phytoplankton production, standing crops and a range of physical and chemical parameters in comparable sections of the Rhode and West rivers and in an adjacent section of the Chesapeake Bay for the 3-year period 1970–1972. We analyzed the data for standing crops and demonstrated that at least at the trophic level of phytoplankton, the judicious application of a paired comparative sampling protocol to the Rhode and West rivers is superior to a study of either system alone. We calculate that the paired comparison sampling protocol requires approximately one tenth the sample size of the single-system sampling technique to achieve the same significance level.

Introduction

The ultimate goal of many estuarine studies is to identify relationships among the factors affecting the viability of the aquatic biota, particularly those species of economic, recreational or aesthetic importance, in order to furnish a basis for most efficient utilization of the system. Suggestions can be made relative to the management of a portion of an ecosystem, i.e., whether or not to direct a chlorinated waste water discharge directly into a spawning area, or to place a cooling water intake in a nursery area. Very often this general knowledge of life-cycle relationships and psysiology can provide the proper advice and so avoid catastrophic consequences. However, where the cause and effect relationship is not so evident or when there is a set of complex trophic level interactions, a predictive model does not yet exist. This is due in part to the complexity of the life cycles of the predators of major importance (shellfish, finfish, crabs), in part to the complexity of the trophic interactions among all of the pelagic and benthic species, and in part to the large experimental variances of the natural systems, daily, seasonal and annual.

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A major concern of our research program has been to study the natural phytoplankton community in a subestuary. We assume that the quantitative relationships among nutrients and nutrient turnover, salinity, temperature, turbidity, species selection and succession, predation and exchange with the bay can be determined. From these quantitative relationships it follows that specific parameters will emerge which can serve as diagnostic indicators of the physiological state and of the previous history and permit the prognosis of the stability of the phytoplankton community. These relationships should permit the prediction of the direction of changes in the community in response to proposed nutrient, sediment or heat loading.

In the study of any natural system the experimenter may remove samples for study in the laboratory under controlled conditions. However, the natural system, with diverse community population is, at any time, the integral of all of the aperiodic climatic, biotic and chemical interactions that have occurred. Thus, experimental reproducibility in the natural system is very difficult to achieve. We are immediately faced with the problem of how to make statistically significant measurements in this variable system.

We have applied the following line of reasoning: Consider any given natural system on which measurements are to be made. The total measured variance will be composed of the variance associated with the "treatment" or the man-introduced stress whose effect it is desired to assess and the large natural variation of the system due to daily, seasonal and annual fluctuations in wind, tide, sunlight, rainfall, etc. In principle therefore a "before" and "after" baseline study of a single system will be subject to both of these sources of uncertainty and only "treatments" which produce sufficiently large mean differences (before minus after) can be assessed with any degree of statistical significance. A further complication exists because statistical parameters such as S. D., tests such as Chi Square, Student's t, F variance ratio, Chauvenet's Criterion and levels of significance have implicit in them the assumption that the data are normally distributed about their mean value. How then are we to assign levels of significance to differences in time averages of these quantities from one season to another or from one year to the next? This latter assignment is at the heart of the baseline study. It should be possible to choose a second system which is comparable (similar) to the first in its response to the natural fluctuations and differs from the first in the absence of the particular "treatment". Under these conditions it should be possible to analyze differences between the two systems and to remove the large natural variations from the statistical analysis. By virtue of the comparability of the systems, the expected value of the mean of the differences, properly normalized, should be zero. Non-homogeneities within the individual systems and their varying responses to localized meteorological changes in addition to measurement error will give rise to a normally distributed spread of difference values which is amenable to statistical analysis. The trick is to work with comparable systems. This is what we have done in the present paper. Our sampling protocol has included temporally coincident (1–2 hours) measurements of comparable sections of the Rhode and West rivers and of an adjacent section of the Chesapeake Bay on approximately a weekly basis for a three year period, 1970–1972. We have asked the following questions: a) Are the mean differences of phytoplankton parameters measured in the Rhode and West rivers statistically significant (95% level of significance, Student's t)? b) How might it be possible to relate such statistically significant differences to man's activities in both systems? c) Might the technique be applied to follow more precisely an annual or seasonal trend in the change of one system with respect to another? d) By how much must the parameters in either of these subestuaries change, presumably as the result of a hypothetical perturbation to one system, in order that the mean differences may be considered statistically significant (95% level of significance, Student's t)? We have analyzed the data both by the comparative technique and by the baseline technique.

Description of the Area

The Rhode and West rivers are small tributary estuaries with a common mouth, which enter the western Chesapeake Bay approximately 5 miles south of Annapolis and the Severn River. The Rhode and West river transects (1 and 2) and the adjacent bay transect (3) are shown in Fig. 1 and have been referred to previously (Loftus et al. 1972; Seliger 1972). Table 1 shows the volume and surface area data at mean low water for the Rhode and West rivers, abstracted from Pritchard and Han (1972). The sections RR1, 2, 3, WR1, 2, 3 referred to in Table 1 and shown in Fig. 1 correspond to those used by Pritchard and Han (pers. commun.) in their preliminary model for exchange rate constants for waters in these subestuaries.

From inspection, and on the basis of salinity transects and phytoplankton sampled in the Rhode and West rivers and in the Chesapeake Bay, we decided that transects 1 and 2 represented approximately equivalent sections of each river, and that transect 3 was representative of the main portion of the Chesapeake Bay adjacent to these rivers.

There were several decided advantages accruing to us by virtue of our location at the Rhode River. 1) Approximately 2500 acres of the watershed surrounding Muddy Creek, the main tributary creek of Rhode River, are conserved by the Smithsonian Institution as the Chesapeake Bay Center for Environmental Studies (CBCES). This consideration together with a relatively low population density in the Rhode River watershed made the Rhode River the least disturbed subestuary on the western shore of the Chesapeake Bay. 2) The phytoplankton study reported here is a part of an interdisciplinary research program on the entire Rhode River watershed-estuarine system (Anon. 1973:Vol. IV). 3) The Rhode and West rivers form a common mouth emptying into the bay. They have similar

TABLE 1. Volumes and surface areas of Rhode and West rivers at mean low water.

Segment	Pritchard and Han Section	Surface Area ($10^6 m^2$)	Volume ($10^6 m^3$)	Mean Depth (m)
	Rhode River			
Cadle Creek	C	0.20	0.29	1.5
	RR1	1.65	3.78	2.29
Bear Neck Creek Whitemarsh Creek	B W	0.61	0.93	1.52
Sellman Creek	S	0.25	0.41	1.65
	RR2	2.38	5.09	2.13
Muddy Creek	RR3	0.81	0.97	1.20
Total Rhode River		5.9	11.47	1.94
	West River			
Cheston Creek Scaffold Creek Popham Creek	PSC	1.06	1.06	1.0
	WR1	2.90	4.25	1.47
Cox Creek Tarthouse Creek	CT	0.92	1.12	1.22
	WR2	2.10	3.28	1.56
Learch Creek Smith Creek Johns Creek South Creek	WR3	2.76	3.37	1.22
Total West River		9.74	13.08	1.34

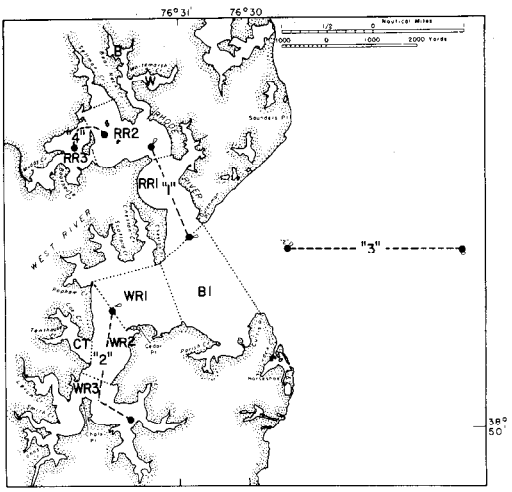

Fig. 1. Chart of Rhode and West rivers showing transects 1, 2, 3 and 4 as thick dashed lines. The sections designated by Pritchard and Han (1972) in their model for exchange rate constants are delineated by the dotted lines.

volumes and essentially form a common system to which nutrients and plankton are delivered by the adjacent bay as the result of exchange due to tidal and density flows.

