

Chapter 6

Nitrogen loading in Buttermilk Bay (MA, USA): Correlations with phytoplankton density, periphyton abundance, and eelgrass (*Zostera marina* L.) distribution

Introduction

The addition of nutrients, particularly nitrogen, has become a serious problem in coastal embayments throughout the world (Nixon, 1983; Nixon, et al., 1987). The sources of added nutrients usually include rainwater, wastewater disposal, fertilizer use, livestock, and street runoff. Nutrient additions may increase planktonic, periphytic, and benthic algae which in turn can cause important changes in coastal ecosystems (Lee and Olsen, 1985). One of the consequences of nutrient loading and increased algal growth is the disappearance of eelgrass (*Zostera marina* L.) meadows, and large scale declines of this and other species of seagrasses due to declining water quality have been reported in Chesapeake Bay and elsewhere (Cambridge and McComb, 1984; Orth and Moore, 1983, Sand-Jensen and Borum, 1985; Nienhuis, 1983).

Elsewhere I reported on the nutrient concentrations and nutrient loading in Buttermilk Bay, a Cape Cod, MA (USA) coastal embayment (Valiela and Costa, in press). In this paper, I examine how dissolved nitrogen concentrations, and patterns of nitrogen loading correlate to phytoplankton density, periphyton abundance, and eelgrass distribution and growth. This work is part of an ongoing study to quantify the impact of nutrient loading on eelgrass distribution.

Nutrient-algae-eelgrass relationships

Increased growth of algae is a common symptom of nitrogen loading in coastal embayments (Valiela, 1984). In coastal waters and estuaries, phytoplankton can increase conspicuously in response to added nitrogen;

and often correlate with nitrogen concentrations (Ryther and Dunstan, 1971; Jarworski, 1981; Monbet et al., 1981). In shallow bays where sufficient light reaches the bottom, benthic algae, especially morphological varieties of unattached "drift" algae may accumulate to a large degree (Lee and Olsen, 1983). Periphyton (microalgae on surfaces) are also more abundant in enriched environments (Sand-Jensen and Borum, 1983), and the accumulation of periphyton on artificial substrates has been used to assess the degree of eutrophication in freshwater systems (Marcus, 1980; Fairchild et al., 1985).

Increased nitrogen loading does not benefit benthic angiosperms such as eelgrass. Part of the reason for this is that in most environments, eelgrass takes up most of its nitrogen through its roots (Dennison et al., 1987; Short, 1983; McRoy and Goering, 1974; Thursby and Harlin, 1982). More importantly, increased growth of epiphytic, planktonic, and drift algae shade eelgrass populations. The lower limit of eelgrass growth is determined by the duration of light intensity above compensation (Dennison, 1987). Hence, in a fundamental way, the distribution of eelgrass is determined by factors that affect water transparency and epiphyte densities (Sand-Jensen and Borum, 1983). In southern New England, eelgrass grows only to 1-2 meters or less in shallow bays with poor water transparency, but grow as deep as 12 m MLW in clear offshore waters (Costa, 1987).

As a result of declining light availability from nutrient loading, eelgrass may show slower growth, recruitment, or death (Sand-Jensen and Borum, 1983; Borum, 1985; Kemp et al., 1983). Eelgrass beds often first

disappear in upper estuaries where nutrient loading is highest, and at the deep edges of beds where light limits growth (Orth and Moore, 1983).

Other factors may contribute to seagrass declines, and need to be considered. Sediment suspension from topsoil runoff or boat propeller wash may also contribute to water transparency decline and loss of eelgrass (Brush and Davis, 1984; Orth and Moore, 1983). This component of water quality decline may be very localized or seasonal. For example, attenuation of PAR was locally important in parts of Chesapeake Bay, but for most parts of Chesapeake Bay, results from artificial estuarine ponds and a computer simulation suggest that nutrient loading effects, rather than suspended sediments, account for most PAR attenuation (Kemp et al., 1983).

In southeastern Massachusetts, eelgrass has been declining in several bays where water quality has declined (Costa, 1987). For example, in Waquoit Bay, a Cape Cod lagoon that has been extensively developed during the last 40 years, eelgrass populations have been declining in most of the Bay since the mid-1960's (Costa, 1988). At that time, eelgrass began to disappear first from the deep central portion of the bay (2-2.4 m), then in shallower areas, especially in the inner half of the Bay. Today eelgrass is limited to the flood delta at the mouth of Waquoit Bay, and covers less than 10% of its peak abundance during the 1950's and 60's.

The loss of eelgrass in enriched environments is not unique and has been reported for other submerged macrophytes in freshwater lakes and ponds (Sondergaard and Sand-Jensen, 1981; Phillips, et. al, 1978), artificial freshwater ponds (Mulligan et al., 1976), tidal estuaries

(Haramis and Carter, 1983), artificial estuarine ponds (Twilley, et al., 1985), and other species in marine embayments (Brush and Davis, 1984; Cambridge and McComb 1984; Littler and Murray, 1975; Orth and Moore, 1983; Kautsky et al. 1986).

