Seasonal variation of vitamin B_{12}, B_{12} analogs, and phytoplankton in a Long Island estuary

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ABSTRACT

Dissolved vitamin B_{12} concentrations in the Peconic Bay estuary, Long Island, were determined over a seasonal period by assay with the diatom *Thalassiosira pseudonana* clone 3H. Concurrently, assays for vitamin B_{12} analog levels were made using clone 13-1 of the same diatom. B_{12} concentrations at several locations ranged from undetectable to 14.3 ng liter^{-1} and averaged 2.2 ng liter^{-1}. B_{12} analog levels varied between undetectable and 29.4 ng liter^{-1} with a mean value of 3.3 ng liter^{-1}. Phytoplankton composition was also analyzed and the flora consisted of 131 species of algae. Regression analyses were made between cobalamin data and phytoplankton population parameters. Significant correlations were observed with some of the phytoplankton parameters but the correlations were scattered among the various stations and no consistent pattern of relationship was observed. Probably vitamin B_{12} is not important in limiting phytoplankton growth rates and this helps explain the lack of consistent correlations between B_{12} and analog levels and any of the tested phytoplankton parameters. Introduction of cobalamins into the bay system may be both authocthonous and allocthonous, and possible mechanisms for each are discussed.

1. Introduction

The role of cobalamins in the marine environment is not fully understood. It is known that vitamin B_{12} or one of its analogs is required for growth by many marine phytoplankters (Provasoli and Carlucci, 1974). The concept that B_{12} may be important in initiating blooms was considered by several investigators (Provasoli, 1963; Carlucci, 1970) while the possibility of vitamins affecting seasonal succession and phytoplankton composition was also proposed (Menzel and Spaeth, 1962). Guillard (1968) suggested that the ratio of B_{12} to its analogs may determine which species of phytoplankton dominate an ecosystem. There have been several studies of the seasonal distribution of dissolved vitamins in the marine environment (Vishniac and Riley, 1961; Menzel and Spaeth, 1962; Cattell, 1969, 1973; Ohwada and Taga, 1972) but seasonal phytoplankton surveys were included only in investigations by Cattell (1969), Propp (1970), and Bruno and Staker (1978). None of

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these field studies included differential assays in order to determine vitamin B_{12} and B_{12} analog concentrations.

Phytoplankton may exhibit one of three responses to B_{12} and its analogs (for definition of analog see Smith, 1965). The most specific is the mammalian type in which an organism responds only to vitamin B_{12} or analogs with benzimidazole nucleotide-like groups. Examples of mammalian type response include the diatom *Chaetoceros lorenzianus* (Guillard, 1968) and the chrysophyte *Ochromonas malhamensis* (Smith, 1965). A second kind of response is the lactobacillus type which elicits a response to all analogs except factor B. Chemically these analogs have a purine substituted in the side chain, usually adenine or a substituted adenine, instead of the benzimidazole nucleotide-like group. The algae *Euglena gracilis* and *Pavlova lutheri* (formerly *Monochrysis lutheri)* are examples of the lactobacillus type response (Droop *et al.,* 1959). The broadest response is the coliform type, with response to all analogs. Algae showing this specificity are *Ditylum brightwelli* (Guillard, 1968) and *Peridinium hangoei* (Iwasaki, 1969). The marine diatom *Thalassiosira pseudonana* clone 3H shows a mammalian type response to vitamin B_{12} whereas *T. pseudonana* clone 13-1 responds to B_{12} and all analogs (coliform response). Thus analog concentration can be determined by the difference between the bioassay results of the two clones.

We describe here the seasonal distribution of dissolved vitamin B_{12} and its analogs in the Peconic Bay estuary, Long Island, New York. The significance of these cobalamins with respect to phytoplankton populations is discussed.

2. Materials and Methods

a. Field sampling. Water samples were collected from nine stations in the Peconic Bay estuary from August, 1976 to April, 1978 (Fig. 1). Only surface samples were collected at stations 1, 2, and 3 because of the shallow nature of the water column (<3.0 m). Surface (0.5 m) and bottom (about 0.5 m above bottom sediment) water samples were collected with 5-liter Niskin bottles (General Oceanics, Inc) at stations 4-9. The sampling dates and kinds of analyses performed are indicated in Table 1. Temperatures were recorded with a bucket thermometer immediately after samples were drawn from the Niskin bottles. Phytoplankton cells from a 1-liter aliquot were concentrated to 10 ml in 3% buffered formalin, using a Kahlsico Continuous Plankton Centrifuge (85% efficient in removing cells). Another liter of water was filtered through a Whatman GF/C glass-fiber filter pad; the pad was frozen (−18°C) and later analyzed for chlorophyll *a*. A 200 ml portion of each water sample was filtered through an HCl washed GF/C pad and 0.45 μm Millipore filter, and then frozen (−18°C) for dissolved vitamin B_{12} and B_{12} analog analysis. Chlorophyll, nutrients, and other hydrographic and biological data collected during this sampling program are presented in Bruno *et al.* (1980).