The bay waters entering these rivers are modified as a result of the physical characteristics of these shallow basins, the nature of the bottom sediments, the delivery of nutrients in fresh water runoff from the uplands, the tidal flushing of the marshes, and the additional effluents due to the human population of the watershed and shorelines. The Rhode and West rivers together with their watersheds are comparable systems, dominated by the exchange with bay water. We have not yet progressed to the stage of introducing our own experimental "treatments". We therefore limit the scope of the present paper to asking whether any significant differences among the transects might be explained by any of the factors listed above.

The Rhode River is a special case of an estuary with two-layer flow; strong vertical mixing (Bowden 1967) due to tidal currents and wind gives rise to vertical and lateral homogeneity. The vertical salinity profiles show monotonic increases from top to close-to-bottom of *ca.* 0.1 to 0.3o/oo with increases in the bottom 0.5–1 m of 0.5–1o/oo. There is a very small gradient of surface water salinity between the mouth of the river and the mouth of Muddy Creek except following a period of heavy rainfall in the watershed. The upper limit of the Rhode River subestuary, where the salinity approaches 0.1o/oo and the chlorinity:total dissolved solids approaches 1:10 to 1:20 (Pritchard 1967a, b), extends a significant distance up into Muddy Creek, the major tributary creek. This is the case for all of the tributary creeks of the Rhode and West rivers.

Because of the small volume of the tidal section of Rhode River, rainfall produces relatively large excursions in the upper limit of the estuary. The tidal section of the subestuary encompasses essentially the remainder of Muddy Creek. There is a negligible "river section" associated with the Rhode River. The land runoff into Rhode River consists of drainage directly into the tidal section of the subestuary. Winds play an important part in maintaining the well-mixed essentially isohaline character of this shallow subestuary. Under proper conditions, a strong northwest wind will rapidly exchange the estuary section of the Rhode River and its plankton populations with the bay.

The delivery of nutrients to the estuary sections of the Rhode and West rivers is the result of tidal action (flushing of the marshes, remixing of soluble nutrients from interstitial water, and resuspension of interstitial sediments) and exchange with the Chesapeake Bay across the mouths of the rivers. However, subsequent to heavy rains the transition zone is subject to major changes in phytoplankton relative species compositions, coinciding with large increases in standing crops of chlorophyll *a*.

Methodology

SAMPLING PROGRAM AND PARAMETERS

The parameters measured in our sampling program are listed in Table 2 together with the techniques used for each measurement, the literature references and the coefficients of variation (C. V.) or the experimental standard deviations (S. D.). Subsequent to July, 1972, when it was desired to exclude small dinoflagellates such as *Prorocentrum minimum* and *Exuviella* sp. from

the "nannoplankton" filtrate, 10 micron netting was used for nannoplankton filtration. We have found that 20 micron netting is sufficient to remove all predators including tintinnids, rotifers, copepod nauplii and veliger larvae when it is desired to examine short-term effects of additions of specific nutrients on rates of primary production. Just prior to Hurricane Agnes (June 21, 1972) a fourth transect was established at the mouth of Muddy Creek in Rhode River (Fig. 1).

AVERAGE OF INTEGRATED TRANSECTS

Water samples were delivered to 5-gallon translucent polyethylene carboys on deck by a peristaltic pump. As the boat proceeded (approx. 2 knots) at constant speed along a transect a vertical tube was raised and lowered at a uniform rate between the surface and the depth of disappearance of a Secchi disc. The water sample collected was designated as the whole integrated or "A" sample.

Early in 1972, continuous *in vivo* chlorophyll fluorescence records were made at 0.5 m and at 1.5 m depths along the regular transects. We were able to demonstrate that the upward and downward motion of the boat with the hose input fixed relative to the boat was equivalent to the manual raising and lowering of the sampling tube. Samples were therefore collected at a fixed "still-water" depth of 0.5 m. The addition of the *in vivo* chlorophyll fluorescence continuous chart records to the sampling protocol provided the further advantage of following the occasional patchiness of the surface waters caused by the differential phototactic migration of the larger dinoflagellate species.

EXPERIMENTAL STANDARD DEVIATIONS

The coefficients of variation and the standard deviations shown in Table 2 are the combined uncertainties due to instrumental variance and the variance of repetitive field sampling. For example, in the case of the determination of dissolved inorganic carbon concentrations the C. V. of repetitive analyses of a single sample was 4%. However the C. V. using field samples was found to be 6%, implying an additional sampling uncertainty of 4%. It is always the combined experimental S. D. or C. V. which is reported.

PHYTOPLANKTON SPECIES IDENTIFICATION

We have been able to identify the larger dinoflagellate species found in this area of the Chesapeake Bay. However since the major phytoplankton biomass, chlorophyll pigments and primary production in Chesapeake Bay are due to phytoplankton which can pass through a 20 micron net (Seliger 1972; Loftus et al. 1972; McCarthy et al. 1974) and whose classification has not been determined, we have used this crude size filtration and extractable chlorophyll pigments to delineate the nannoplankton.

In Vivo FLUORESCENCE

The technique used and its limitations are described in Loftus et al. (1972).

DATA HANDLING

The storage, retrieval, treatment and plotting of data were made compatible with the Hewlett Packard 9800 Series Programmable Calculator. The yearly data were stored on magnetic cards in pairs consisting of the day of the year and the measured or calculated value of the parameter.

Programs were written to operate on as well as to print out the data or to plot the results on the 9800 Series Plotter, either for individual years or for a multiple year interval. The abscissa is the day of the year.

There are irreducible variations between our so-called comparable sections of the rivers due to short-term differential effects of local climate. One would therefore expect to observe a high frequency ($t \simeq$ days) jitter superimposed on the seasonal variations of parameters. We integrated this high frequency component by means of a smoothing program for drawing lines in the plotted data. A smoothed curve between data ordinate i, corresponding for example to day 120 and data ordinate i + 1, corresponding to day 127 is actually a line drawn between the ordinate

$$\tfrac{1}{4}\,[(i - 1) + 2i + (i + 1)]$$

for day 120 and the ordinate

$$\tfrac{1}{4}\,[i + 2(i + 1) + (i + 2)]$$

TABLE 2. Parameters in sampling program.

Parameter	Measurement	Method of Measurement	Units	Reference	C. V. or S. D.
00	Turbidity	Secchi disc disappearance	m^{-1}		10%
01	Salinity	Beckman induction salinometer	o/oo		0.2 o/oo
02	Temperature	Thermistor probe of salinometer	°C		0.2°C
03	Dissolved oxygen	Yellow Springs Model 51 O_2 probe	mg m^{-3}		5%
04	Inorganic carbon	Beckman carbon analyzer	mg m^{-3}		6%
04	Inorganic carbon	Alkalinity titration	mg m^{-3}		6%
05	Dissolved NO_2^-	Optical density (543 nm)	μg atom $liter^{-1}$	Benshneider and Robinson 1952	5%
06	Dissolved NO_3^-	Cd reduction, optical density (543 nm)	μg atom $liter^{-1}$	Morris and Riley 1963	7%
07	Dissolved NH_4^+	Optical density (640 nm)	μg atom $liter^{-1}$	Solórzano 1969	10%
08	Dissolved PO_4^{---}	Optical density (883 nm)	μg atom $liter^{-1}$	Murphy and Riley 1962	4%
09	Total dissolved N	Ultraviolet oxidation to NO_3	μg atom $liter^{-1}$	Strickland and Parsons 1968	
10	Total dissolved P	Ultraviolet oxidation to PO_4	μg atom $liter^{-1}$	Strickland and Parsons 1968	8%
11	Extractable chlorophyll *a*	90% Acetone extraction; fluorometry	μg $liter^{-1}$	Loftus and Carpenter 1971	7%
12	Extractable chlorophyll *b*	90% Acetone extraction; fluorometry	μg $liter^{-1}$	Loftus and Carpenter 1971	—
13	Extractable chlorophyll *c*	90% Acetone extraction; fluorometry	μg $liter^{-1}$	Loftus and Carpenter 1971	10%
14	Extractable pheophytin *a*	90% Acetone extraction; fluorometry	μg $liter^{-1}$	Loftus and Carpenter 1971	—
15	Rate of carbon uptake	^{14}C bicarbonate tracer technique	μgC $liter^{-1}$ hr^{-1}	Steemann Nielsen 1952	6%
16	Gross O_2 evolution	Winkler technique (modified)	μgC $liter^{-1}$ hr^{-1}	Carpenter 1965	
17	Rate of respiration	Winkler technique (modified)	μgC $liter^{-1}$ hr^{-1}	Carpenter 1965	20%
18	Stimulable bioluminescence	Mechanical stirrer	[10^8 photons]	Seliger and McElroy 1968; Biggley et al. 1969	
19	Rainfall at Rhode River	Rain gauge Σ over 5 day intervals	mm per 5 days		
20	Hour of day at high water slack				
21	Surface sunlight intensity during incubation	Eppley pyroheliometer (integral/hr) foot candle meter	Ly hr^{-1}		
22	pH	pH probe			
23	Chlorophyll *a* from *in vivo* fluorescence	Continuous flow fluorometer	μg $liter^{-1}$	Loftus et al. 1972; Lorenzen 1966	20%
30–39	Derived parameters				
30	Assimilation rates	(15)/(11)	mgC mg Chl a^{-1} hr^{-1}	Yentsch and Lee 1966	
31	Ratio Chl *c*/Chl *a*	(13)/(11)		Humphrey 1963	
32	Ratio Pheo *a*/Chl *a*	(14)/(11)		Beers and Stewart 1969	—
33	Dissolved inorganic N	Σ (05), (06), (07)	μg atom $liter^{-1}$		
34	Dissolved organic N	(09) – (33)	μg atom $liter^{-1}$		
35	Dissolved organic P	(10) – (08)	μg atom $liter^{-1}$		~12%
36	Ratio $\frac{\text{Chl } a < 20\,\mu}{\text{Chl } a \text{ whole sample}}$				