It is not always clear if periphyton or phytoplankton are more important in causing macrophyte loss. For example, in enriched artificial estuarine ponds, submerged angiosperms were nearly eliminated at the high loadings because epiphytes attenuated 80% of incident photosynthetically active radiation (PAR) at leaf surfaces (Twilley et al., 1985). Nonetheless, nutrient loading would not have caused the observed loss without co-occurring increases in phytoplankton (Twilley et al., 1985).

Along a nutrient gradient in a Danish estuary, biomass of eelgrass algal epiphytes increased 50-100 fold, whereas phytoplankton abundance increased only 5 - 10 fold (Borum, 1985). Light attenuation by epiphytes on eelgrass shoots was 90% on older leaves in these enriched areas (Borum, 1985). Besides shading, algal epiphytes can slow photosynthesis by forming a barrier to carbon uptake (Sand-Jensen, 1977). These observations suggest that epiphytic algae are more important in limiting eelgrass growth, but the problem is complex.

Epiphyte biomass is highest on old, slow growing plant material which contribute less to production (Borum, 1985; Sand-Jensen and Borum, 1983). Furthermore, eelgrass declines also often occur where both phytoplankton and epiphytes increase (Sand-Jensen and Borum, 1983). In less enriched habitats, epiphyte abundance is less important than water transparency in affecting eelgrass growth and photosynthesis (Mazella

and Alberte, 1986). Accumulation of epiphytes in general may only slow macrophyte growth during periods of light intensity near compensation (Sand-Jensen and Revsback, 1987).

Alternate explanations have been offered for some eelgrass declines. For example, Nienhuis (1983) suggested that the recent disappearance of eelgrass in a Danish coastal pond was not due to epiphyte abundance, but "toxification" of the sediments from decomposing drift algae that accumulated because of nutrient loading. This mechanism has not been well studied, but decomposing drift material can change appreciably pore water chemistry in seagrass beds (Zimmerman and Montgomery, 1984).

Identifying the impact of nutrient loading is complicated because eelgrass populations here and elsewhere in the Atlantic have been recovering for decades from massive declines induced by disease during the early 1930's (den Hartog, 1987; Costa, 1987). Consequently, in some polluted, poorly flushed bays in Southeastern Massachusetts, eelgrass populations never recovered from the wasting disease or showed new declines in subsequent decades (Costa, 1987, 1988).

Buttermilk Bay has been studied to determine stream and groundwater flow (Moog, 1987), water circulation (Fish, 1987), and fecal coliform pollution (Heufelder, 1987). Elsewhere I examined nutrient concentrations and inputs in Buttermilk Bay and its surrounding watershed (Valiela and Costa, in press). We reported that nitrogen concentrations were highest along shore, especially near groundwater and streams inputs carrying high DIN loads (generally from human inputs). Concentrations of DIN were variable in different parts of the Bay;

concentrations were highest in Hideaway Village Cove, Millers Cove, and near Red Brook, intermediate in Queen Sewell Cove, Skunk Cove, and Little Buttermilk, and lowest in the central portions of the Bay (see Fig. 1). In effect, a large scale natural "experiment" on nitrogen loading is underway in Buttermilk Bay.

The impact of the added nitrogen in Buttermilk Bay is not fully apparent, but there have been modest declines in eelgrass cover in the deepest parts of the Bay during the last 15 years (Costa, 1987). In this paper I present data on the relationships between nitrogen concentrations and abundance of phytoplankton, periphyton, and eelgrass depth distribution, and discuss how these data corroborate other studies of nitrogen loading in marine ecosystems.