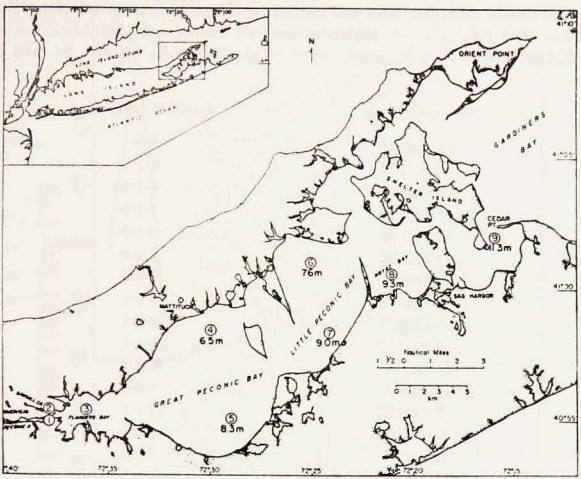

Figure 1. Location of the sampling stations in the Peconic Bay estuary. Mean high tide water depths are indicated for stations 4-9. Water depths at stations 1, 2, and 3 are 3.0, 2.0, and 1.6 m respectively.

b. Laboratory procedures. Phytoplankton cell counts and identifications were made using a nanoplankton counting chamber (Palmer and Maloney, 1954). Species diversity index (H′) was determined using the Shannon and Weaver (1963) formula. Concentrations of chlorophyll *a* were measured using a Perkin-Elmer double beam spectrophotometer (Strickland and Parsons, 1972).

Concentrations of dissolved vitamin B_{12} and B_{12} analogs were determined for each sample by bioassay using the diatom *Thalassiosira pseudonana* Hasle et Heimdal, clones 3H and 13-1 (Ryther and Guillard, 1962; Carlucci, 1973). Both assay organisms were obtained from R.R.L. Guillard, Woods Hole Oceanographic Institution. Vitamin-free seawater for the assays was prepared according to methods described in Strickland and Parsons (1972). Samples for B_{12} assay were dispensed in 5 or 10 ml aliquants into 50 ml micro-fernback flasks (Bellco, Inc.) with stainless steel caps. Each sample was diluted with 5 or 10 ml of vitamin-free seawater enriched with nutrients as described by Carlucci (1973). External standards (Calibration curve) for the bioassay contained: 0, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, and 3.0 ng liter^{-1} of vitamin B_{12}. Vitamin B_{12} was added to duplicate sets of each sample resulting in an addition of 1 ng liter^{-1}. These flasks served as internal standards to determine possible inhibition of the seawater to the assay organism. Samples,

Table 1. The sample collection dates and analyses performed on water samples collected from the Peconic Bay estuary (+ = phytoplankton cell counts and identification only; ++ = phytoplankton and vitamin B_{12} assay; +++ = phytoplankton, vitamin B_{12}, and B_{12} analog assay).

Sampling Date	Analysis Performed
16 Aug 76	++
15 Sept 76	++
19 Oct 76	+++
16 Nov 76	+++
15 Dec 76	+++
9 Mar 77	+++
30 Mar 77	+++
15 Apr 77	+++
5 May 77	+++
25 May 77	+++
21 Jun 77	+++
14 Jul 77	+++
2 Aug 77	+++
18 Aug 77	+++
30 Sep 77	+++
21 Oct 77	+++
11 Nov 77	+++
29 Nov 77	+++
27 Dec 77	+++
27 Jan 78	+
20 Mar 78	+
17 Apr 78	+

external standards, and internal standards were prepared in duplicate and inoculated with approximately 10^4 cells ml^{-1} of B_{12} depleted *T. pseudonana* cells.

The bioassay series were incubated in a temperature-controlled room at 20 ± 1°C below a bank of G.E. fluorescent lamps emitting 6000 lux on a 14:10 h L:D cycle. Each assay was terminated after 4 days and vitamin B_{12} (or B_{12} +analogs) concentrations were calculated on the basis of final cell yield. Cell counts were made using a hemacytometer. A least squares regression line calculated from the mean standard values of each bioassay series was used to estimate vitamin B_{12} (from *T. pseudonana* clone 3H) and vitamin B_{12} + analogs (from *T. pseudonana* clone 13-1) concentration of the samples.

c. Standardization of B_{12} analog assay. Because utilization of B_{12} analogs by *T. pseudonana* (13-1) is not at 100% effectiveness (based on B_{12} concentrations as 100%), it was necessary to determine at what level of effectiveness the analogs were being utilized by the diatom under our experimental conditions. Standardization assays were carried out with clone 13-1 in which effective utilization of pseudo-