37	Water density σ_T	Data from (01), (02)		
40–49	Dinoflagellates	Microscopic identification, live and preserved	liter^{-1}	10%
50–59	Diatoms	Microscopic identification, live and preserved	liter^{-1}	10%
60–69	Nannoplankton	No suitable taxonomy	μg Chl a liter^{-1} $<20\,\mu$	10%
70–79	Non-resident species	Microscopic identification, live and preserved	liter^{-1}	
80–89	Zooplankton	Microscopic identification, live and preserved	liter^{-1}	

for day 127, where (i − 1) represents the parameter value for the week prior to day 120 and i + 2 represents the value for the week subsequent to day 127. For the end points (1, . . . n) the smooth ordinates are

$$\tfrac{1}{3}\,[2 \text{ datum } 1 + \text{datum } 2]$$

and

$$\tfrac{1}{3}\,[\text{datum } (n-1) + 2 \text{ datum } n]$$

respectively. Provision in the plotting program was made so that solid lines were not drawn between parameter values separated by more than two weeks. We drew dashed lines in these cases to extrapolate the trend and to indicate the absence of specific data. A smoothed plot consists of the actual data points through which the smoothed lines were drawn and is so described in the caption. In some cases, the lines were drawn through the data points directly.

Results and Discussion

PHYSICAL CHARACTERISTICS

The salinities and temperatures of surface waters in the Rhode River (transect 1) over the period 1969 through 1972 are shown in Figs. 2 and 3, respectively. Similar data were obtained for both West River (transect 2) and the Chesapeake Bay (transect 3) over the period 1970 through 1972. The data of Fig. 2 indicate the aperiodic component of the seasonal and annual salinity patterns in this area during the past four years. However, general features can be observed. There is a sharp spring drop, a more gradual rise beginning in May and reaching a maximum (in 1969 as high as 16°/$_{oo}$) around October. The curves of Fig. 2 indicate the increasingly larger spring flows of the Susquehanna in 1970, 1971 and 1972, resulting in successively deeper troughs. The effect of Hurricane Agnes, subsequent to June 21, 1972 is seen as a drop from approximately 7°/$_{oo}$ to 2°/$_{oo}$, followed by a recovery during July and August. A more intensive time sequence of the salinity just prior to Agnes and extending to November, 1972 is shown in Fig. 4 (A. Place, pers. commun.), for the mouth of Muddy Creek (transect 4, see Fig. 1).

The major troughs in Fig. 2 are the result of the delivery of spring runoff by the Sus-

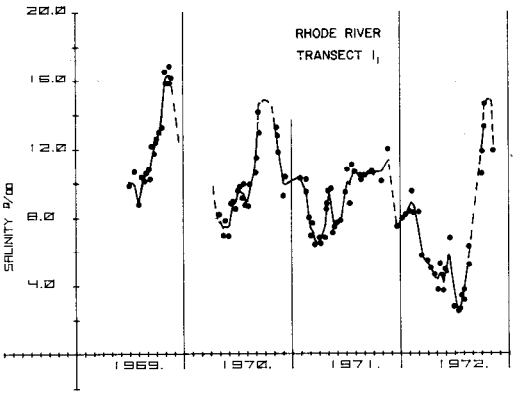

Fig. 2. Surface water salinities (0.1m) at the beginning of transect 1, designated by 1_1, in Rhode River for the period 1969 through 1972. The solid dots are true data points. The solid lines are smoothed curves as described in the text. The dashed lines are extrapolated and are shown between data points separated in time by more than 2 weeks.

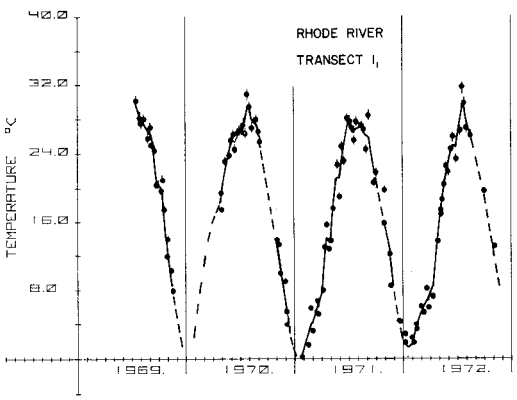

Fig. 3. Surface water temperatures (0.1m) at the beginning of transect 1, designated by 1_1, in Rhode River for the period 1969 through 1972. The solid dots are true data points. The small vertical bars represent the estimated standard error. The solid lines are smoothed curves as described in the text. The dashed lines are extrapolated and are drawn between data points separated in time by more than 2 weeks.

quehanna River. The smaller oscillations are the result of a combination of the above with the effects of local winds and rainfalls. We have referred to the effect that a strong northwest wind can have on the exchange of Rhode River water. As can be seen from Fig. 1, a northwest wind will not affect the West River in the same way. It is possible therefore that under certain local wind conditions, even though phytoplankton growth conditions in both rivers were optimal and would lead to high standing crops, one or the other would exchange its waters more rapidly with the bay and not reflect this increase. These local and temporary differences in exchange rates are part of the irreducible experimental variance when standing crops are measured by the paired comparison technique.

A second source of variance occurs because of the finite time required for the system to approach a new steady state as the result of a change in any part. Fig. 5 shows the rainfall in units of cm per 5 day interval at the CBCES. In this section and the following we shall use the abbreviations RR, WR and CB for Rhode River, West River and Chesapeake Bay, respectively. In February, 1972 there were heavy local rains in this area resulting in a marked lowering of the RR and WR surface water salinities in March. This can be seen by comparing Fig. 6, in which RR

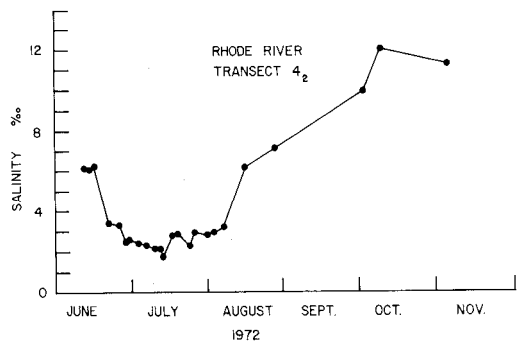

Fig. 4. Surface water salinities (0.1m) for transect 4 (mouth of Muddy Creek) prior, during and subsequent to Hurricane Agnes.

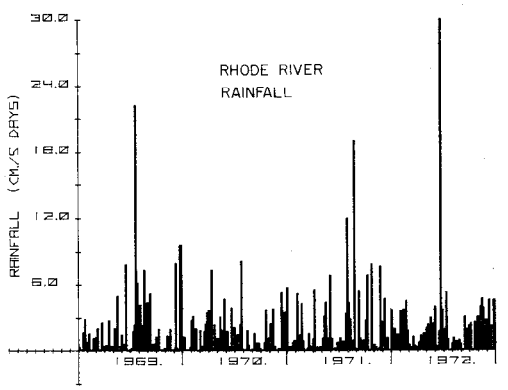

Fig. 5. Rainfall in Rhode River area over the time period 1969–1972 shown as sum over 5 day intervals (cm/5 days).