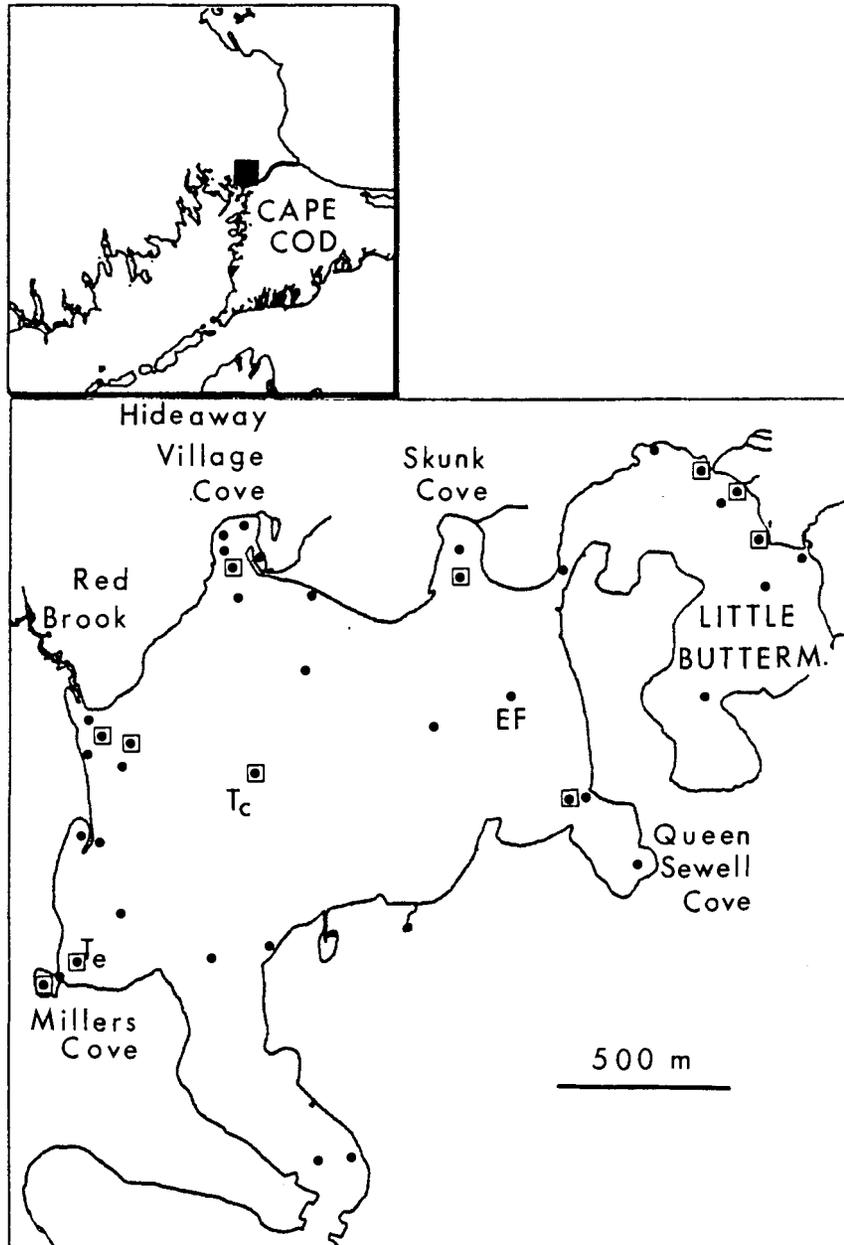
Methods

Water sampling methods and sites

Water samples (250 ml) were taken from bay, stream, and groundwater stations located in and around Buttermilk Bay (Fig 1). The water sampling methods and nitrogen analysis protocols are described elsewhere (Valiela and Costa, in press). The bay water samples were taken during ebbing, at least two hours after high tide and at least 2 days after any major storm.

The impact of nitrogen loading was assessed by examining depth of eelgrass growth, phytoplankton, and periphyton abundance. The depth of eelgrass growth at 9 stations was compared to mean DIN observed during the most active growing period for eelgrass (March- November; 5 to 15 sample dates per station). The accumulation of periphyton on eelgrass

Figure 1. Map of Buttermilk Bay showing site names and stations sample for nutrients and phytoplankton chl_a (●), similar stations but with settlement substrate floats set out (◻), transplant stations (T_c= control, T_e= enriched stations), and position of enrichment floats (EF). For purposes of clarity, not all stations measured for nutrients and phytoplankton chl_a are shown.



(11 stations) and artificial substrate (15 stations) was compared to mean DIN observed within one month of sampling (1-8 water samples dates per station).

To measure chlorophyll content, Bay water samples were filtered (Whatman GF/C), and the filter extracted in 90% acetone, placed in the dark at -15°C until analyzed, generally within 48 hr. The extract was then sonicated, centrifuged, and the absorbance read at 480, 630, 645, 650, and 750, and converted to chl_a, chl_b, and chl_c, by the trichromatic method (Strickland and Parsons, 1972).

Periphyton sampling

Periphyton attached to eelgrass was sampled by collecting 3 randomly selected shoots at 9 water sampling stations where eelgrass grew. Each shoot was placed in a bag, then later placed in a tray of filtered seawater and epiphytes were removed with a razor blade. The suspension of epiphytes was filtered and extracted as described above. The abundance of periphyton was calculated as $\mu\text{g chl}_a \text{ cm}^{-2}$ of eelgrass leaf surface.

Periphyton on eelgrass leaves may not be a reliable indicator of nitrogen exposure because of eelgrass depth of growth (thus variable light), herbivore grazing, and differences in leaf age. Consequently, periphyton was also measured on artificial settlement strips (3 strips, 1 cm x 10 cm) attached to floats and placed in different parts of the Bay. Pilot experiments on float design showed that settlement surfaces that were textured or made of screen minimized the effects of local differences in wave and current action on the colonization of epiphytes.

and in the experiments reported here, polyester screening (100 μm) was used. These floats were left out for 1 - 2 weeks so that the periphyton consisted primarily of benthic diatoms. A preliminary study showed that the diatoms on the artificial substrate were the same species that settled on eelgrass. Longer duration exposure of the strips resulted a larger algal taxa whose composition varied among the different habitats. Hence, the short settlement strip exposure minimized variability in the chl composition (see results). When the strips were harvested, they were immediately placed into centrifuge tubes containing buffered 90% acetone, and stored in the dark on ice until analysis.

