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INTRODUCTION

Ecological studies of edaphic salt marsh diatoms have been few in number and limited in scope. Carter (1933), Salah (1952 and 1955), and Obeng-Asamoa (1968) identified the species present at their respective salt marshes and correlated this with season and marsh habitat. Williams (1962) identified diatoms from the mud where the grass Spartina alterniflora was found and measured the standing crop of diatoms and their productivity. Gallagher (1971) investigated productivity and respiration of edaphic algal communities of a salt marsh in Delaware, the majority of these communities being mainly composed of diatoms. The standing crop of edaphic algae was also measured and was correlated with productivity patterns. A significant feature of this study included manipulation of light intensity and temperature to test its effect on productivity of the edaphic algal communities. Sullivan (1971), working in the same salt marsh as Gallagher, identified a total of 104 species belonging to at least one of five edaphic diatom assemblages under investigation. Occurrence in a particular diatom assemblage for a species was easier to explain than periodicity in the assemblage. It was suggested that light, temperature, desiccation, and salinity play the dominant roles in influencing diatom growth and abundance on the marsh. According to Hendey (1964) the mud-dwelling diatoms may have access to an inexhaustible supply of nitrogen and phosphorus from below; therefore, the role of these two elements is in question on the salt marsh as far as the edaphic diatoms are concerned. A better understanding of the effect of these ecological parameters on the salt marsh diatom assemblages and associated soil algae seems imperative at this point in time.

OBJECTIVES

The chief objective of the research is to test the hypothesis that light intensity and nutrients are significant factors in the structure of the edaphic diatom assemblage and in the abundance of edaphic soil algae. The effect of temperature on diatom periodicity was discussed in some detail by Sullivan (1971). It was also pointed out in this work that salinity and desiccation seem to be more important in determining occurrence of a particular diatom species rather than its periodicity, although desiccation may have a stronger role than salinity in predicting periodicity. The importance of light and nutrients were less understood and it is these parameters that may have important effects on the dynamic structure of the edaphic diatom assemblage and abundance of soil algae.

The questions that the research program is designed to answer are as follows:

1. What is the effect of altering light intensity in the field on the structure of the edaphic diatom assemblage of the dwarf Spartina alterniflora and the total standing crop of edaphic algae that exists there?
2. What is the effect of adding phosphorus and nitrogen to the mud surface on this same diatom assemblage and the standing crop of edaphic algae?

3. Is there an interaction between altered light intensity and nutrients on this diatom assemblage and the total standing crop of edaphic algae?

MATERIALS AND METHODS

Manipulation of Field Sites

This research was carried out in the Canary Creek salt marsh, Lewes, Delaware, and was restricted to a pure stand of dwarf *S. alterniflora*. This particular grass covers approximately 50% of the total area of the marsh, the edaphic algae have the highest gross annual production in this area of the marsh (Gallagher, 1971), and a high diversity and distributional uniformity of diatom species is known to exist here (Sullivan, 1971).

Levels of light and nutrient addition to the mud surface for the various treatment combinations are summarized below:

Light	No nutrients	N added	P added
Natural	X	X	X
30% shade	X	X	X
60% shade	X	X	X
Clipped	X	X	X

There are four levels of light and in a sense three levels of nutrients for a total of 12 experimental combinations or treatments. In addition, two replicates of each experimental combination were set up.

The treatment sites were isolated enough from each other so that no "leakage" of nutrients occurred from one treatment site to another. Addition of the fertilizer during low tide periods also helped to eliminate this problem. All treatment sites were located between the same two drainage ditches in close proximity with one another.

The natural areas are untouched and served as controls for the research. Clipped areas have been clipped down to the mud-water interface so that the edaphic algae receive full ambient sunlight.

Saran shade cloth was employed to provide a shade cover of 30% and 60%, in addition to the shade already provided by the untouched spermatophyte cover. The shade cloth is suspended over the tips of the spermatophyte shoots using two-by-fours as supporting posts. The spermatophyte cover has only been shaded at these experimental sites, along with the edaphic marsh algae.

The purpose of clipping the spermatophytes down to ground level is to produce an environment like that found at the bare bank and panne, where the marsh surface always receives full ambient sunlight and desiccation becomes an important factor. Adding a shade cover, to that already existing due to the presence of spermatophyte cover, will create an environment more like that of the tall *S. alterniflora* and *Distichlis spicata*. At these areas of the marsh, light intensity reaching the marsh surface is less than that reaching the marsh surface under dwarf *S. alterniflora* shoots. It is already known what diatom species are found in these other four areas throughout the year (Sullivan, 1971), and what the standing crop of edaphic algae approximates at these same areas (Gallagher, 1971). It is obvious that this information may be of great significance in the interpretation of any significant changes in the structure of the diatom assemblages exposed to different combinations of light intensity and nutrients.

Nitrogen was added in the form of ammonium nitrate - a common commercial fertilizer. Both ammonia and nitrate nitrogen was thus available to the edaphic algae. Another commercial fertilizer, super phosphate, was the source of phosphorus for the experiment. Each study area was fertilized on the first of the month and collections made near the fifteenth of the month.