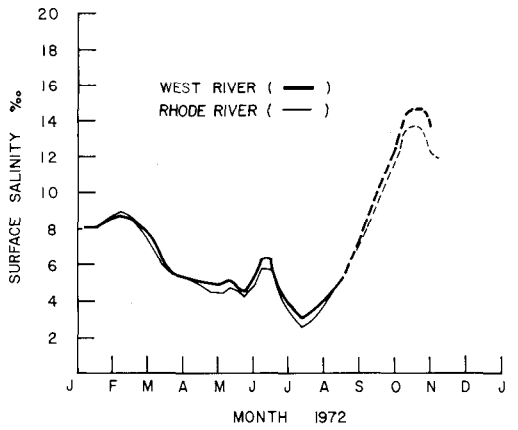

Fig. 6. Smoothed curves of surface water salinities of transect 1 in Rhode River (thin line) compared with those of transect 2 in West River (thick line), showing very close agreement.

surface water salinities for 1972 are superimposed upon the same parameter for WR with Fig. 7 where RR is compared with CB. In Fig. 6, the similarity between RR and WR salinities is quite evident, while in Fig. 7 differences between RR and CB occurred during March-April.

The means of the paired salinity difference measurements, $\overline{\Delta S}$(RR-WR), for 1970, 1971 and 1972 were $0.2^{o}/_{oo}$ (*; n = 20), $0.1^{o}/_{oo}$ (NS; n = 29) and $-0.2^{o}/_{oo}$ (*; n = 26), respectively, where the asterisk indicates a $P \leq 0.05$ level of significance, based on the Student's t test of means to be described in the section on standing crops of chlorophyll. The symbol NS indicates "not significant", a $P > 0.05$ level of significance. We ordinarily take $P \leq 0.05$ as the cutoff for assigning statistical significance. F variance ratio tests between the 3-year combined data and any single year were not significant. For the complete set of data, therefore, $\overline{\Delta S}(RR\text{-}WR)^{3\ years} = 0.02^{o}/_{oo}$ (NS; n = 75) with a standard deviation S. D. = $0.5^{o}/_{oo}$ and a standard error of the mean of $0.06^{o}/_{oo}$.

The large natural variations in surface water salinities in the Rhode and West rivers are temporally coincident in transects 1 and 2. Therefore these transects represent comparable salinity sections.

The temperatures in the subestuaries are more periodic than the salinities. From Fig. 3, except for the slight fluctuations, the surface water temperatures at any day x in the Rhode River can be fitted approximately by the relation

$$T = 28 \sin^2 \frac{\pi(x - 30)}{365} \qquad (1)$$

The means of the paired temperature difference measurements $\overline{\Delta T}$ (RR-WR) for 1970, 1971 and 1972 were 0.5 C (NS; n = 20), −0.1 C (NS; n = 30) and 0.1 C (NS; n = 25), respectively. The pooled 3-year data gave

$$\overline{\Delta T}\ (RR\text{-}WR)^{3\ years} = 0.1\ C\ (NS;\ n = 75)$$

with a standard deviation, S. D. = 0.8 C and a standard error of the mean, 0.09 C.

The data indicate that temporally coincident measurements of salinity and temperature in comparable sections of the two rivers show no differences or trends in salinity or temperature between the two rivers. The rivers are approximately the same depth and the exchange of both mesohaline sections with the bay is approximately the same. This has been confirmed recently by Pritchard and Han (pers. commun.) on the basis of a preliminary model of the sections shown in Fig. 1.

It would therefore be expected that the similar phytoplankton communities in the Rhode and West rivers would respond similarly to temperature and salinity changes.

Since the bay exerts the major influence on the rivers, salinity differences between the

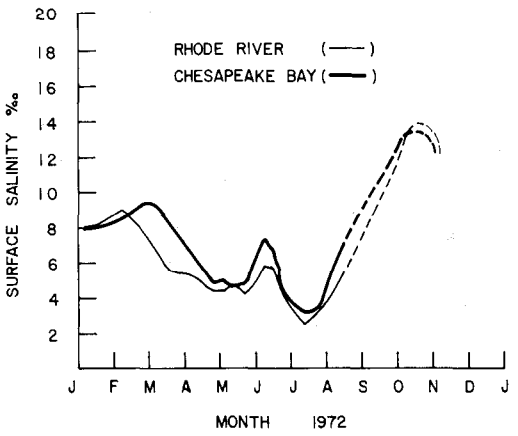

Fig. 7. Smoothed curves of surface water salinities of transect 1 in Rhode River (thin line) compared with those of transect 3 in Chesapeake Bay (thick line) showing the large spring dilution of the river water as the result of local rainfall.

surface waters of the rivers and the bay will largely reflect phase differences due to finite exchange times and only occasionally will be due to heavy local rainfalls. However the shallow and more protected rivers will heat up faster than the bay. As shown in Fig. 8, the river is significantly warmer than the bay for a major portion of the spring and summer. The temperature difference is relatively greater in the early spring. One might expect, therefore, that the differential effects of insolation would be manifested as an early spring increase in production rate in the rivers, leading to an earlier increase in the standing crop of chlorophyll relative to the bay. Fig. 9 shows smoothed curves for extractable chlorophyll *a* for both RR and CB for 1971. In 1971 we did not observe the "usual" spring pulse of production and standing crops in the bay which occurred in 1969, 1970, 1972 and very markedly in 1973. However, what we shall call the spring insolation increase in RR was very marked in February and March as shown in the figure.

The seasonal flow patterns of surface waters in the two rivers can be inferred from the summary data on the differences in surface water densities, σ_T, between RR and CB and between WR and CB, in Fig. 10.

These plots of $\Delta\sigma_T$ over 3 survey years show that from March to December the river surface waters tend to overlay the adjacent bay surface water on most survey dates.

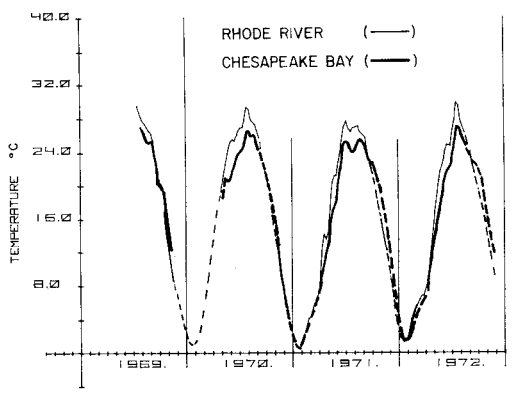

Fig. 8. Surface water temperatures at the beginning of transect 1 in Rhode River (thin line) compared with temperatures at the end of transect 3 in Chesapeake Bay (thick line) for the time period 1969–1972.

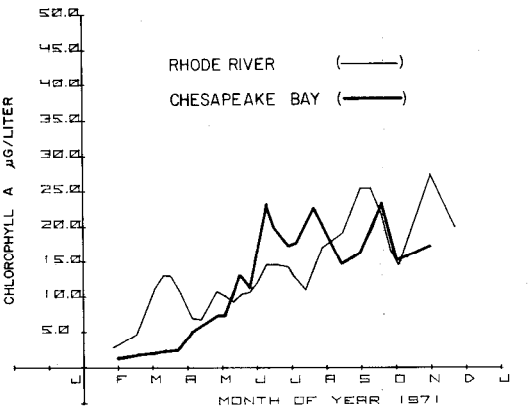

Fig. 9. Extractable chlorophyll *a* in transect 1 of Rhode River (thin line) as compared with the transect 3 of Chesapeake Bay (thick line) during 1971, showing a spring insolation increase in the river during February and March, 1971.

These surface density gradients imply a two-layer flow also evident in vertical profiles of temperature and salinity. Since similar patterns in $\Delta\sigma_T$ existed between the bay surface waters and surface waters of both rivers, both would receive phytoplankton inocula from the same common source, via transport of near-surface bay water to deeper layers in the rivers. During sunlight hours these surface waters may contain relatively higher concentrations of vertically-migrating, positively-phototactic dinoflagellate species such as *Gymnodinium nelsoni* and *Prorocentrum minimum*. In these cases, there will be an increased delivery of these dinoflagellates to the rivers relative to the smaller nannoplankton which do not migrate appreciably and are therefore more uniformly distributed in the water column.