To test whether small increases in nitrogen loading can cause an elevation in periphyton abundance comparable to enriched parts of Buttermilk Bay, chambers that released nutrients were attached to other floats that held settlement strips. The nutrient chamber consisted of a corked PVC pipe with perforated with holes. A slow-release fertilizer "tree spike" (Jobes, 16:8:8, N as ammonium) was added to the chamber. To slow the dissolution of the fertilizer stick and lower nutrient concentrations that the strips were exposed to, the sticks were wrapped in dialysis membrane. The strips were attached to a current vane on the float to keep them downstream of the nutrient chamber. Eight floats (4 control, 4 experimental), each with 3 settlement strips, were placed in the east central portion of Buttermilk Bay, an area of low nutrient concentrations (see Valiela and Costa, in press). Two trials were performed: one for 6 days and one for 14 days. In the longer experiment, the fertilizer sticks were replaced on the 6th day. In both experiments, nutrient concentration in the water near the strips was

measured 3 days after fertilizer sticks were added and at the end of the experiment.

Eelgrass growth and transplantation

To determine if nutrient loading slows growth of endemic populations, the growth of shoots in 3, 625 cm⁻² quadrats were measured in two areas: an enriched and a less enriched part of the Bay, both at 30-40 cm MLW. The less enriched site was located on the north lobe of an eelgrass bed in center of the bay (Fig. 1) which generally had the lowest nitrogen concentrations of any station during summer months. The enriched site was located in Millers Cove, which typically had high concentrations of DIN (Valiela and Costa, in press).

To measure eelgrass growth, randomly selected shoots within each quadrat were marked by inserting an insect pin through the top of the leaf sheath (c.f. Jacobs, 1979). After 9 days, the outgrowth of hole scars were measured to calculate the Plastochrone Interval (PI; the number of days between successive new leaves).

To rule out growth differences between the endemic populations due to sediment quality or population differences, clumps of eelgrass were collected from another locale (Ram Island in Great Harbor, Woods Hole, MA), and transplanted to these two sites in Buttermilk Bay. These shoots were collected with sediment, potted in peat fiber pots (10 cm diameter), and transplanted adjacent to the quadrats described above after an equal area of plants were removed to accommodate them. After the plants were acclimatized for two weeks, the growth of these plants were measured as described above.

The growth of the endemic populations were measured in mid-September, and the growth of the transplants were measured in Mid-October. Since the observations were not concurrent, only relative growth rates between endemic and transplanted eelgrass were considered.