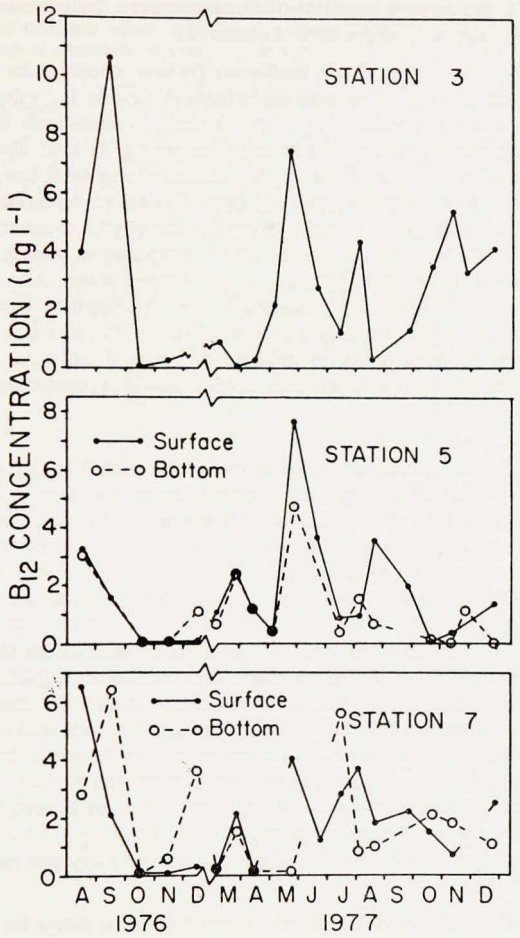

Figure 2. Dissolved vitamin B_{12} levels at stations 3, 5, and 7 during the study period.

B_{12} and factor B was measured as a percentage of cyanocobalamin. The analogs were obtained from L. Provasoli, Haskins Laboratories at Yale University. The assay for all vitamin B_{12}-like compounds was carried out as described in

section *(b)* for the external standards (Calibration curve). Culture conditions and calculation of results were as described in section *(b)*.

d. Regression analyses. Correlation coefficients (r) were calculated for stations 1, 3, 4, 5, 6, and 7. Regressions were made between vitamin B_{12} values and the following variables: 1) standing crop of *Skeletonema costatum* (cells liter^{-1}), the dominant phytoplankter during the investigation period; 2) total diatom standing crop (cells liter^{-1}); 3) total dinoflagellate standing crop (cells liter^{-1}); 4) the ratio of diatom to dinoflagellate standing crop; 5) total phytoplankton standing crop (cells liter^{-1}); 6) species diversity index; 7) chlorophyll *a* concentration. Correlation coefficients were also computed for these 7 variables vs. vitamin B_{12} analog values and the ratio of vitamin B_{12}:B_{12} analog concentrations. Also correlation coefficients were calculated for temperature with: dinoflagellate standing crop; vitamin B_{12} concentration; B_{12} analog concentration. In total, 24 linear regressions were computed for each station in order to determine if significant correlation existed between cobalamin concentrations and phytoplankton populations.

3. Results

a. Vitamin B_{12} data. Standard curves were run for each bioassay series. The typical B_{12} standardization assay gave a straight line with a slope of 0.6 ± 0.05 (cells ml^{-1}-B_{12}). A total of 190 B_{12} bioassays was made and results are presented graphically for stations 3, 5, and 7 only (Fig. 2) for the period from August, 1976 through December, 1977. Only surface data are presented for station 3, as the water column is but 3.0 m at this station, located near the headwaters of the Peconic Bay system.

Table 2 presents mean vitamin B_{12} measurements for each station sampled over the entire sampling period. The mean values were calculated using 0.05 ng liter^{-1} for minima (limit of detection). Station 3 had the highest mean B_{12} concentrations, while station 5 (bottom) had the lowest. In general, the north shore stations of the estuary were lower in vitamin B_{12} concentration than the south shore stations. The mean value for the entire study period at all stations was 2.2 ± 2.3 ng liter^{-1}. Summer concentrations ($\bar{x}$ = 3.2 ± 2.5 ng liter^{-1}) were the highest, followed by spring, winter, and fall. Fall values averaged lowest for the year at 1.2 ± 1.4 ng liter^{-1}. Sampling during the months of January and February was restricted due to ice over the bay.

b. Vitamin B_{12} analog data. Results of the standardization assays for pseudo-B_{12} and factor B, expressed as percentage of effective utilization compared to cyanocobalamin, are presented in Table 3. A total of 170 seawater samples was bioassayed for vitamin B_{12} + B_{12} analog concentration and the average B_{12} analog results for the period October, 1976 through December, 1977 are presented in Table 2 for stations 1 and 3-7, and are illustrated in Figure 3 for stations 3, 5, and 7.