The density discontinuity in the rivers occurs near 2 m depth at below 1% surface light. The nannoplankton delivered to the river at this depth must depend upon vertical mixing to reach optimum light levels for photosynthesis. Again the positively phototactic dinoflagellates would appear to have an advantage over the nannoplankton in being able to migrate to surface waters. Therefore density driven exchange between the bay and the rivers can promote the delivery and retention and even occasional dominance of the larger dinoflagellates in the rivers. The larger dinoflagellates are absent in the winter

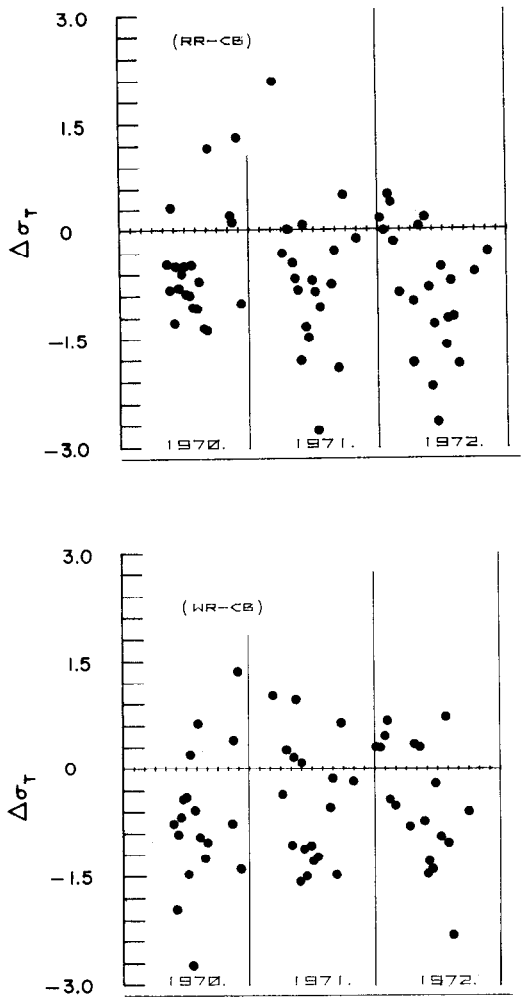

Fig. 10. Differences in surface water densities between Rhode River and Chesapeake Bay (top) and West River and Chesapeake Bay (bottom), suggesting a two-layer flow.

months when little or no density gradient exists.

LIGHT INTENSITIES

The photic zone in the subestuaries is quite limited in depth. The depth of visual disappearance of a Secchi disc, corresponding to two tenths[2] of the surface sunlight intensity, varies during the year between 0.5 m and 1.5 m. There is a general pattern of increases in absorption during spring and fall. Turbidities in Rhode River and West River showed a strong overlap and were consistently higher than in the bay. This may be the result of runoff or marsh flushing in which case the compositions of sediment and detrital suspensions could conceivably differ from those in the bay. Alternatively, the increased river turbidities could be due to the higher steady state resuspension of sediments by tidal action in the shallower river bottoms.

This estimation of absorption by Secchi disc is at best a crude one. Since the method is a visual one, it measures the relative absorption of a range of wavelengths for human photopic vision. The peak sensitivity for photopic vision, 555 nm, lies between the absorption peaks for chlorophyll and most of the blue-absorbing accessory pigments. One must therefore make the tenuous assumption that absorption coefficients do not change with depth or with sediment load. We have just begun a study of the spectral distributions of underwater sunlight in estuarine waters using a 4 π diffuser-detector in combination with a pressurized underwater spectrometer designed by W. G. Fastie (see Seliger and Fastie 1968). In Fig. 11 we show the relative spectral distribution of sunlight at the water surface and at a depth of 0.8 m corresponding on that day to the depth of disappearance of a Secchi disc. The surface sunlight intensity was 0.55 Langleys per minute. These studies will be presented separately.

The major significance of Fig. 11 is to demonstrate the marked contraction of the photic zone in the subestuary as compared with coastal and oceanic waters. There is also a significant distortion of the original sunlight spectrum. The mean path length for absorption (true absorption and scattering) of blue light changes from 33 m in the clearest ocean waters (Jerlov 1968) to 0.5 m in the Rhode River.

During the months of April through October light appears to be a limiting factor for phytoplankton growth in both rivers. Phytoplankton mixed in the water column spend a significant fraction of their daylight hours at light intensity below their photosynthesis saturation values. A decrease in sediment load should in principle result in an increase in

[2] The assumption is made that the 1% light intensity level is equal to three times the Secchi disc depth.

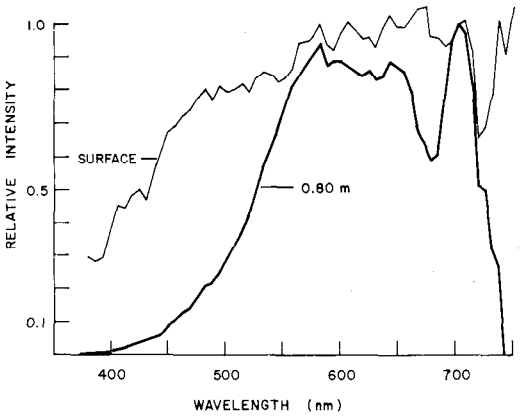

Fig. 11. Relative spectral photon intensities of sunlight incident on the surface (0.55 Langleys per minute) of the water and at a depth of 0.8 m in the Rhode River on 27 July 1972. The intensity data are normalized at 710 nm.

production in the water column. However, the relationships between nutrient delivery, which accompanies sediment delivery, and the direct and indirect effects of sediments (including detritus) in providing nutrients to the phytoplankton and zooplankton are not completely understood.

UNIFORMITY OF TRANSECTS

In general in all three transects, the surface waters which comprised the photic zone were reasonably well mixed, as evidenced by the uniformity of depth profiles of salinity. The nannoplankton were uniformly distributed throughout the photic zone and any minor taxis or flotation related to light or nutrients was insufficient to produce significant differential vertical distributions. Under these conditions grid sampling comparisons by extraction of chlorophyll pigments of surface water samples (0.5 m) and as functions of depth showed the same shallow horizontal negative gradient of phytoplankton concentrations along transect 1 and in fact all the way out to the end of transect 3 (see Fig. 1). This was verified in much more detail by continuous *in vivo* chlorophyll fluorescence sampling along these same transects.

At intervals throughout the year inocula of the larger dinoflagellates originating in the bay grow up in significant concentrations in the saline portions of the tributary creeks and drift as patches into the main river sections. We shall discuss in a subsequent paper the summer blooms of *Prorocentrum minimum* which occur in this area of the bay. This phenomenon occurred subsequent to Agnes during July and August, 1972. A subsequent blooming and dissipation of the dinoflagellate *Gymnodinium nelsoni* in Rhode River is shown in the *in vivo* chlorophyll fluorescence transect records of Fig. 12. The measurements were made at 0.5 m depth along the axis of the river, from the beginning of transect 4 straight through to the end of transect 1, a distance of 4.5 km. The axial stations on the abscissa refer to specific buoys or landmarks. Thus transect 4 comprises axial positions from 0 to 4; transect 1 comprises axial positions 6 through 10. It is important for continuous *in vivo* fluorescence measurements to parallel any plankton sampling program, in order to avert the large statistical variations possible in grab samples due to patchiness. The continuous, or integral sampling technique acts to average out the patchiness in the area.

STANDING CROPS OF PHYTOPLANKTON

The measured standing crops of total chlorophyll *a* in the Rhode River, West River, and Chesapeake Bay for the period 1969 through 1972 are shown by the solid lines in Fig. 13. In these graphs the solid lines have been drawn through the actual weekly data points. Each point represents an average value of total chlorophyll *a* liter^{-1} for a complete transect. Several points can be made:

1) Except for a trend toward high standing crops in summer and low standing crops in winter there existed no apparent reproducibility of standing crops of phytoplankton from one year to the next.