Results

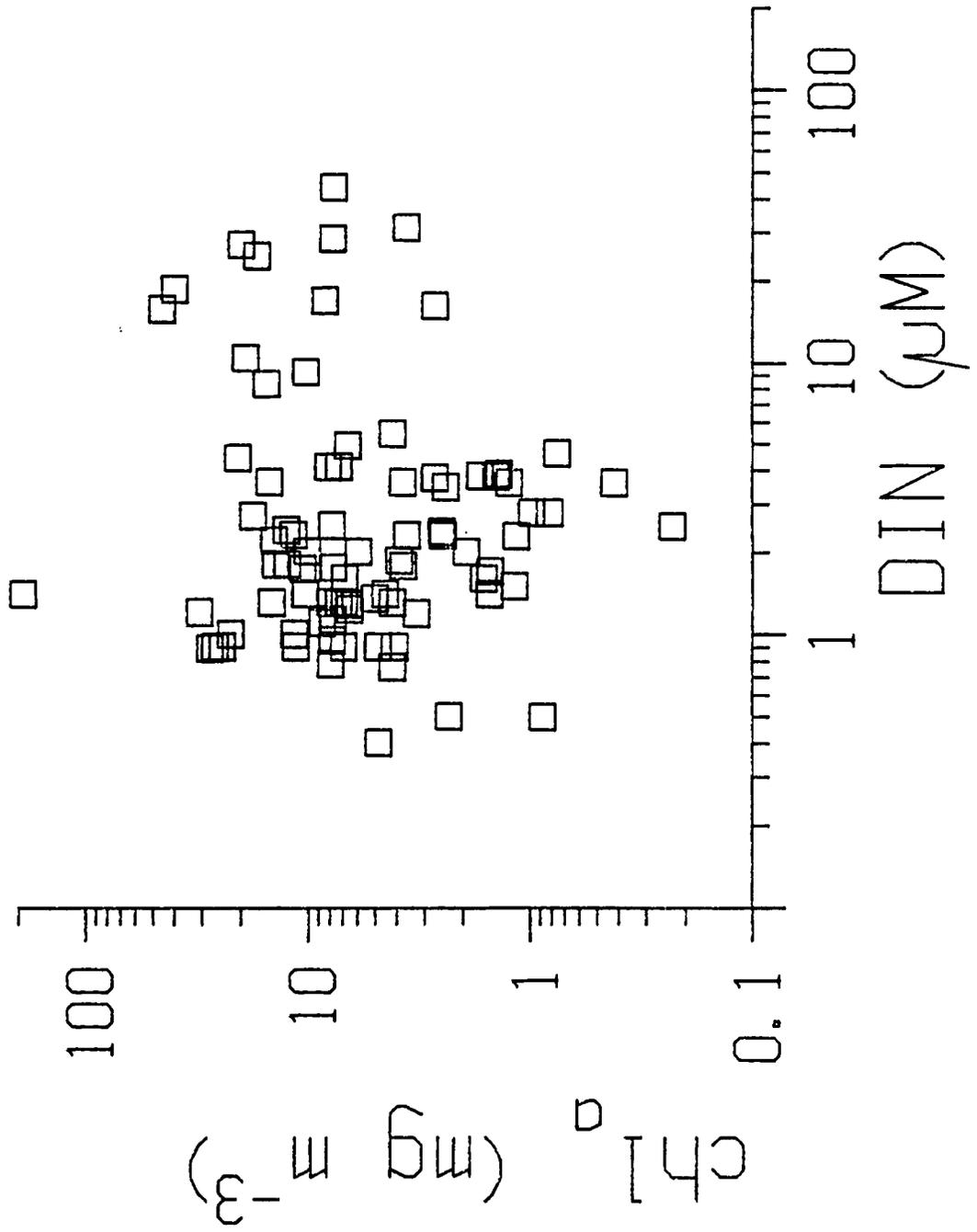
Chl_a correlated well with chl_c in periphyton from artificial substrates, eelgrass leaves, and water samples (Fig. 2). Because chl_c estimation is unreliable using the trichromatic method under some conditions, (Strickland and Parsons, 1972), all comparisons were made using chl_a. The closer correlation between chl_a and chl_c of periphyton on the artificial substrate (Fig. 2) was due to a similar taxa composition (primarily diatoms) after colonization and growth.

Chl_a concentration in the water column during June, July, and August did not correlate with surrounding water nitrate + nitrite, ammonia, total dissolved inorganic nitrogen (DIN), phosphate, or N/P ratio (DIN shown only, Fig. 3). This is because nitrogen gradients do not remain established long enough for phytoplankton abundance to build up (Valiela and Costa, in press). The absence of nitrogen gradients in Buttermilk Bay is due both to uptake by phytoplankton and benthic macrophytes and microphytes, and because the Bay is well flushed (50% of the water is exchanged with each tide (Valiela and Costa, in press). Uptake of nitrogen by benthic producers may be appreciable because this component account for 60% of the production in the Bay (Costa, 1987).

In contrast to phytoplankton, both periphyton (as chl_a) on eelgrass and periphyton on settlement strips correlated with DIN at each

Figure 2. Chl_c vs chl_a on settlement strips, eelgrass leaves, and seawater. The slopes were not statistically different ($p < 0.05$).

Figure 3. Chl_a in the water column vs DIN on each date. There was no significant correlation.



station ($r^2 = 0.62$, $p > 0.05$, $r^2 = 0.44$, $p > 0.05$ respectively, Fig. 4). In both instances, periphyton correlated better with DIN than with ammonia or nitrate + nitrite alone (not shown).

The floats containing slow release fertilizer elevated DIN concentrations ≈ 1.0 μM DIN over background concentrations (mean = 2.5 μM DIN), but increases in phosphate were not measurable. These added nutrients enhanced the growth of periphyton on strips attached to these floats at levels comparable to enriched parts of the Bay (Fig. 4).

The eelgrass shoots transplanted in pots had poor survival, and one pot in each treatment disappeared. Of the surviving marked plants, the growth rate of eelgrass transplanted to the enriched area was slightly slower (higher PI) than observed in the unenriched area, but this difference was not significant. Similarly, endemic populations of eelgrass grew slightly slower in Millers Cove, but these results also were not significant (Table 1). These results also indicate that larger scale, or longer running growth experiments are necessary to resolve whether high nutrient concentrations can lower eelgrass growth.

Depth of eelgrass growth significantly correlated with mean DIN concentration at each station (Fig. 5). The few data points is due to the limited number of deep sites in Buttermilk Bay near sampling stations. Some stations showed more variability in nitrogen concentrations than others.

Discussion

Assessing nitrogen loading impacts

Table 1. Growth rate (as PI \pm SE) of endemic and transplanted eelgrass at an enriched (Millers Cove) and less enriched (mid-Bay) areas. Growth rate of endemic and transplanted eelgrass was not measured concurrently (see text).