Table 2. Average cobalamin values found at the Peconic Bay stations with standard deviation, variance, and maximum values. Assays at each station revealed cobalamin concentrations below the limit of detectability at some time during the study (0.05 ng liter^{-1}).

Station	Cobalamin	Mean (ng l^{-1})	Standard Deviation	Variance	Maximum Value	Number of Samples
1	B_{12}	2.6	1.6	2.4	5.6	18
	Analogs	3.5	4.7	20.7	13.8	13
3	B_{12}	2.8	2.9	7.8	10.7	18
	Analogs	4.1	8.2	62.7	29.4	13
4-S	B_{12}	2.4	1.9	3.3	7.9	18
	Analogs	1.5	2.0	3.7	5.1	15
4-B	B_{12}	2.5	2.4	5.6	10.6	19
	Analogs	0.97	2.9	7.6	10.6	14
5-S	B_{12}	1.7	1.9	3.5	7.7	18
	Analogs	2.4	4.3	17.3	17.3	16
5-B	B_{12}	1.1	1.3	1.6	4.8	16
	Analogs	4.2	4.3	16.8	14.9	14
6-S	B_{12}	2.6	3.0	8.3	10.8	18
	Analogs	4.5	6.3	36.4	20.7	13
6-B	B_{12}	2.4	3.3	10.3	14.3	19
	Analogs	4.5	6.6	41.4	23.0	15
7-S	B_{12}	1.9	1.7	3.2	6.5	17
	Analogs	3.3	4.3	17.3	15.6	15
7-B	B_{12}	1.8	2.0	3.7	6.4	15
	Analogs	4.4	4.7	20.2	16.6	15

Vitamin B_{12} analog concentration for each sample was calculated by subtracting the corresponding vitamin B_{12} concentration and then multiplying the difference by a correction factor of 2.3. This factor was obtained from the results of the standardization assay. Since the mean responses, as a percentage of cyanocobalamin, for pseudo-B_{12} and factor B are 50% and 38% respectively, the average

Table 3. Result of standardization assays for pseudo-B_{12} and factor B, expressed as percentage of effective utilization compared to various concentrations of cyanocobalamin.

Cyanocobalamin (ng liter^{-1})	% Utilization pseudo-B_{12}	% Utilization factor B
0.1	44	31
0.2	49	38
0.4	49	28
0.8	50	37
1.0	47	37
2.0	54	44
3.0	57	52
$\bar{x}$	50 ± 4.3	38 ± 8.0

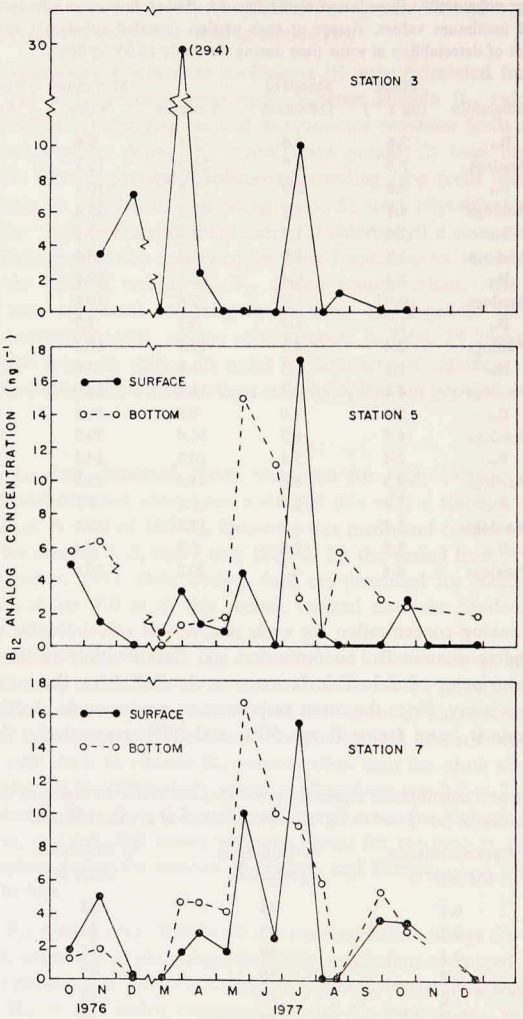

Figure 3. Vitamin B_{12} analog concentrations at stations 3, 5, and 7 during the study period.

response to both will be 44%, yielding a correction factor of 2.3. This assumes that the two analogs are equally mixed in the sample and that response to all other analogs is similar. The range of concentrations for all of the analog data was undetectable to 29.4 ng liter^{-1}. As observed for the vitamin B_{12} levels, highest analog concentration occurred during the summer months ($\bar{x}$ = 4.6 ± 6.4 ng liter^{-1}) followed by spring ($\bar{x}$ = 3.6 ± 4.4 ng liter^{-1}), fall ($\bar{x}$ = 3.0 ± 3.0 ng liter^{-1}), and winter ($\bar{x}$ = 2.2 ± 6.0 ng liter^{-1}). Average concentration for all stations during the investigation was 3.3 ± 5.1 ng liter^{-1}.