Ammonium nitrate was added in the amount of 60 grams per square meter and super phosphate added in the amount of 35 grams per square meter each month. These amounts of fertilizer are equivalent to rates of utilization or uptake by the spermatophytes of nitrogen and phosphorus respectively per year. Such a rate of fertilization provides an additional source of nitrogen and phosphorus over what is normally available to the edaphic algae.

Collection, Cleaning, and Mounting of Diatoms

Cores of the marsh were taken at random in each experimental site, using a random number table to designate one of eight quadrats. Each quadrat measured one by one-half meter in dimension. Sampling took place every two months. More frequent sampling was not deemed advisable since it would irreversibly destroy the entire area and the diatoms had been sampled at least once a month for two years on this marsh. The work of Sullivan (1971) has shown that no profound changes occur in the diatom assemblage of the dwarf S. alterniflora in the course of a month's time. The schedule of sampling was set up to coincide with changes observed in previous work.

Upon return to the laboratory, a sheet of filter paper was laid on the top centimeter of each core and moistened with filtered sea-water. At 10 A.M. the next morning the diatoms trapped in the filter paper were ready to be cleaned. Eaton and Moss (1966) describe this method, collecting almost 90% of the cells, and more important, only the live onces. The diatoms were then cleaned and mounted according to the procedure described by Patrick and Reimer (1966), except that nitric acid was used instead of sulphuric acid.

Estimation of Standing Crop of Edaphic Algae

Three chlorophyll cores were taken at random each month at each study area for a total of six replicates for each experimental combination, again using a random number table to designate quadrats. Pigments from each soil sample are extracted using 90% acetone and magnesium carbonate suspension as described for phytoplankton pigments by Strickland and Parsons (1968) with correction for phaeo-pigments. The terminology of Gallagher (1971) will be followed and the measurement of chlorophyll a corrected for phaeo-pigments will thus be called edaphic chlorophyll.

Soil Chemistry

The rest of the core not used for collection of the diatoms was analyzed chemically for magnesium, calcium, total phosphorus, potash, and soil pH by the Soil Chemistry Laboratory of the College of Agricultural Science, University of Delaware.

RESULTS

EDAPHIC DIATOMS

The permanent slides made to date have not been counted and, therefore, this report will concentrate primarily on the edaphic algal standing crop and the chemistry of the marsh soil.

EDAPHIC ALGAL STANDING CROP

Edaphic Chlorophyll

Due to the presence of large quantities of degraded chlorophyll, the trichromatic method of determining chlorophyll a gives values that are much greater than the active portion existing in the soil. Absorbance values were first measured at 750 and 665 millimicrons for the chlorophyll extracts. The solutions were then acidified with dilute hydrochloric acid and remeasured at the same wavelengths. The readings at 750 millimicrons are turbidity blanks and are subtracted from the 665 millimicron readings for both non-acidified and acidified extracts. The difference between the absorbance value of the non-acidified extract corrected for turbidity blank (665*) and the corrected absorbance value of the acidified extract (665a*) is used to calculate the concentration of active chlorophyll a per square meter of marsh surface. This estimate of algal biomass or standing crop is thus a measure of the chlorophyll a of cells that are capable of photosynthesis if environmental conditions are favorable. We therefore have in the edaphic chlorophyll measurement, an estimate of the primary productivity potential, which is the most important ecological feature of the organisms under study.

The corrected absorbance values (665* and 665a*) can also be used to calculate an absorbance ratio which will be discussed later.

The concentration of edaphic chlorophyll per square meter for each study area during the study is presented in Table 1. In Table 2 can be found the mean value of edaphic chlorophyll at each study area during the descendis and ascendis periods. Gallagher (1971) divided the year into three segments: thermis, mid-May to mid-September; descendis, mid-September to mid-January; and ascendis, mid-January to mid-May. It was felt that these year segments reflected the light and temperature patterns and metabolic activities of the edaphic algae more closely than the traditional four seasons. In adopting these time divisions for Table 2, the descendis period included the 9/17/71 to 2/15/72 collections, and the ascendis period included the 3/17/72 - 5/15/72 collections. The reason for including the 2/15/72 collection in descendis rather than ascendis was because of cold winter temperatures existing into early March and delaying the normal development of the marsh flora by approximately one month. The start of the ascendis period used in this study coincides with the appearance of the first seedlings of dwarf Spartina plants in all study areas, which further strengthens the argument for excluding the 2/15/72 collection from ascendis.

The most obvious feature of both Tables 1 and 2 is the much larger amount of edaphic chlorophyll in the three clipped areas throughout most of the study. The maximum value of edaphic chlorophyll for natural dwarf Spartina reported by Gallagher (1971) was 183 mg/M^2 during ascendis.

TABLES

Explanation of Study Area Codes

N - O = natural area, no fertilizer

N - N = natural area, ammonium nitrate fertilizer

N - P = natural area, super phosphate fertilizer

C - O = clipped area, no fertilizer

C - N = clipped area, ammonium nitrate fertilizer

C - P = clipped area, super phosphate fertilizer

30 - N = natural area, 30% shade cover, ammonium nitrate fertilizer

30 - P = natural area, 30% shade cover and super phosphate fertilizer

60 - O = natural area, 60% shade cover, no fertilizer

60 - N = natural area, 60% shade cover and ammonium nitrate fertilizer

60 - P = natural area, 60% shade cover and super phosphate fertilizer

It should be remembered that the study areas with 30% and 60% shade cover have not been clipped and therefore the dwarf *Spartina alterniflora* plants are intact and shade cloths are suspended just over the shoot tips.