	PI (days)	
	Endemic	Transplanted
Mid-Bay:	17.7 \pm 1.04n = 8	33.6 \pm 3.0n = 17
Millers Cove:	18.7. \pm 3.7n = 14	36.3 \pm 5.1n = 26

In order to quantify the levels and impacts of nitrogen loading, and to regulate pollution in coastal embayments, environmental managers must have a way of objectively determining to what degree a bay is polluted by nutrients. Frequently chl_a and nutrients in the water column concentrations are used to assess the effects of enrichment. Valiela and Costa (in press) showed that nitrogen concentrations in the water column of a shallow, well-mixed coastal embayment like Buttermilk Bay, are not always a good indicator of nutrient loading because of tidal flushing and nitrogen uptake by plants and algae. In particular, nutrient data collected on a single date was often unrepresentative of long term patterns at many stations. In this study, it is also clear that chl_a in water of a well-mixed and flushed Bay, do not always correlate with nitrogen concentrations. Thus, low nitrogen concentrations or phytoplankton abundance in the water column, particularly on single sampling dates, do not necessarily imply low nutrient exposure.

In this study, stationary biological indicators correlated well with long-term nitrogen exposure. I should also note that benthic drift algae accumulated in enriched parts of Buttermilk Bay, but were not studied because patterns of abundance seemed equally affected by the topography and hydrography of the Bay.

Depth of eelgrass growth correlated well with DIN, but depth of eelgrass growth is often influenced by other factors such as water turbidity, thus other data are necessary to estimate nitrogen impact. Periphyton growth on artificial substrates and eelgrass show similar degrees of correlation with DIN. Periphyton growth on artificial

Figure 4. Chl_a on settlement strips (top) and eelgrass (bottom) vs. DIN during the experimental period. Standard errors of the mean are shown for three replicate strips for chl_a , and a variable number of nutrient samples for an extended period at each station (see text).