c. Seasonal phytoplankton composition and standing crops. Analysis of 330 water samples for phytoplankton species composition revealed a total of 131 species with the Bacillariophyta (diatoms) comprising the dominant group based on the number of species (88/131). The genus *Chaetoceros* exhibited more species than any other genus (15); however, the standing crops of *Chaetoceros* were usually small. Based upon the number of times a taxon was dominant, the diatom *Skeletonema costatum* was most important, followed by *Leptocylindrus danicus, Thalassiosira nordenskiöldii,* and *Lithodesmium undulatum.* Populations of *T. nordenskiöldii,* occurred almost exclusively during the month of April, when water temperatures are in the 8-9° C range, while *L. undulatum* generally appeared during the months of July to September when temperatures were > 18° C. The centric diatom *L. danicus* usually preceded the occurrence of *L. undulatum* while *Detonula confervacea* was sometimes very important in the winter flora, often preceding *T. nordenskiöldii.*

The Pyrrophyta (dinoflagellates) represented the second most important group of phytoplankton and were generally found during the warmer months of the year (cf Table 4). Important among the dinoflagellates were *Prorocentrum redfieldi, P. minimum,* and *Peridinium trochoideum.* The largest standing crop observed (6.46×10^7 cells liter^{-1}) was during a bloom of the dinoflagellate *P. trochoideum* in the estuary headwaters in March, 1977 (station 2).

Fourteen species of Chlorophyta were noted, many of which were freshwater forms found only at the less saline stations 1, 2, and 3. Several species of *Scenedesmus* are included here. The dominant species of phytoplankton are listed in Table 4 along with the highest cell densities observed and the season when a taxon was usually prevalent. Seasonal variation in standing crops of several important phytoplankters at station 7, which was typical of the seasonl cycle, is shown in Figure 4.

d. Regression analyses. The significant results ($P<0.05$) of the various regressions calculated are shown in Table 5. Temperature vs. dinoflagellate standing crop had the most number of significant correlations with respect to the various sampling stations and depths. Other correlations were scattered and show no real pattern; 17 of 24 regressions showed no significance at $P < 0.05$. The ratio of vitamin

Table 4. Summary of dominant phytoplankton data from all stations and all sampling times in the Peconic Bay estuary. The total sample number was 330.

Dominant Phytoplankter	Frequency of Dominance-% of Total	Highest Cell Density-cell l^{-1}	Period of Dominance
Skeletonema costatum (Grev.) Cleve	39.39	2.57×10^6	Jul.-Nov., Mar.
Leptocylindrus danicus Cleve	8.79	5.93×10^5	May-Jun.
Thalassiosira nordenskiöldii Cleve	7.88	2.26×10^6	Mar.-Apr.
Lithodesmium undulatum Ehr.	7.88	5.72×10^5	Aug.-Sep.
Asterionella glacialis Korner	6.06	9.61×10^5	Nov.-Dec.
Chaetoceros ingolfianum Ostenf.	4.24	7.40×10^4	Mar.
Thalassiosira pseudonana Hasle et Heimdal	3.94	1.50×10^7	Jul.-Aug.
Rhizosolenia delicatula Cleve.	3.64	2.27×10^5	May
Melosira sp.	3.33	2.40×10^3	Dec.
Eutreptiella sp.	2.12	8.53×10^3	Aug.-Dec.
Prorocentrum minimum Martin	2.12	2.70×10^5	May-Oct.
Peridinium trochoideum (Stein) Lemmerm.	1.82	6.46×10^7	Mar.-Aug.
Prorocentrum redfieldi Bursa	1.52	5.15×10^7	Aug.-Sep.
Prorocentrum micans Ehr.	1.21	1.31×10^4	Aug.
Ebria tripartita (Schum.) Lemmerm.	1.21	3.00×10^3	Dec.
Detonula confervacea (Cleve) Gran	0.91	1.66×10^5	Jan.-Mar.
Exuviaella sp.	0.61	1.29×10^7	Sep.

B_{12}/B_{12} analog concentration showed more significant correlation with the various phytoplankton parameters than either B_{12} or B_{12} analog concentration by themselves. With the exception of temperature vs. dinoflagellate standing crop, most regressions showed significance only at one or two stations, if at all, indicating no very strong relationship between vitamins-analogs and phytoplankton population parameters.