Table 1. Edaphic chlorophyll (mg/M^2) in the upper centimeter of the marsh surface at each study area.

STUDY AREA	9/17	10/15	11/12	12/15	2/15	3/17	4/14	5/15
N - O	79	88	103	126	100	115	133	115
N - N	130	118	119	110	111	155	185	149
N - P	110	120	154	142	162	160	158	177
C - O	144	164	149	136	162	229	210	232
C - N	175	188	156	97	112	165	202	201
C - P	168	194	117	144	145	216	244	244
30 - O	84	110	116	130	152	166	135	149
30 - N	75	112	149	177	137	161	194	184
30 - P	86	101	131	122	138	193	148	164
60 - O	91	95	103	125	140	132	147	123
60 - N	93	78	134	108	114	135	132	118
60 - P	69	66	103	106	100	151	169	139

Table 2. Mean concentration of edaphic chlorophyll (mg/M^2) in the upper centimeter of the marsh surface at each study area during descendis and ascendis periods.

STUDY AREA	DESCENDIS	ASCENDIS
N - O	99	121
N - N	118	163
N - P	138	165
C - O	151	224
C - N	146	186
C - P	154	235
30 - O	118	150
30 - N	130	180
30 - P	116	168
60 - O	111	134
60 - N	105	128
60 - P	89	153

During this same time period in the present study, the maximum concentration for the N - 0 area was 133 mg/M^2 (4/14/72), and for the C - 0 area 232 mg/M^2 (5/15/72). Increasing the light intensity reaching the marsh surface to a maximum value by clipping the dwarf Spartina plants not only increased the standing crop of edaphic algae, but also caused a change in the natural algal flora and in the salinity regime as well.

Filamentous green (Rhizoclonium sp.) and blue green algae were much more abundant in all three clipped areas than in any other area. In fact, on 3/17/72 an algal mat formed in the C - 0 and C - P areas similar to those found in salt pannes. In some cases the large standing crop of filamentous algae seemed to reduce the diatom populations in the clipped areas. The study areas with shade covers (30 - 0, N, P and 60 - 0, N, P) and the natural areas (N - 0, N, P) without shade covers seemed to have a reduced crop of filamentous algae, this reduction being most extreme in the 60% shaded areas.

Both the salinity of the standing water and interstitial water of the marsh surface were increased in the clipped areas relative to the other treatment sites. The interstitial water had a salinity of 76 ^{o}/oo on 6/7/72 and 150 ^{o}/oo on 4/14/72 in the C - 0 area, while the highest recorded such salinity recorded by Sullivan (1971) for natural dwarf Spartina was 53 ^{o}/oo on 5/16/70. The salinity of the standing water on the three clipped areas ranged from 45 - 55 ^{o}/oo on 6/7/72 while the corresponding values for all other study areas ranged from 31 - 38 ^{o}/oo. This is a good example of the moderating effect of the dwarf Spartina plants in reducing environmental fluctuations in physical parameters such as salinity and temperature. It would seem that the increased light intensity reaching the marsh surface, rather than increased salinity, is responsible for the large concentrations of filamentous algae in the clipped areas. The algal flora of tall Spartina areas is almost exclusively composed of diatoms, where light intensity reaching the marsh surface is usually less than that reaching the marsh surface of the natural dwarf Spartina areas.

Although the edaphic chlorophyll concentration was increased substantially by clipping the spermatophyte cover, the opposite corollary - namely, that shading the dwarf Spartina plants should reduce the edaphic algal standing crop -- did not appear to hold true for this study.

If one groups all three natural areas (N - 0, N - N, and N - P), the three clipped areas, etc., and calculates a mean value of edaphic chlorophyll for all natural, clipped, etc. areas, the following would be the result:

STUDY AREA	DESCENDIS	ASCENDIS
N	118	150
C	150	215
30	121	166
60	102	138

This table makes the assumption that nutrients have no effect on the edaphic algal standing crop. Although this does not seem to be the case, such a grouping is useful to compare light treatments in the different areas. It can be seen that the 30% shade cover had an associated increase in edaphic chlorophyll while 60% shade cover tended to have a decreased concentration. It seems that suspending shade cloths over the spermatophytes has no significant effect on the total biomass of edaphic algae and perhaps on its productivity. When a statistical analysis of the data has been completed, a better understanding of the effect of reducing the light intensity reaching the marsh surface on edaphic chlorophyll, if any, will result. Initially, it was thought that reducing the light intensity would decrease the rate of photosynthesis and hence energy available for cell division. However, this does not seem to have occurred in the shaded dwarf *Spartina* areas. Possible explanations are that the light intensity reaching the marsh surface is adequate to support the normal edaphic chlorophyll concentrations found in natural dwarf *Spartina* areas, or that those algae adapted to low light intensities have become dominant in the shaded areas and reproduced at more rapid rates than normal. It is more probable that the interaction of both explanations for the different algal species is involved. The latter explanation will be tested when the permanent slides of diatoms are counted.