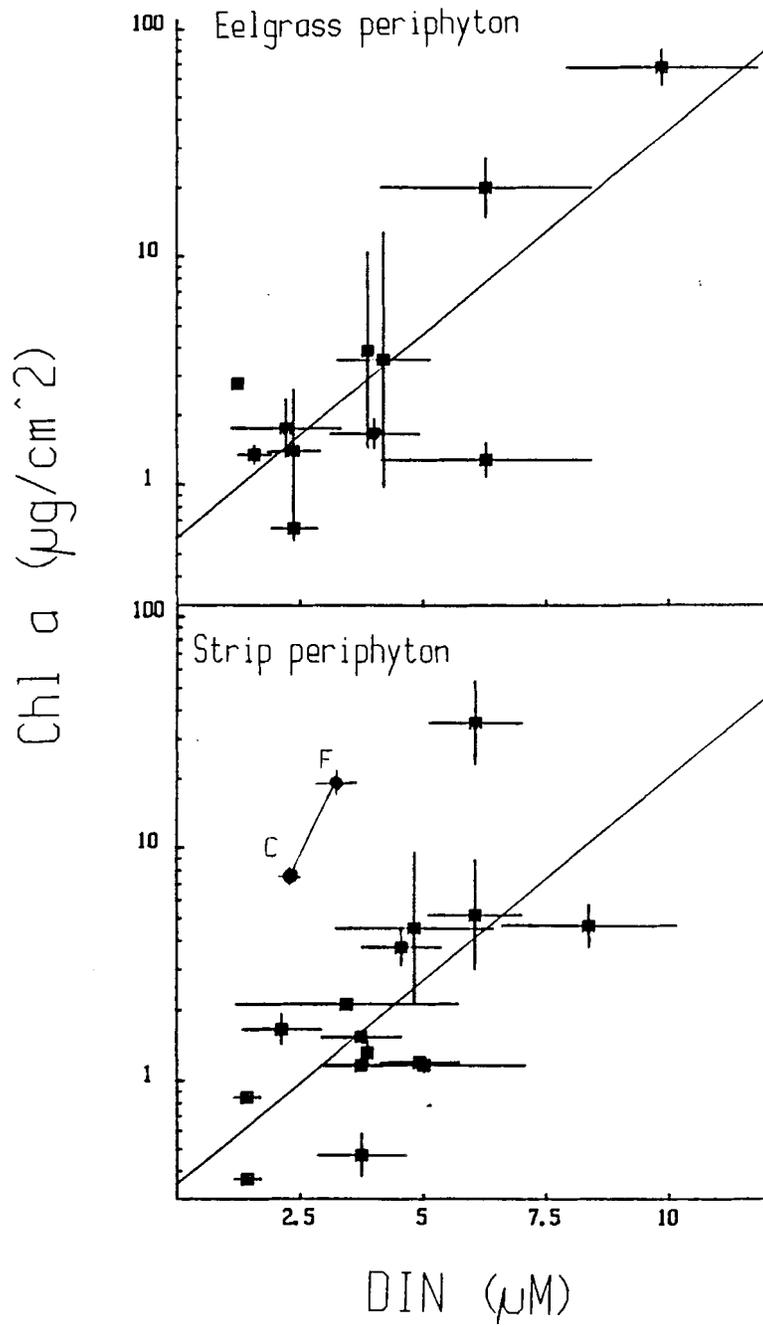
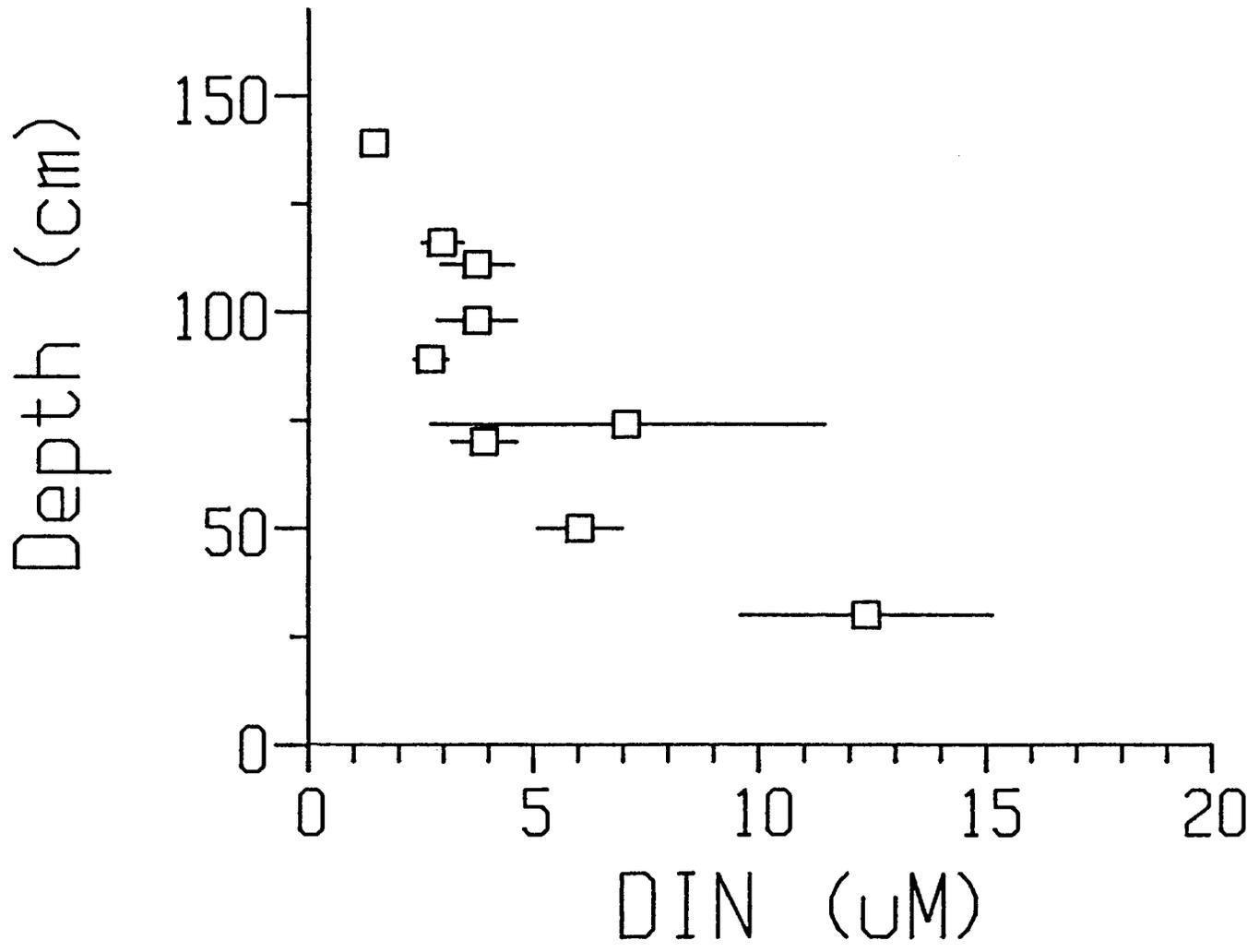


Figure 5. Depth of eelgrass growth vs DIN (\pm SE).



substrates as an assay offers advantages over eelgrass periphytes because eelgrass does not grow in all areas, and differences in periphyton abundance on eelgrass may be due to other factors controlling epiphyte abundance, such as invertebrate grazing, leaf age, and plant depth. Periphyton growth on settlement strips is a relative index of nitrogen exposure because growth rates of periphyton vary with light and temperature as well as nutrients (Sand-Jensen and Borum, 1983). Consequently interpretation of periphyton growth requires the simultaneous deployment of many floats with settlement strips.

The results of the growth experiment are inconclusive because of the large variability among shoots in relation to differences among between the two sites. Elsewhere I have seen that loss of eelgrass is gradual, and often occurs over 5 to 15 years. If the loss of eelgrass beds in an enriched bay results from lower vegetative growth or recruitment so that maintenance of populations cannot keep up with attrition from physical disturbance and death, then annual differences in eelgrass growth between enriched and unenriched areas may be small, and larger sample populations may be needed to resolve growth differences.