4. Discussion

The observed dissolved vitamin B_{12} concentrations ($\bar{x} = 2.2$ ng liter^{-1}) for the entire study period in the Peconic Bay estuary is similar to levels found in other coastal waters by some investigators (Carlucci, 1970; Propp, 1970; Ohwada and Taga, 1972; Inoue *et al.*, 1973; Bruno and Staker, 1978) but is considerably

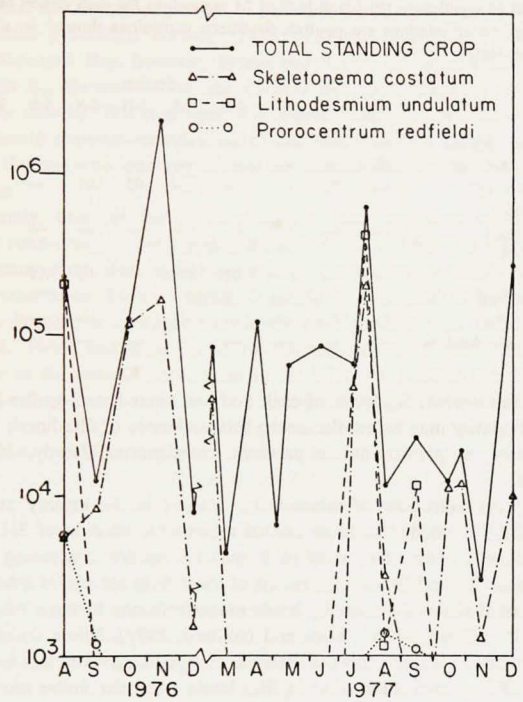

Figure 4. The dominant phytoplankton pattern during the study period at station 7 (surface samples). Vertical axis represents cells liter^{-1} and not biomass units.

less than concentrations found by Vishniac and Riley (1961) in Long Island Sound and Cattell (1973) in the Straits of Georgia, British Columbia. The annual mean vitamin B_{12} concentration in Long Island Sound was about 8.0 ng liter^{-1}; this is significantly different from levels observed in the Peconic Bay estuary considering the close proximity of these two bodies of water. Vishniac and Riley (1961) used a different technique in measuring cobalamin concentrations and this may explain some of the discrepancy. Bruno and Staker (1978) observed mean annual B_{12} concentrations of 1.8 ng liter^{-1} for the Napeague Bay region of Block Island Sound. This body of water lies just outside the Peconic Bay estuary, separated from it only by Gardiner's Bay. There was no significant difference between

Table 5. Level of significance (P) found in 7 of 24 regressions for each station in the Peconic Bay estuary. All correlations are positive. Seventeen regressions showed no significance at $P < 0.05$ (see text).

	Station									
Regression	1	3	4-S	4-B	5-S	5-B	6-S	6-B	7-S	7-B
Dinoflagellate Crop. B_{12}	—	—	—	—	—	—	—	.01	.01	—
S. costatum: Analogs	.05	—	—	—	—	—	—	—	—	—
Dinoflagellate crop: B_{12}/Analog Ratio	—	.01	—	—	—	.02	.01	—	—	—
Species Diversity: B_{12}/Analog Ratio	—	—	—	—	—	—	—	—	—	.05
Temperature: Dinoflagellate crop	—	—	—	.01	.05	—	.05	.05	.01	.05
Temperature: B_{12}	—	—	—	—	—	—	—	—	.02	—
Temperature: B_{12} Analogs	—	—	—	—	—	—	—	—	.02	.05

observed mean vitamin B_{12} levels of each body of water based on the *t*-test (95% CL). The similarity may reflect the strong tidal influence of Gardiner's Bay waters on the estuary, as pointed out in previous investigations (Hardy, 1976; Bruno *et al.*, 1980).

Dissolved concentrations of vitamin B_{12} analogs in the estuary are generally higher than B_{12} levels and have an annual mean concentration of 3.3 ng liter^{-1}. Although there are no other data of a seasonal nature concerning differential assays for both B_{12} and its analogs, results of short term surveys in other bodies of water indicate that dissolved analog levels range from one to three times the level of vitamin B_{12} (Cowey, 1956; Swift and Guillard, 1977). These observations are consistent with the results for the Peconic Bay estuary with B_{12} analog levels averaging 50% greater than vitamin B_{12} levels over the entire survey period. Analog levels may even be larger in the bottom muds and sediment of estuaries as well as on suspended solids (Burkholder and Burkholder, 1956; Cattell, 1973). This is likely due to greater production of analogs than of B_{12} by marine bacteria (Haines and Guillard, 1974).