The inorganic nitrogen and phosphorus added to the marsh surface tended to increase the edaphic algal standing crop in the unclipped dwarf *Spartina* areas, particularly during ascendis. The addition of nitrogen as both ammonia and nitrate seemed to have an inhibitory effect in the C - N area in the 12/15/71 and 2/15/72 collections, coinciding with a period of high nitrate concentrations found in Canary Creek.

Edaphic chlorophyll increased substantially in the N - N and 30 - N study areas during ascendis (Table 2), while the 60 - N area had approximately the same value as the 60 - 0 area. The increase may be significant in view of the fact that nitrate concentrations in Canary Creek tend to reach a minimum as summer nears, and this particular marsh is considered to be low in nitrogen and high in phosphorus.

The effect of inorganic phosphorus was seen to be much greater in the N - P than 30 - P and 60 - P areas. Edaphic chlorophyll was particularly high in the N - P area during descendis and ascendis when compared to the N - 0 area. The concentration of inorganic phosphorus reaches a minimum during the winter months, and may be a limiting nutrient for edaphic algae during this time period. Phosphorus addition to the marsh surface seemed to have no effect during descendis in the 30 - P and 60 - P study areas, but the edaphic algal standing crop did increase somewhat in these areas during ascendis.

The addition of inorganic nitrogen and phosphorus to the clipped areas seemed to have no stimulatory effect, and may have even been inhibitory in the C - N area during the winter months.

The following comparisons between the present study and that of Gallagher (1971) can be made:

(1) the edaphic chlorophyll in the N - 0 area followed the same pattern from September to May as Gallagher's natural dwarf Spartina area, but its concentration was reduced in the present study.

(2) the edaphic chlorophyll was substantially higher in ascendis than descendis in all areas under study, just as Gallagher found in natural dwarf and tall Spartina and Distichlis areas.

Phaeo-pigments

Table 3 gives the concentration of phaeo-pigments at each study area during the sampling period. The phaeo-pigments, as measured in the procedure previously discussed, are degradation products of chlorophyll a, and the edaphic algae are a major source of these pigments. Of interest is the fact that the N - N area seemed to have a fairly consistent higher concentration of phaeo-pigments than the other eleven areas under study. Except for this observation (Table 3) no real trend can be seen in the other areas. Measurement of phaeo-pigments in the marsh surface may give an idea of the turnover rate in the edaphic algal communities, but evidence for this has not surfaced in the present study.

665*/665a* Absorbance Ratio

The figures contained in Table 4 represent the ratio of the absorbance value at 665 millimicrons corrected for turbidity of the unacidified chlorophyll extract and the absorbance value at 665 millimicrons corrected for turbidity of the acidified chlorophyll extract.

Table 3. Phaeo-pigments (mg/M^2) in the upper centimeter of the marsh surface at each study area.

STUDY AREA	9/17	10/15	11/12	12/15	2/15	3/17	4/14	5/15
N - O	306	330	322	312	269	253	283	284
N - N	464	440	401	433	447	367	441	434
N - P	327	406	359	345	340	339	347	358
C - O	406	395	370	352	318	344	347	366
C - N	369	431	374	488	414	359	404	401
C - P	356	398	352	333	300	389	413	396
30 - O	301	307	303	308	362	317	319	343
30 - N	304	336	323	327	290	332	385	352
30 - P	318	304	345	311	286	376	323	374
60 - O	333	349	314	306	340	292	329	314
60 - N	346	349	386	379	277	348	325	334
60 - P	257	258	317	292	260	304	292	339

Table 4. 665*/665a* absorbance ratio for upper centimeter of the marsh surface at each study area.

STUDY AREA	9/17	10/15	11/12	12/15	2/15	3/17	4/14	5/15
N - O	1.14	1.14	1.17	1.20	1.19	1.22	1.22	1.20
N - N	1.15	1.14	1.16	1.14	1.13	1.20	1.21	1.18
N - P	1.17	1.16	1.21	1.20	1.23	1.22	1.23	1.23
C - O	1.18	1.21	1.20	1.19	1.24	1.28	1.26	1.28
C - N	1.22	1.21	1.21	1.12	1.15	1.22	1.23	1.24
C - P	1.22	1.23	1.18	1.21	1.23	1.25	1.26	1.27
30 - O	1.15	1.18	1.20	1.21	1.21	1.24	1.21	1.21
30 - N	1.14	1.17	1.22	1.25	1.22	1.23	1.24	1.24
30 - P	1.14	1.17	1.19	1.20	1.22	1.24	1.22	1.21
60 - O	1.14	1.16	1.18	1.20	1.20	1.22	1.22	1.20
60 - N	1.15	1.13	1.18	1.15	1.20	1.20	1.20	1.19
60 - P	1.15	1.14	1.17	1.18	1.19	1.23	1.26	1.20

Tietjen (1968) calculated such ratios for estuarine sediments using the same general extraction procedure discussed in this paper, but made the measurements of unacidified and acidified extracts with a fluorometer instead of a spectrophotometer. His ratio was thus based on the fluorescence of chlorophyll a and phaeo-pigments and was reported as the $F_o:F_a$ ratio.