Other factors may explain the lack of distinct growth rates between the two sites data. The bed and transplants in Miller Cove were not at the limit of eelgrass depth distribution there (which was 50 cm deeper). This small difference may be critical in terms of observing reduced growth in enriched areas. Also, declines of eelgrass populations may involve some seasonal declines or slightly higher rates

of shoot death that can only be measured with large numbers of plants over long periods.

Even though the results of the growth experiments are unclear, eelgrass distribution in Buttermilk Bay shows that eelgrass grows to lesser depths in enriched areas (Fig 5). Since periphyton is more abundant in these areas, I concur with Sand-Jensen and Borum (1983) and others that the distribution of eelgrass beds may be controlled by nitrogen inputs and concentrations.

In the deeper parts of the Bay where the slope of the bottom is small, eelgrass has receded by ≈ 200 m during the last 20 years (Costa, 1987). While the areal declines of eelgrass in Buttermilk Bay have been small, these losses indicate this Bay will be sensitive to additional loading. The mean depth of a bay is a critical component in estimating the impacts of nitrogen inputs. For example, Waquoit Bay, which has similar levels of development (in prep), but has a mean depth greater than 1.5 m, has lost 90% of its eelgrass population (Costa, 1987). It is likely that if the mean depth of Buttermilk Bay were 0.5 m deeper, the impacts of nitrogen loading would have been greater.

The depth of Buttermilk Bay may only partly explain why eelgrass has not declined appreciably. The nutrient concentration data from Buttermilk Bay (Valiela and Costa, in press) and phytoplankton chl data (here) illustrate the importance of tidal mixing when assessing the effects of nutrient loading. At any level of nutrient loading, the effects of that nutrient loading will be more pronounced if only 10% of bay water is exchanged with each tide than if 50% is exchanged. To assess the impact of nitrogen loading on eelgrass distribution, the

residence period of water in the bay must be considered. In Table 2 I ranked nitrogen loading of some well studied bays and coastal lagoons based on volume, area, and tidal flushing (data from Nixon, 1983; Giblin et al., 1983; Gaines, 1985, Valiela and Costa, in press). On a volume basis, Buttermilk Bay is one of the most polluted bays shown. In contrast, when nitrogen additions are considered on a volume basis during the residence time of water in each bay (Table 2, 4th column), Buttermilk Bay is one of the least enriched systems. This may explain both the absence of large declines in eelgrass, or large accumulations of drift algae in Buttermilk Bay as has occurred elsewhere in the region (Costa, 1987, 1988).

The results reported here, in Valiela and Costa (in press), and in other studies, suggest that many parameters need to be examined or monitored together to assess the impact of nitrogen additions. The most practical assays with the best correlations to nitrogen concentrations in Buttermilk Bay were depth distribution of eelgrass, and growth of periphyton on artificial substrates. These types of observations, together with long-term sampling of nitrogen concentrations in the water column and measurement of tidal flushing should be a fruitful approach for studying the impact of added nutrients in shallow coastal lagoons.

Table 2. Nutrient loading (per m^3 , per m^2 , and turnover-weighted) for various estuaries and embayments. Data taken from Nixon (1983), Nixon and Pilson (1983), Lee and Olsen (1985), and Gaines (1985). Method of calculation of turnover times indicated as "a" are described in Valiela and Costa (in press); other values as reported in literature by various methods.

	Loading		Turnover times (d)	Turnover-weighted
	$m \text{ mol N } m^{-3} \text{ yr}^{-1}$	$m \text{ mol N } m^{-2} \text{ yr}^{-1}$		loading ($m \text{ mol N } m^{-3} \text{ yr}^{-1}$)
Long Island Sound	30	400	27	2.2
Kaneohe Bay	40	230		
Lagoon Pond	57	261	21.5	3.4
Chesapeake Bay	80	510	166	36.4
Narraganset Bay	100	950	25	6.8
Town Cove	100	860	26	7.1
Patuxent Estuary	110	600	51	15.4
Delaware Bay	140	1350	97	37.2
Potomac Estuary	140	810	45	17.3
Apalachicola Bay	213	315	10 ^a	5.9
Point Judith Pond	240	560	6	3.9
Pamlico Estuary	250	430	12.5 ^a	17.1
Ninigret Pond	280	340	24.5 ^a	37.6
Barataria Bay	290	570		
North San Francisco Bay	290	2010		
South San Francisco Bay	310	1600	320	271.8
Raritan Bay	330	1460		
Buttermilk Bay	390	543	5.0	5.6
Mobile Bay	400	1280		
Green Pond	500	1144	20 ^a	27.4
Green Hill Pond	780	620	56.5 ^a	121
Potter Pond	1050	710	25 ^a	72
New York Bay	4550	31930	3	37.4