2) Despite the averaging involved in the individual sampling transects the weekly measurements of standing crops showed large oscillations. Variations in average phytoplankton concentrations can be introduced as the result of sampling at different phases of the tide, since there do exist gradients of phytoplankton concentrations along the transects. These variations are averaged out somewhat by virtue of the fact that the linear

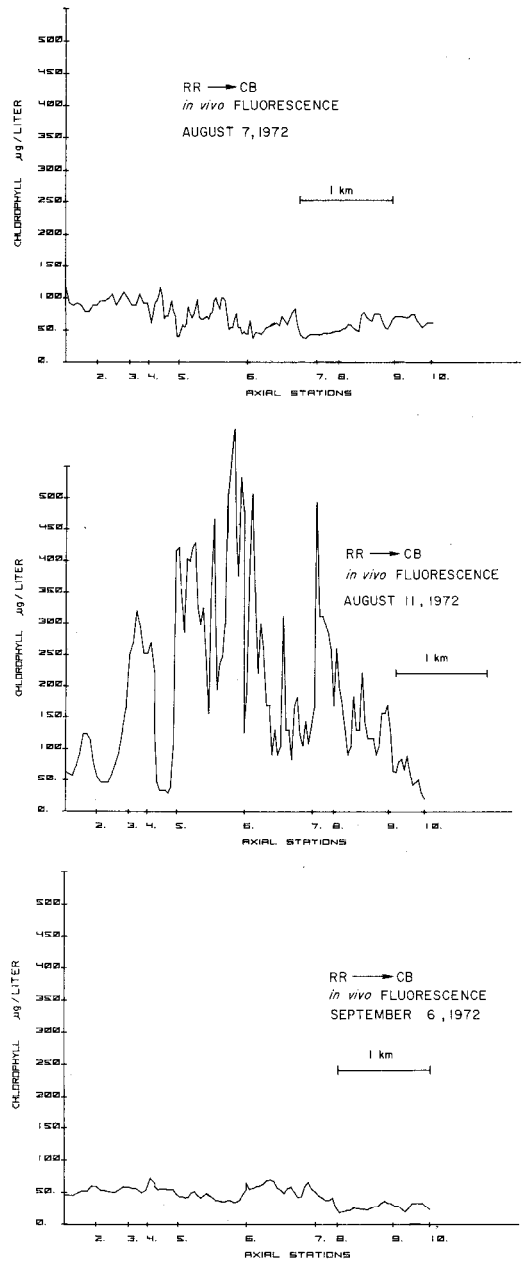

Fig. 12. *In vivo* fluorescence in units of extractable chlorophyll *a* (µg liter^{-1}) preceding (top), during (middle), and following (bottom) a bloom of *Gymnodinium nelsoni* in Rhode River. Recordings were made from samples drawn through fluorometer from 0.5m depth by means of a peristaltic pump. The axial distance from the origin to position 10 was 4.5 km.

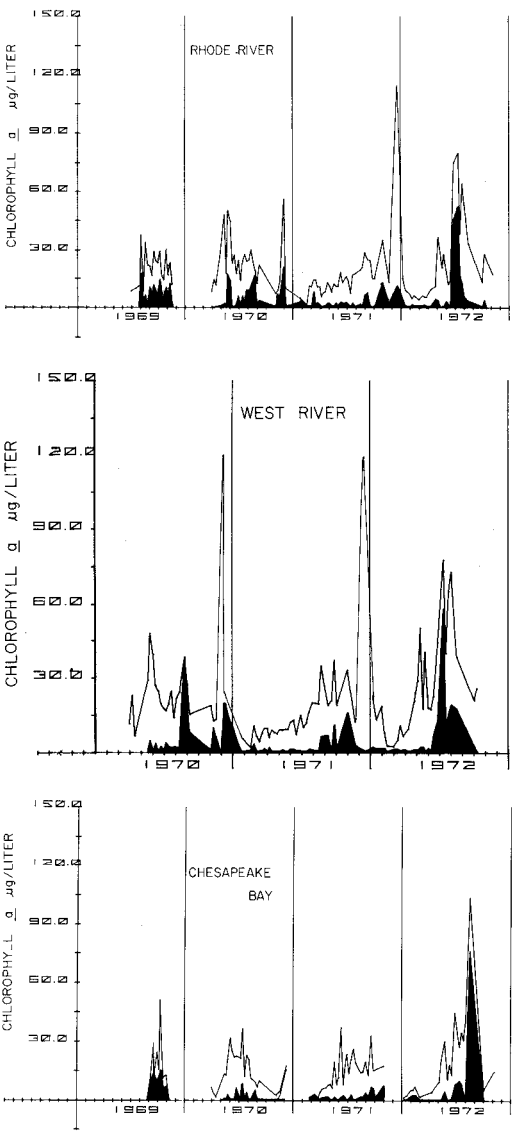

Fig. 13. Standing crops of total chlorophyll *a* (unshaded) and the chlorophyll *a* contained in a size fraction greater than 20 µ (darkened areas) for the time period 1969–1972 for Rhode River (top), West River (middle), and Chesapeake Bay (bottom). The blank area enclosed between the solid lines and the darkened areas represents the nannoplankton.

extent of each of the integrated transects in the rivers was more than twice the tidal excursion. In the determination of a hydrographic model of conservative quantities such as salinity, it is reasonable and necessary to make measurements at the same arbitrary phase of the tide. However the phases of sunlight intensities, heating and turbulent mixing due to insolation and wind mixing are solar phases. In phytoplankton sampling one must choose between sampling at the same solar time, and sampling at the same lunar

time. We chose the former since sunlight is the primary source for free energy and regulation in phytoplankton communities.

3) The solid black portions in the figures show the contributions to the total chlorophyll *a* pigments in the surface waters, of dinoflagellates greater than 20 μ in cross sectional linear dimension. As can be seen, the major contribution to chlorophyll pigments is due to phytoplankton which pass through a 20 μ mesh net (Seliger 1972; Loftus et al. 1972; McCarthy et al. 1974). In July and August, 1972, significant fractions of rapidly growing *G. nelsoni* were able to pass through a 20 μ net and in these cases a 10 μ mesh size was used for filtration. The difference in timing between the appearance of large dinoflagellate standing crops in RR and WR, and the later appearance in CB, can be seen by examining the solid black areas of Fig. 15 for July through September, 1972. In the rivers the dinoflagellate blooms began to grow immediately following Agnes while in the bay the blooming did not occur until August, when the river standing crops were past their peak.

A pattern, if any, which might be developed from these blacked areas is a minor appearance of larger dinoflagellates in summer and a significant growth in the fall. The relationship of these dinoflagellate successions and nutrients will be discussed in a later paper.

Despite the rather chaotic appearance of the 3-year chlorophyll *a* standing crop data it has been possible to obtain some valid statistical analyses by virtue of the temporal coincidence of the measurements. Owing to the residence times of the phytoplankton in the rivers and the increased nutrient availability we might expect that even in the presence of exchange with the bay, the steady state standing crops of phytoplankton, and consequent chlorophyll *a* concentrations, would be higher in the rivers than in the bay (RR − CB > 0). The same line of reasoning applied to the RR − WR differences would predict a zero average difference provided that a) the exchange rates relative to total volume in each river section were the same, b) local effects such as wind differences, creek contributions, depth differences, etc. tended to average out, and c) the chemical inputs and nutrient delivery from the marshes including all of man's activities in both river sections were the same.

The reversal of the trend of RR − CB > 0 occurs during May–June when *Prorocentrum* blooms originate in the bay north of Rhode River (Seliger 1972). In our statistical analysis we have divided the time periods of the RR − WR comparison into winter (day 1–90) spring-summer (day 91–240) and fall (day 241–365). The division corresponds to the general trend in Fig. 13 of winter low, spring-summer rise and peak and fall decline. In the analysis of the RR − CB data we have separated the times of the *Prorocentrum* blooms (day 151–200) from the rest of the data and therefore have winter (day 1–90), spring-summer minus the Bloom Period (day 91–150; 201–240), Bloom (day 151–200), and fall (day 241–365).

We asked whether, during any of the time periods, the differences between RR and WR and between RR and CB were significantly different from zero. When there are two sets of measurements x_i (Rhode River) and y_i (West River) we may ask whether $\bar{x}$ is significantly different from $\bar{y}$. In this case the Student's t is defined as

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{S_x^2}{n} + \frac{S_y^2}{m}}} \qquad (2)$$

where S_x and S_y are the respective standard deviations of the measurements $(x_1, x_2 \ldots x_n)$ and $(y_1, y_2 \ldots y_m)$. As can be seen from Fig. 13 the large variations in x_i and y_i throughout the year as well as from one week to the next will give rise to large values of S_x and S_y. This in turn will require the numerator of equation (2) to be large in order to be statistically significant. The random method of analysis of variance therefore tends to mask true differences which may exist between systems x and y (see Sokal and Rohlf, 1969: 328).