The highest absorbance ratios usually existed in the clipped areas (Table 4), reaching a maximum value of 1.28. Whether or not significant differences exist between the different areas in regard to absorbance ratios has not yet been determined.

A $F_o:F_a$ ratio of 1.35 or less indicates that 50% or more of the total sediment pigment content is represented by phaeo-pigments (Tietjen, 1968). The low absorbance ratios found in the dwarf *Spartina* areas agree well with the predominance of phaeo-pigments over chlorophyll a in all extracts prepared during the course of the study. The highest percentage of chlorophyll a was 40% in the C - 0 area on 3/17/72, with an absorbance ratio of 1.28. The low absorbance ratios calculated in Table 4 are representative of estuarine sediments that are sites of deposition, where tidal currents are weak, organic matter is high, and silt and clay predominate in the sediment grain composition (Tietjen, 1968).

CHEMISTRY OF THE MARSH SOIL

Magnesium

Fluctuations in magnesium concentration in the marsh soil did not follow any detectable pattern (Table 5). Magnesium is an essential component of the chlorophyll molecules of the spermatophytes and edaphic algae, but is not assumed to be a limiting nutrient on the salt marsh.

Potassium

This essential plant nutrient was measured as water soluble potash (K_2O) in the marsh soil at each study area. Table 6 lists the results and again no detectable pattern seems to be evident. Potassium acts as a balancing force between the effects of nitrogen and phosphorus on plant growth, and is necessary in the synthesis of chlorophyll (Lyon and Buckman, 1943).

Calcium

There seems to be a trend of increasing marsh soil calcium concentrations in most study areas with time (Table 7). Calcium is an important element in controlling soil pH as it is the most freely active metal in the soil, due to the ease with which it forms mineral and organic colloidal complexes (Lyon and Buckman, 1943). Ionic exchange in the soil occurs most readily with calcium ions (exchanged for H^+) and this can exert large effects on soil pH.

Soil pH

The data show the dwarf Spartina soils to be generally acidic, with pH values becoming as low as 5.2 (Table 8). According to Lyon and Buckman (1943), acidity occurs in soils that lose appreciable amounts of calcium and magnesium or by dissociation of hydrogen-carrying soil colloids. Carbon dioxide pressure was also cited as being important as carbonic acid tends to be formed and this will generally lower the soil pH. High rates of photosynthesis by edaphic algae increase the pH of the standing water on the marsh surface by uptake of dissolved carbon dioxide (Gallagher, 1971). pH has long been recognized as a major factor in the distribution of algae since it determines what ionic form various nutrients will assume in the water that bathes the algal cells.

The preceeding effect of pH on ionic solubility is important in determining what happens to the phosphorus fertilizer applied to the marsh surface in four of the twelve study areas. Lyon and Buckman (1943) state that addition of super phosphate ($CaH_4(PO_4)_2$) at a soil pH of approximately 5.6 to 6.5 will result in the formation of $HPO_4^{=}$ and $H_2PO_4^{-}$ ions, which are the ionic form of phosphate readily available to the edaphic algae and dwarf <u>Spartina</u> plants. These ions are held in the soil colloids by anion adsorption, the phosphate ions possibly replacing the silicate or hydroxyl radicals. Lyon and Buckman go on further to say that above a pH of 7 the $PO_4^{\equiv}$ predominates, which is unavailable to plants and forms insoluble calcium phosphates. Below a pH of 5 insoluble aluminum and iron phosphates are formed, again unavailable to the plants.

In looking at the pH values for the areas fertilized with super phosphate (N - P, C - P, 30 - P, and 60 - P), one sees that the pH for the most part falls in the range favorable for formation of the ionic forms available to the plants and edaphic algae - $HPO_4^{=}$ and $H_2PO_4^{-}$.

Phosphorus

Up until the 1/17/72 collection, it can be seen that phosphorus was not particularly high in the areas fertilized with super phosphate relative to the non-phosphorus areas (Table 9). The situation changed during the 3/17/72 and 5/15/72 collections. Phosphorus was much higher in the former relative to the latter areas even though phosphorus concentrations had increased substantially in the latter areas. Two facts must be kept in mind in trying to interpret the data:

(1) phosphorus concentrations reach a maximum in the summer and a minimum in the winter in Canary Creek, and

(2) the time period from March through May (ascendis) is one of great phosphorus utilization by the new crop of dwarf *Spartina* plants and increased biomass of edaphic algae in the unclipped areas.

Phosphorus addition to the unclipped marsh surface did increase the edaphic algal standing crop in all areas relative to unfertilized areas during ascendis, but such an effect was only seen in the N - P area during descendis. If phosphorus is to be deemed responsible for increasing edaphic chlorophyll during ascendis, then it should be scarce during this time period. The data show the opposite situation existing on the marsh. Phosphorus concentrations are lowest during the winter months, but only the N - P area showed an increase in edaphic chlorophyll in response to super phosphate. One possible explanation for this seemingly contradictory data is that although phosphorus concentrations increase substantially in ascendis due to higher

Table 5. Magnesium (in parts per million) in marsh soil at each study area during 1971-72 in Canary Creek Marsh.