Seasonal variation of dissolved vitamin B_{12} and its analogs show a similar pattern with both groups having seasonal highs during the summer months and with the lowest mean levels for each being observed during fall and winter respectively. Dissolved levels of both cobalamin groups were occasionally undetectable, particularly during fall and winter months. These results are similar to those of Bruno and Staker (1978), giving further evidence that the cobalamin cycle in the estuary may to some extent be influenced by tidal exchange through Gardiner's Bay. Our seasonal observations of vitamin B_{12}-B_{12} analog fluctuation in temperate waters differ from those of other investigators who observed higher levels during winter months, apparently in relation to the cycle of inorganic nutrients (nitrate and

phosphate) and the normal regenerative processes associated with the breakdown of the seasonal pycnocline (Cowey, 1956; Vishniac and Riley, 1961; Cattell, 1973). In Napeague Bay, however, Bruno and Staker (1978) observed that temporal vitamin B_{12} fluctuation did not parallel inorganic nutrients which peaked during winter months. The Napeague Bay region of Block Island Sound does not show any strong seasonal stratification of the water column (Staker and Bruno, 1978) and this may be one reason for the observed difference from previous investiagtions.

Unfortunately, none of the previous studies have any data concerning ammonium ion concentration along with cobalamin levels and data on other inorganic nutrients. Ammonium often makes up a large percentage of dissolved inorganic nitrogen concentration (DIN = nitrate + nitrite + ammonia) at higher temperatures. In the Peconic Bay estuary DIN levels are high during the summer months (Bruno *et al.*, 1980) and Bruno *et al.* (in preparation) have shown that the percentage of N in the form of ammonium in the DIN is a positive function of temperature indicating that this estuary is a nitrogen regenerative system throughout most of the year. Temporal variation of vitamin B_{12} and B_{12} analogs in the bay system somewhat parallels seasonal variation of DIN with higher levels observed during the summer months and lower concentrations during fall and winter. However, regression analyses between temperature and cobalamin levels show significant correlation only at station 7 (see Table 5). Patches of phytoplankton were not observed in this study, perhaps because of rapid movement of water (hydrodynamics., see Bruno *et al.*, 1980). This lack of correlation at most stations is probably the result of somewhat erratic increases and decreases in cobalamin levels as opposed to the consistent pattern of temperature variation. In general, however, the higher cobalamin levels along with higher temperature and DIN concentration indicate that cobalamin increases are at least partially the result of authochthonous, or intrinsic, metabolic processes of the ecosystem. There are two likely sources of authochthonous cobalamin supply: 1) production by bacteria, both in the bottom sediments and in the water column; 2) liberation into the environment by cobalamin-producing phytoplankters. The second possibility as a continuous source of high environmental levels of cobalamins is unlikely for several reasons. First, during the summer months, when cobalamin levels are highest, the dominant phytoplankters are composed of a mixture of vitamin B_{12} auxotrophs which include *Skeletonema costatum* and a variety of dinoflagellates. Secondly, laboratory experiments have demonstrated that facultative autotrophic marine phytoplankters only liberate vitamin B_{12} into the environment when the surrounding medium is depleted of the vitamin (Carlucci and Bowes, 1970; Swift and Guillard, 1978). Vitamin B_{12} levels in the Peconic Bay during the summer and most other seasons were always detectable.

It is more likely that bacterial production is an important authocthonous source

of vitamin B_{12} and its analogs in this estuary. Bacterial cell numbers and metabolic rate would be increased with higher summer temperatures and Berand *et al.* (1976) have shown that most coastal bacteria are producers rather than consumers of vitamins. Release of bacterially produced cobalamins from the bottom sediment is very likely an important source for the estuary. Burkholder and Burkholder (1956) found high levels of cobalamin in bottom sediments along the coast of Georgia. Any cobalamins released from bottom sediments of the Peconic Bay estuary would be rapidly mixed through the water column as this estuary is essentially vertically homogeneous (Bruno *et al.*, 1980). The observation that most of the B_{12}-like compounds in this estuary are analogs of vitamin B_{12} rather than B_{12} itself provides further evidence for their bacterial production. Starr *et al.* (1957) found that marine bacteria were responsible for release of vitamin B_{12} and its analogs into the medium but released more of the latter. No marine phytoplankters have yet been shown to release vitamin B_{12} analogs into their environment, although this possibility has not yet been investigated. Bacterially produced cobalamins have been shown by Haines and Guillard (1974) to be utilized by marine phytoplankters.

There are certain instances, however, when phytoplankton-produced vitamin B_{12} may be important as an authochthonous source of environmental levels of this growth factor. For instance, the diatom *Thalassiosira nordenskiöldii* is the dominant phytoplankter during March and April throughout most of the bay system. Cobalamin levels (both B_{12} and its analogs) are observed to be increasing during period and the increase in vitamin B_{12} at least, may be the result of its release into the environment by *T. nordenskiöldii*. Swift and Guillard (1978) have demonstrated in culture that some clones of this diatom will liberate the vitamin into the surrounding medium when the medium is depleted of B_{12}. Liberation occurs even if there is a considerable amount of B_{12} analog present. Assays for the months preceeding the *T. nordenskiöldii* bloom period show very low and even undetectable levels of vitamin B_{12} and its analogs. It is conceivable then, that during this bloom period, and even at other instances, phytoplankters may produce a small portion of the observed dissolved environmental levels of vitamin B_{12} in the Peconic Bay estuary.