By the expedient of making essentially simultaneous measurements of x_i and y_i we can ask whether

$$\bar{z} = \frac{1}{n} \sum_{1}^{n} (x_z - y_z) \qquad (3)$$

is significantly different from zero. In this case the Student's t is defined (Sokal and Rohlf, 1969) as

$$t = \frac{\bar{z} - \mu}{S_{\bar{z}}} \quad (4)$$

where μ is the parametric mean of the population with which we are concerned and $S_{\bar{z}}$ is the standard deviation of the mean of the difference measurements. The null hypothesis asks whether the data z_i belong to the population (H_0:$\mu = 0$).

There are two sources of variance which contribute to the total variance S_z^2,

$$S_z^2 = \frac{1}{n-1}\left[\sum z_i^2 - \frac{1}{n}(\sum z_i)^2\right] \quad (5)$$

The first can be defined as the result of a large number of repetitive measurements made on x and y separately. The *a priori* variance of z_i, assuming only measurement error, is

$$S_\Delta^2 = \sigma_x^2 + \sigma_y^2 \simeq 2\,\sigma_x^2 \quad (6)$$

where the σ's are used to indicate the standard deviations of repetitive measurements as we have discussed above. The second experimental variance is due to the patchiness of the systems, caused by local variations in mixing and in exchange rates caused by the wind, blooms of organisms developing in some creeks and not in others, local rainfalls, etc. The data on standing crops of chlorophyll *a* in RR, WR and CB were analyzed in the following ways: To conform with equation (3) we let $z_i = RR_i - WR_i$ in one analysis and $z_j = RR_j - CB_j$ in the second. In order to give equal relative weight to periods of the year when standing crops were low we also analyzed the relative differences z_i-$(RR_i - WR_i)/(RR_i)$ and $z_j = (RR_j - CB_j)/(RR_j)$.

The summary of the paired variate statistical analyses of the chlorophyll *a* standing crop data for 1970, 1971 and 1972 is given in Table 3 for both the relative differences (RR − WR)/RR, (RR − CB)/(RR) and the absolute differences RR − WR, RR − CB. The years have been subdivided into winter, spring-summer and fall periods in an attempt to detect seasonal patterns.

There are several results from Table 3 which can be summarized.

a) The standing crops of chlorophyll *a* in West River appear to be consistently higher than those in Rhode River. However only in spring-summer, 1972 did the mean differences and the mean relative differences reach the 5% significance level. If we assume a normal distribution of error for the paired comparisons ($RR_i - WR_i$), we obtain, for 1972:

$$\bar{z} = \frac{1}{26}\left[\sum_1^{26}(RR_i - WR_i)\right] = -3.0$$

$$S_z = 9.69$$
$$S_{\bar{z}} = 1.90$$
$$t_{25} = -1.57^{ns}$$

The mean difference was not statistically significant. A further advantage of the paired comparison sampling protocol comes from the ability to treat individual data. A frequency table (Sokol and Rohlf, 1969:549) of the twenty-six original z_i values for 1972 is shown in Table 4. A Chi Square test of these data indicates a non-normal distribution. However upon inspection the datum in the $+3.5\ S_z$ class appears to be the major source of the high Chi Square value. The justifications for excluding this paired difference value within the $+3.5\ S_z$ class were twofold: Chauvinet's criterion (Wang and Willis[3] 1965:192) *and* the fact that a Chi Square test on the remaining frequency distribution indicated that except for this datum the data are normally distributed. The adjusted values for 1972 became

$$(RR - WR)_{1\text{-}365}^{1972} = -4.3\ \mu g\ liter^{-1} \quad (*;\ n = 25)$$

and

$$(RR - WR)/(RR)_{1\text{-}365}^{1972} = -.33\ (*;\ n = 25).$$

It should be noted that while the data exclusion criteria we have used permit a better estimate of the average value, the variance of the data remains unchanged and the denominator in the Student's *t* test is the same $S_{\bar{z}}$ as was calculated for the complete set of data. The paired sampling protocol applied to comparable systems makes it possible not only to treat the experimental design and the data by the powerful techniques of analysis of variance but to establish probability distributions which justify the exclusion of "bad" data points as we have demonstrated above. We can now say that in 1972 there was a

[3] Note that there is a typographical error in this reference. On page 192 the sentence should read "...is equal to, or less than $\frac{1}{2N}$,..." instead of ½ N.

TABLE 3. Summary of paired variate analyses for Rhode and West rivers for 1971 through 1972. There are three entries for each year and each class. The top entry is the calculated mean difference followed by symbols indicating the level of significance. The middle entry is the sample size. The bottom entry in brackets is the absolute value of the mean difference which would be significant at the P = 0.05 level, based on $S_{\bar{z}}$ of equation (4).

Comparison	Period	Days	1970	1971	1972
$\frac{RR-WR}{RR}$	Winter	1–90	—	−.1 NS	−.46 NS
			—	n = 7	n = 9
			—	[.91]	[.62]
	Spring-Summer	91–240	−.08 NS	0.0 NS	−.25*
			n = 17	n = 19	n = 14
			[.21]	[.15]	[.22]
	Fall	241–365	−.82 NS	−.19 NS	−.28 ID
			n = 6	n = 7	n = 2
			[.93]	[.59]	—
	Whole Year	1–365	−.27 NS	−.07 NS	−.30*
			n = 23	n = 33	n = 26
			[.28]	[.20]	[.22]
RR − WR [μg liter^{-1}]	Winter	1–90	—	2.7 NS	−3.2 NS
			—	n = 7	n = 9
			—	[3.9]	[3.7]
	Spring-Summer	91–240	−.17 NS	.07 NS	−5.2*
			n = 17	n = 19	n = 14
			[3.9]	[2.1]	[4.9]
	Fall	241–365	−6.5 NS	−1.8 NS	−2.9 ID
			n = 6	n = 7	n = 2
			[9.1]	[8.9]	—
	Whole Year	1–365	−1.8 NS	.24 NS	−4.3*
			n = 23	n = 32	n = 25
			[3.6]	[2.1]	[3.9]
$\frac{RR-CB}{RR}$	Winter	1–90	—	.69**	.36 NS
			—	n = 6	n = 6
			—	[.35]	[.50]
	Spring-Summer less Bloom	91–150, 201–240	.50***	.09 NS	.34*
			n = 11	n = 12	n = 7
			[.19]	[.24]	[.28]
	Bloom	151–200	−.16 NS	−.56 NS	.20 NS
			n = 6	n = 6	n = 5
			[.39]	[.93]	[.58]
	Fall	241–365	.59***	.15 NS	.38 ID
			n = 5	n = 6	n = 2
			[.11]	[.38]	—
	Whole Year	1–365	.34***	.1 NS	.32**
			n = 22	n = 30	n = 20
			[.18]	[.24]	[.18]

TABLE 3. (con't).

Comparison	Period	Days	1970	1971	1972
RR – CB [μg liter^{-1}]	Winter	1–90	— — —	6.6* n = 6 [4.3]	3.6 NS n = 6 [5.8]
	Spring-Summer less Bloom	91–150 201–240	12.7** n = 11 [7.4]	1.0 NS n = 12 [3.2]	12.6* n = 7 [12.5]
	Bloom	151–200	–1.3 NS n = 6 [9.7]	–6 NS n = 6 [9.5]	17 NS n = 5 [30]
	Fall	241–365	7** n = 5 [4]	4.9 NS n = 6 [10]	5.1 ID n = 2 —
	Whole Year	1–365	7.6** n = 22 [4.8]	1.4 NS n = 30 [3]	10.3** n = 20 [6.8]

ID:insufficient data for statistical analysis
NS:not significant P > .05
*:significant .05 > P > .01
**:very significant .01 > P > .001
***:extremely significant .001 > P

TABLE 4. Frequency table of 1972 (RR – WR) chlorophyll *a* concentrations.