STUDY AREA	9/17	11/12	3/17	5/15
N - O	160	156	161	212
N - N	146	151	148	204
N - P	94	140	184	157
C - O	188	128	265	138
C - N	153	152	170	188
C - P	149	157	160	157
30 - O	147	176	186	224
30 - N	156	187	208	182
30 - P	112	189	208	235
60 - O	164	110	171	184
60 - N	126	183	164	130
60 - P	156	196	244	173

Table 6. Potash (in parts per million) in marsh soil at each study area during 1971-72 in Canary Creek Marsh.

STUDY AREA	9/17	11/12	3/17	5/15
N - O	121	135	110	134
N - N	120	111	98	140
N - P	56	94	128	121
C - O	148	90	175	102
C - N	106	100	105	130
C - P	88	121	110	100
30 - O	130	120	134	166
30 - N	150	125	126	116
30 - P	113	130	135	172
60 - O	172	86	110	135
60 - N	108	129	100	86
60 - P	150	173	154	126

Table 7. Calcium (in parts per million) in marsh soil at each study area during 1971-72 in Canary Creek Marsh.

STUDY AREA	9/17	11/12	3/17	5/15
N - O	125	165	144	224
N - N	116	129	144	205
N - P	140	130	152	198
C - O	125	109	159	125
C - N	112	154	148	170
C - P	112	156	132	185
30 - O	138	142	146	182
30 - N	128	156	144	168
30 - P	100	163	135	211
60 - O	132	131	106	203
60 - N	132	178	105	212
60 - P	110	147	180	200

Table 8. pH of marsh soil at each study area during 1971-72 in Canary Creek Marsh.

STUDY AREA	9/17	11/12	1/17	3/17	5/15
N - O	6.6	7.2	6.2	6.4	6.4
N - N	6.4	7.0	6.6	6.4	6.2
N - P	6.5	7.0	-	6.2	6.5
C - O	6.4	6.9	-	5.8	5.2
C - N	6.8	6.9	-	6.7	6.6
C - P	6.6	6.9	-	6.2	6.5
30 - O	6.5	7.3	6.2	6.6	5.8
30 - N	6.5	7.3	6.1	6.6	5.6
30 - P	5.9	7.2	-	6.3	5.6
60 - O	6.6	7.5	5.9	6.4	5.8
60 - N	6.3	7.4	6.6	6.2	6.4
60 - P	6.5	7.1	6.3	6.8	6.4

Table 9. Phosphorus (in parts per million) in marsh soil at each study area during 1971-72 in Canary Creek Marsh.

STUDY AREA	9/17	11/12	1/17	3/17	5/15
N - O	1.62	.61	1.80	7.0	8.4
N - N	1.72	.98	1.60	8.6	8.0
N - P	2.00	.93	-	18.1	14.2
C - O	0.80	.88	-	6.2	8.0
C - N	2.00	.59	-	7.0	12.1
C - P	2.25	.59	-	15.9	23.1
30 - O	2.02	.98	2.50	9.0	8.2
30 - N	2.30	.98	2.08	8.6	9.3
30 - P	3.45	1.13	-	20.6	35.0
60 - O	2.70	.68	1.85	7.3	9.2
60 - N	1.75	.49	1.66	6.8	7.4
60 - P	2.80	.49	3.00	17.0	18.0

concentrations in Canary Creek, the extra demand placed upon this supply by the dwarf Spartina plants renders this increase to a status of phosphorus deficiency and not abundance. The counting of the permanent slides of diatoms may shed new light on this complex but interesting phenomenon.

Path of Applied Phosphorus and Nitrogen

The results of this study indicate that the application of phosphorus as super phosphate and nitrogen as ammonium nitrate tend to increase the edaphic algal standing crops in the unclipped study areas. The question of what paths the phosphorus and nitrogen take after fertilizer application naturally arises. An experiment was conducted during June, 1972 to elucidate some facts concerning this problem.

Natural Spartina areas were fertilized with the usual amounts of super phosphate and ammonium nitrate. Plastic cores with a 6.8 cm. diameter were then inserted to a depth of approximately 10 inches in the following manner: one core in the phosphorus fertilized areas, two cores in the nitrogen fertilized area, and three cores were inserted in an unfertilized control area for comparison. The three natural Spartina areas were set up to be as close together as possible without danger of contamination occurring. The six cores were left out on the marsh for a period of 24 hours. At the end of this incubation period, the cores were dug out of the marsh soil and their ends stoppered. Upon return to the laboratory, three one inch sections were cut from the top of each core and each was placed in a glass jar. After the addition of 200 mls. of distilled water to sections that were to be analyzed for total phosphorus and nitrate and 300 mls. of distilled H_2O to samples to be analyzed for Kjeldahl nitrogen, each of the 18 sections were homogenized in a Waring blender for 30 seconds. The sample was then poured into a flask and let settle for another 24 hours. The liquid fraction was then decanted and filtered through .45u glass filters. The following analyses on the clear sediment-free liquid fraction were completed:

(1) total phosphorus was determined for the core fertilized with super phosphate and one of the control cores using the method of Menzel and Corwin (1965).

(2) nitrate nitrogen was determined for one of the cores fertilized with ammonium nitrate and a second core from the control area using the acid-brucine procedure of Jenkins (1968).