External sources of B_{12}-like compounds could include influx from the Peconic River and smaller streams entering Flanders Bay at the headwaters of the estuary; runoff from various non-point sources surrounding the bay system; and advection of seawater into the estuary through Gardiners Bay. The latter possibility as a source of increased levels in the estuary is unlikely as tidal action tends to flush out the system and is probably more important in transporting B_{12}-like compounds out of the estuary. The possibility of B_{12}-like compounds being brought into the estuary with freshwater influx is very likely as previous studies have shown that the B_{12} content of suspended particles is high (Burkholder and Burkholder, 1956, 1958; Starr, 1956). Cattell (1973) has suggested particle runoff as an important

source of B_{12} during the summer months in the Straits of Georgia, British Columbia. One problem with this suggestion is that dissolved B_{12} + B_{12} analog levels in the headwaters of the Peconic Bay estuary were generally similar to levels at other stations with the exception of late March when B_{12} analog concentrations were very high (12.0-29.4 ng liter^{-1}). However, the release of cobalamins from suspended particles may take place very slowly and Burkholder and Burkholder (1956, 1958) have shown that B_{12} is released from suspended particles by microbial activity.

Results of the regression analyses (Table 4) do not show any consistent correlation of phytoplankton population parameters with dissolved B_{12} and/or B_{12} analog levels. The significant correlations which are observed seem to be related to one or two stations only, thus indicating that phytoplankton populations, taken in a seasonal context, are not dramatically influenced by vitamin B_{12} and/or B_{12} analog concentrations in the environment. This is probably the result of their generally high levels in the ecosystem, providing a sufficient amount of the growth factors for high rates of primary productivity and high biomass yield. It is unlikely that vitamin B_{12} or one of its analogs ever limit phytoplankton biomass yield or the growth rate of the phytoplankton population as a whole. Investigations in this estuary have determined that light and less often temperature, are the two factors which are overwhelmingly important in regulating rates of primary productivity for phytoplankton populations (Bruno *et al.*, 1980, in preparation). This, however, does not rule out the possibility that the growth rates of individual phytoplankton species may be affected by low concentrations of dissolved cobalamins in the estuary or other coastal waters. If this is so, cobalamin levels in the environment may play an important role in seasonal succession of phytoplankton species. During the fall and winter months vitamin B_{12} and B_{12} analog levels are often undetectable (<0.05 ng liter^{-1}) and may limit growth rates of organisms with mammalian and/or coliform type response to vitamin B_{12}-like compounds. These dissolved levels are below the half-saturation constants (K_s) determined for vitamin B_{12} auxotrophic marine phytoplanters studied in culture (Droop, 1968, 1970; Swift and Taylor, 1974). Unfortunately, no data are available on the effect of varying concentration of vitamin B_{12} analogs on phytoplankton growth rate.

Mean concentration for vitamin B_{12} plus B_{12} analogs in the Peconic Bay estuary for the entire investigation was measured at 5.5 ng liter^{-1}. This figure only represents the amount available to the assay organisms and the actual concentration in the environment may be much higher. Vitamin B_{12} (and probably its analogs) is also present in the environment as an organic complex with B_{12} binding factors (Droop, 1968) which appear to be proteinaceous in nature (Pintner and Altmeyer, 1979). These binding factors can be degraded by bacteria and the bound B_{12}-like compounds are therefore potentially available to the phytoplankton population (Swift and Guillard, 1977). Also, Sharma *et al.* (1979), using a radioisotope dilu-

tion technique to measure B_{12} and B_{12} analogs in seawater, have observed levels 4-10 times higher than results obtained by standard microbiological assays. The disparity between results obtained by the biological and isotopic methods may be explained by the fact that the latter technique measures both biologically active and inactive forms indiscriminately. Such inactive B_{12}-like compounds may be made up of corrinoids, such as lactam-B_{12} and lactone-B_{12}, which are potentially available to phytoplankters through further microbial transformation and which result from break-down of B_{12}, other cobalamines, and cobamides. Considering the tedious nature of bioassay techniques, any improvement in the already very rapid radioisotopic method which would make this technique specific for vitamin B_{12} and individual B_{12} analogs would be a significant contribution.

The interaction of B_{12} and/or B_{12} analogs with marine microbial populations appears to be quite complex. The results and observations presented in this paper reflect this complexity in one estuarine ecosystem. Any study designed to fully unravel this complexity must include: 1) simultaneous investigations of seasonal variations in the distribution of cobalamins in the water column and sediments; 2) the *in situ* rates of cobalamin production and removal by both biological and non-biological processes; 3) the relationship between seasonal variation in the distribution of cobalamins and phytoplankton species; 4) the identification of cobalamin producers and consumers; and 5) the presence and biological activity of cobalamin binders which may render a variety of cobalamins unavailable for microbial use.

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