Frequency class of units of S_z	Observed f	Expected $\hat{f}$	$(f - \hat{f})^2 / \hat{f}$
–2.5	0	.24	.240
–2.0	1	.73	.100
–1.5	1	1.70	.288
–1.0	2	3.15	.420
–0.5	7	4.54	1.333
0	5	5.13	.003
+0.5	8	4.54	2.637
+1.0	1	3.15	1.467
+1.5	0	1.70	1.70
+2.0	0	.73	.73
+2.5	0	.24	.24
+3.0	0	.06	.06
+3.5	1	.01	98.0

$\Sigma f = 26$

Summary:

n = 26; $\bar{z} = -3.0$ μ gram liter^{-1} (NS); $S_{\bar{z}} = 1.90$ μ gram liter^{-1}; n = 25; Hz = –4.3 μ gram liter^{-1} (*): $\chi_6^2 = 8.68$.

small but significant difference between the standing crops of phytoplankton in the Rhode River and the West River, a result not attainable from the baseline technique. If we used the very same yearly data and treated them as though the measurements had been made independently, the difference of the means remains the same,

$$(\overline{RR})_{1\text{-}365}^{1972} - (\overline{WR})_{1\text{-}365}^{1972} = -3 \ \mu\text{g liter}^{-1}$$

but the standard error of the means, the denominator of equation (2) becomes 6.0 as compared with 1.90 for the paired comparison. Since the precision varies as $1/\sqrt{n}$ it follows that the application of the paired comparison sampling protocol can reduce the number of samples required to achieve a particular precision; in this case by a factor of 10.

Consider the following hypothetical case which will become apparent from observation of Fig. 13. Let us assume that some sewage or industrial effluent or other perturbation had been initiated in West River in late 1970. In 1971 we set about to measure, among other things, the spring pulse in phytoplankton standing crop in the West River and compare it to the 1970 data. Considering the *before* and *after* baseline our data for these two years give

$$(\overline{WR})_{91\text{-}150}^{1970} - (\overline{WR})_{91\text{-}150}^{1971} = 17.4 \ \mu\text{g liter}^{-1}$$
$$(*;\ n + m = 16).$$

Since

$$(\overline{WR})_{91\text{-}150}^{1970} = 25.6 \ \mu\text{g liter}^{-1},$$

the West River has "suffered" a 68% decrease in the spring phytoplankton standing crop compared with 1970. However from Table 3 the values of (RR − WR) and (RR − WR)/(RR) for spring-summer 1970 and spring-summer 1971 show that, compared with the Rhode River, which presumably did not have the hypothetical effluent or perturbation, there was no significant difference in phytoplankton standing crops between RR and WR in spring-summer 1970 *and* definitely not in spring-summer 1971; the 68% decrease observed in WR between 1970 and 1971 was reflected in RR as well. Therefore the hypothetical man-made perturbation in WR was not responsible for the observed changes in WR phytoplankton in 1971.

The advantages of the paired comparative sampling protocol are thus not only in reducing the sample size, but in separating changes due to natural variability which relate to both systems from changes in a system due to specific perturbations in one system.

b) The analyses tell us something about the natural variability of phytoplankton standing crops in these sections of the Rhode and West rivers. On the basis of the 3-year composite data for RR − WR we find that

$$(RR - WR)^{1970\text{-}1972} = -1.7\ \mu g\ liter^{-1}$$
$$(*;\ n = 81).$$

The fact that a small difference of only 1.7 μg liter^{-1} of chlorophyll *a* is statistically significant is an indication of the precision of the comparative sampling protocol. More relevant, the standard deviation S_Z = 7.14 μg liter^{-1}. Since SΔ, the instrumental and calibration error of a difference measurement of extractable chlorophyll *a* is equal to less than 2 μg liter^{-1} it follows that the patchiness or irreducible natural variations still give rise to the major component of the total uncertainty, even when the total uncertainty is reduced. It would therefore be unfruitful to attempt to improve the assay technique for chlorophyll *a*. Rather it should be possible to relax the precision requirements of the assay technique in favor of increasing the sampling frequency.

The *in vivo* chlorophyll *a* fluorescence assay is, despite its uncertainties (Loftus et al. 1972) the method of choice for assays of phytoplankton standing crops. It can readily increase the sample size since it can be adapted to routine measurement of large areas by technical personnel and to unattended continuous operation *in situ*.

c) The phytoplankton standing crops in Rhode River were generally higher than in the bay proper. However this was not the case in 1971. The mean differences RR − CB for the seasonally combined 1970, 1971 and 1972 data were 7.6 μg liter^{-1} ** (n = 22); 1.4 μg liter^{-1} NS (n = 30) and 10.3 μg liter^{-1} ** (n = 20) respectively.

d) The statistical analysis of the January-March, 1971 data, $\left(\frac{RR - CB}{RR}\right)^{1971}_{1\text{-}90}$ supports the insolation effect shown in Fig. 8. The effect occurred slightly later in 1972. If days 1–150 are analyzed, $\left(\frac{RR - CB}{RR}\right)^{1972}_{1\text{-}150}$ = 0.37* (n = 10). In 1970 our first data point was day 91. From day 91 through day 150, $\left(\frac{RR - CB}{RR}\right)^{1970}_{91\text{-}150}$ = 0.58** (n = 6).

Conclusions

The application of the paired comparison sampling protocol to a baseline study can represent an improvement in experimental design and in the statistical analysis of data. In the sample calculation contrasting the precision of the paired measurements with the separate system technique we arbitrarily calculated a standard deviation for the latter even though there was no *a priori* reason to assume a normal distribution for the annual data. This we justified only to compare the same data (see Sokal and Rohlf, 1969:333). It would be more reasonable to assume that the differences of parameters in comparable sections are normally distributed since the irreducible experimental variance is the result of non-homogeneities in both sections. The relative differences (A − B)/A are not symmetric. For example (A − B)/A $\rightarrow$ 1 for A $\gg$ B and approaches $-\infty$ for A $\ll$ B. The distribution becomes important when A is quite different from B.

The concentrations of chlorophyll *a* at any time are the result of previous production,

predation and water exchange. The standing crop data in this paper are by themselves not sufficient for a comparative study of the Rhode River and West River phytoplankton communities. The parallel data on nutrients, primary production, species composition, succession and predators will be presented in a subsequent paper. The standing crop data have been used to demonstrate the degree of precision that can be obtained by the paired comparison technique. We have concluded that based on the combined data for 1970 through 1972 the mean annual phytoplankton standing crop in West River is higher than that in Rhode River; 1.7 μg liter^{-1} at the 5% probability level. This is a small value and therefore the lack of exact comparability between the river sections could just as easily be responsible for the difference as some increased stress on the West River. It does not appear therefore that the relatively greater human population and boat use in the West River watershed have changed the phytoplankton concentrations in the West River. It is possible that further upstream in West River there may be some small sections which have been disturbed by septic tank leakage or effluents from the boat marinas or silt runoff as the result of increased density of use of the watershed. The strong coupling and feedback in the phytoplankton-zooplankton-detritus-bacteria-protozoa food chain tends to damp out many of these stresses, and the effectiveness of this damping is a measure of the stability or assimilatory capacity of the system. If a local effect is extreme, if there are many small local effects or if a portion of the food web is interfered with, the recovery may not be complete. In this case the river section outside of the local areas may be affected. The present comparison was between river sections outside of the "local effect" areas, i.e. outside of the creeks. However the extent of a "local effect" depends on the size of the subestuary. In the Potomac River the local effects of blue-green algae due to eutrophication can be extensive, as can the results of power plant predation by entrainment or inhibition by heat or chemical effluents. It is conceivable that in these extensive areas the comparable river sections chosen for the "paired" comparison protocol could also include adjacent sections of the river above and below the perturbation whose effect is to be measured.

The standing crops of chlorophyll *a* in both rivers were higher than in the bay, except during the summer when there were *Prorocentrum* blooms in the bay (unpublished data). In 1971 however there was no spring pulse and therefore no significant difference between either river and the bay.

Only in 1972 did the standing crops of chlorophyll *a* in the Rhode River differ significantly from those in the West River. We have no explanation for this. We know of no major man-introduced perturbations which might have changed conditions in either RR or WR from 1970 and 1971 when the phytoplankton populations were essentially the same.

The precision demonstrated for the paired sampling protocol should make it feasible to study the response of the Rhode River subestuary to defined nutrient or biocide inputs, simulating concentrations observed or proposed for other, larger subestuaries. The effects should be measurable at a series of levels of additions which will not be exorbitantly expensive or impractical to handle physically, and from which the assimilatory capacity of the system can be estimated.

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