(3) Kjeldahl nitrogen was analyzed for in the second core fertilized with ammonium nitrate and the third control core using the method outlined in Standard Methods for the examination of water and waste water (1965).

Table 10 gives the results of these chemical analyses.

The core fertilized with super phosphate was significantly higher in phosphorus in all sections except perhaps the 1-2 inch section. It can be seen that six times more phosphorus was found in the upper one inch section of the fertilized core than in the corresponding section of the control core. The upper one inch section probably contains all edaphic algae that are carrying out photosynthesis and engaged in some form of reproduction. Phosphorus seems to be transported downward in the fertilized core, as its concentration increases almost two-fold beneath the upper one inch section. The concentration of phosphorus in the 1-2 inch section of the control core may be an anomalous value because of the trends exhibited by nitrate and Kjeldahl nitrogen in the other two control cores.

The core fertilized with ammonium nitrate and analyzed for nitrate nitrogen had substantial amounts of this anion in the upper two inch sections of the core. Approximately 16 times more nitrate was found in both of the upper two sections of the fertilized core over the control core. The fact that only three times more nitrate was found in the 2-3 inch section of the fertilized core may mean that the upper marsh surface may be deficient in nitrate during the period of time the experiment was carried out.

In the analysis of total Kjeldahl nitrogen ammonia and organically combined nitrogen are measured, but nitrate and nitrite nitrogen are ignored. The results in Table 10 show that we are dealing for the most part with large amounts of ammonia supplied by the fertilizer to the upper marsh surface. Downward transport of ammonia is definitely occurring after fertilizer application but substantial amounts remain in the upper two inch section after the 24 hour period. Diatoms are known to take up ammonia in preference to nitrate when both are available, therefore, the ammonia portion of the fertilizer rather than the nitrate portion may be responsible for increasing the edaphic algal standing crop when such occurred.

It should be remembered that the incubation period for the cores in the field was 24 hours, and further transport of the various chemical species may occur after this time period. The important finding of the experiment is that the phosphorus and nitrogen fertilizers are moving into the marsh soil fairly rapidly and seem to be providing additional sources of these essential elements for the edaphic algae and dwarf Spartina plants.

Effect of Nitrogen on Dwarf Spartina Plants

Although the measurement of the height of the dwarf Spartina plants was not an original objective of this study, the dramatic effect nitrogen had on the unshaded spermatophytes warrants its inclusion in this report. Height measurements began with the onset of the growing season in March and the results are listed in Table 11.

It can be seen that the height of the plants receiving nitrogen fertilizer increased substantially more than the height of the control plants receiving no fertilizer. Field observations during May and June revealed that the growth of the spermatophytes in the N - N area was much denser and luxuriant than the corresponding growth in the N - 0 area. This resulted in a decrease in the amount of the light reaching the marsh surface, but did not seem to affect the edaphic algal standing crop during this time period. Lyon and Buckman (1943) state that the addition of nitrogen tends to encourage above-ground vegetative growth and to impart to the leaves a deep green color. This is exactly what happened in the N - N area, and to a lesser extent the 30 - N area,

Table 10. Concentration of phosphorus (ug-at/l), nitrate (ug-at/l), and Kjeldahl nitrogen (mg/l) in one inch sections of cores both unfertilized and fertilized with super phosphate and ammonium nitrate (NH_4NO_3) in the dwarf Spartina alterniflora on June 6, 1972.

	Phosphorus		Nitrate		Kjeldahl Nitrogen	
Core Section	Control	Fertilized	Control	Fertilized	Control	Fertilized
0 - 1"	4.0	26.4	2.0	31.5	1.33	182.7
1 - 2"	31.6	46.8	12.5	204.0	2.00	49.7
2 - 3"	5.3	49.5	35.1	110.0	0.00	9.9

Table 11. Height of dwarf *Spartina* plants in the natural unfertilized area (N - 0) and natural area fertilized with ammonium nitrate (N - N) in cms.

Date	N - 0	N - N
3/17/72	Tiny seedlings in both areas 1-2 cms. high	
4/14/72	5-7	5-7
5/15/72	25-28	41
6/15/72	37	59

where shoot height and plant density was greater than that found in the 30 - 0 area. The 60 - N area showed no difference from the 60 - N area. This sequence seems to indicate that shading of the dwarf Spartina plants tends to reduce the beneficial effect of additional nitrogen, nullifying the effect completely in the 60 - N area. In all areas where phosphorus fertilizer was supplied to the dwarf Spartina plants, no differences could be detected in such areas when compared to the unfertilized control areas. The results give very strong evidence that nitrogen in the form of ammonia or nitrate is a limiting nutrient as far as the dwarf Spartina plants of the Canary Creek salt marsh are concerned. This is also in agreement with the finding that this particular salt marsh is a high phosphorus - low nitrogen area. Tyler (1967) found that the addition of ammonia supplied as NH_4Cl and phosphorus as NaH_2PO_4 brought about an increase in the production of a Juncus gerardi shore meadow amounting to approximately 30%, while the addition of nitrate as $NaNO_3$ had very little effect. The addition of the former two nutrients also resulted in an increase of the nitrogen and phosphorus contents of the shoots respectively. It was suggested that since the clayey soils had a lower capacity for nitrate than ammonium ions, the effect of the latter would be more pronounced because the clay colloids serve primarily as acidoids, absorbing or releasing cations. This discussion of the relative effects of ammonia and nitrate lend support to the hypothesis that the dwarf Spartina plants are responding primarily to ammonia. The data from the present experiment justifies future investigations into the effectiveness of ammonia and nitrate, acting in concert and alone, in promoting the growth of dwarf Spartina plants.

The maximum height of the dwarf Spartina plants during the 1969 growing season was approximately 30 cms. during the month of October (Gallagher, 1971). The fact that the spermatophyte height recorded on 6/15/72 was 37 cm. in the present study deserves some attention. The Canary Creek salt marsh has not been ditched at or near the investigation area since studies of the edaphic algae began in July, 1969. Field observations during the three years of investigation show the lateral ditches to be filling in quite rapidly. The tall Spartina plants now cover a much wider area of the ditch, and the bare bank areas have disappeared as dwarf Spartina plants now grow to the edge of the shallow ditches. Since the ditches have filled in to a considerable extent, flooding of the marsh proper occurs more frequently. It is generally agreed that the Spartina plants will grow taller in response to increased inundation. An almost inescapable conclusion from the above discussion is that taller dwarf-form Spartina plants are growing on the marsh proper in 1972 than those that grew in 1969 because of the increased frequency of tides flooding the marsh surface. The productivity of the marsh has thus been increased by the taller dwarf-form Spartina plants and the presence of these forms on areas that were once bare banks. The diversity of diatoms is much greater in the spermatophyte areas than on the bare bank (Sullivan, 1971) and edaphic algal productivity in the bare bank is the lowest for the Canary Creek salt marsh (Gallagher, 1971). Therefore, this report makes the recommendation that the most productive marsh management is no ditching management. Interference by man in the form of ditching causes a decrease in the productivity of the salt marsh when compared to the greatly increased productivity of the grasses and edaphic algae found in more natural salt marshes.

SUMMARY

(1) Four levels of light (natural, clipped, 30% shade, and 60% shade) and three levels of nutrients (none, nitrogen fertilizer, and phosphorus fertilizer) were set up in the field in the dwarf *Spartina alterniflora* of Canary Creek Marsh during 1971-72. Responses to these treatments were determined by measuring edaphic chlorophyll and carrying out soil chemistry analyses in each of the twelve study areas. Permanent slides of diatoms were made, but have not been evaluated.

(2) Edaphic chlorophyll was found to be highest in the clipped areas. Increased interstitial water salinities and density of filamentous algae were also found in this study area.

(3) Shading the dwarf *Spartina* plants did not decrease the edaphic algal standing crop to any substantial degree. If anything, it tended to increase it. Filamentous algae were less prevalent in the shaded areas than in natural unshaded areas.

(4) Addition of inorganic nitrogen tended to increase the edaphic algal standing crop in the unclipped dwarf *Spartina* areas, particularly during the ascendis period.

(5) The inorganic phosphorus fertilizer had a greater effect on increasing the standing crop of edaphic algae in the N - P area than in the 30 - P and 60 - P areas during ascendis. No such increase was seen in the latter two study areas during the descendis period when phosphorus is at a minimum concentration in Canary Creek.

(6) Nitrogen and phosphorus had no stimulatory effect in the clipped areas on the edaphic algal standing crop. Nitrogen may have had an inhibitory effect in the C - N area during the winter months.

(7) Results of this study agreed with those of Gallagher (1971) in showing that edaphic algal biomass was higher in ascendis than descendis in the dwarf Spartina.

(8) Phaeo-pigments showed no detectable trend during the course of the study.

(9) Absorbance ratios calculated for the marsh showed that more than half of the edaphic pigments were degraded chlorophyll and characterized the dwarf Spartina area as one of deposition.

(10) No detectable trends in the concentrations of magnesium and potassium in the marsh soil were observed. Calcium tended to increase with time. Phosphorus was much higher in the areas fertilized with super phosphate in the March and May collections.

(11) Soil pH showed the dwarf Spartina soils to be mainly acidic and conditions favorable for allowing orthophosphate ions to be available for uptake by edaphic algae and spermatophytes after fertilizer application.

(12) Experiments undertaken to trace the path of applied nitrogen and phosphorus fertilizer indicated that ammonia, nitrate, and phosphate move into the soil quite rapidly in fairly large concentrations.

(13) Dwarf Spartina plants in natural areas responded by greatly increased shoot height and density when compared to natural plants receiving no fertilizer. This effect was less dramatic in the 30 - N

area and non-existent in the 60 - N area. Phosphorus had no effect on plant growth in the N - P, 30 - P, and 60 - P areas. These results were in agreement with the low nitrogen-high phosphorus status of Canary Creek salt marsh found in previous studies. It was hypothesized that the spermatophytes and edaphic algae were responding to the ammonium ion, rather than the nitrate ion, because of the nature of the soil colloids and other work cited in this report.

(14) Field observations over the last three years indicate the productivity of Canary Creek salt marsh has increased due to increased flooding of the marsh proper, as the formerly deep lateral ditches fill with trapped sediment. Due to this beneficial effect, as the marsh returns to a more natural state, it was recommended that this situation continue and ditching be terminated.

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