

Analysis of Agricultural Nonpoint Source Runoff to Baffin Bay and its Possible Effect on the Brown Tide Phytoplankton Bloom

CCBNEP Publication August 1999

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The views expressed herein are those of the authors and do not necessarily reflect the views of CBBEP or other organizations that may have provided funding for this project.

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TNRCC Interagency Cooperation Contract Contract Number: 62-000000-39

prepared by: University of Texas Marine Science Institute

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ACKNOWLEDGMENTS

Many people played critical roles in helping to successfully complete this project. Dr. Bobby Eddleman, Texas A&M Agricultural Experiment Station, helped determine sampling locations and generally gave us advice on working on King Ranch. Hudson DeYoe, University of Texas-Pan American, was research program coordinator for the Corpus Christi Bay National Estuary Program and was instrumental in make this project a reality.

The mesocosm experiment was set up with the help of Lynn Tinnin, Brian Wysor and Christopher Collumb. Hydrographic and nutrient studies were aided by Lynn Tinnin, who performed sampling and nutrient analyses in the laboratory. Zooplankton studies were performed by Brian Wysor and Christopher Collumb.

The benthic studies were aided by several people. Rick Kalke performed the benthic sampling in the field and performed the macrofauna benthic laboratory analyses. Steve Jarvis analyzed meiofaunal samples. Jyotsna Sharma, University of Texas-San Antonio, identified the nematode feeding groups. Carrol Simanek organized and managaed benthic data.

The final report was completed with help from many people. Susan Schonberg created the map of the study area. Carrol Simanek reformatted and prepared most of the figures in the report.

EXECUTIVE SUMMARY

There is a strong theoretical basis to predict that non-point agricultural runoff has a stimulating affect on phytoplankton populations. There are two main components, which act in the same way: fertilizers and pesticides. The fertilizers can supply limiting nutrients while the pesticides can limit grazers. The net effect would be blooms of phytoplankton.

The purpose of this research project was to assess the direct effect of crop land runoff on growth and maintenance of phytoplankton in general, and brown tide alga (*Aureoumbra lagunensis*) in particular, in Baffin Bay and its associated tertiary bays. Two approaches were used: experimental introduction of runoff into mesocosms, and an observational survey of stable isotope tracers in bay organisms.

The first experimental approach was to add runoff collected from drainage ditches to mesocosms containing bay water in various mixtures and compare biological responses to untreated mesocosms and ambient bay water. The hypothesis was that agricultural runoff could contain nutrients that might positively influence biomass and primary productivity of brown tide algae, or pesticides (or herbicides) might negatively influence phytoplankton, zooplankton and benthic organisms. Changes in phytoplankton population size could also cause indirect food chain effects on herbivorous zooplankton and benthic organisms.

The second approach was conduct a survey of stable isotope tracers in Baffin Bay. Stable isotopes integrate effects over longer time periods and can resolve partitioning between new and regenerated productivity. Stable nitrogen isotope ($\delta^{15}N$) values of estuarine organisms can be characterized by large inputs of terrestrially derived nitrogen from agricultural sources. Fertilizers have low $\delta^{15}N$ values, so if they promote phytoplankton growth, then $\delta^{15}N$ values of phytoplankton should decrease after freshwater inflow events due to high runoff. The $\delta^{15}N$ values in filter feeders should also decrease. Samples were collected in three sites in Baffin Bay in April 1996 (during a period of minimal freshwater inflow and precipitation), and again in April 1997 (after the flood and runoff event).

The mesocosm experiment was setup on 7 April 1997 in a small embayment located between Sandy Hook and Kleberg Point bordering Cayo del Grullo, a tertiary bay of Baffin Bay, . The location was chosen because it was near a King Ranch road (approximately 0.5 mile), brown tide was present in Cayo del Grullo, it was sheltered, and had a flat bottom of sand covered by 6-12 inches of mud. About three inches of rain fell between 2 and 3 April 1997 on King Ranch. This produced a significant runoff event, flooding roads surrounding cultivated fields on King Ranch. On 4 April, about 600 gallons of runoff water was collected from the Texas Agricultural Experiment Station monitoring site #3 using a gasoline powered pump. The runoff water was stored in clean 100-gallon polyethylene tanks for 3 days after which time the flooded dirt roads and pastureland were passable by a 4-wheel drive truck. The mesocosms are tanks consisting of a fiberglass ring with a diameter of about 1.3 meters and a height of about 1.6 meters. The ring was pushed into the sediment using the weight of several people and anchored with line tied to 8foot steel fence posts driven into the sediment. The mesocosms were aligned along the bay bottom to maintain a relatively constant depth of about 1 meter about 50 meters from the shoreline. The day after the mesocosms were installed, the experiment was initiated by introducing runoff water. A gasoline powered pump was floated to the mesocosms installed in the embayment and salt water was pumped out to allow space for addition of runoff water. Runoff water was added to five of seven mesocosms. It was estimated that mesocosms contained about 1200 liters of water based on the penetration depth and water column depth. A series was set up at an estimated 0%, 10%, 20%, 40% and 50% dilution. These estimates closely resembled the actual final dilutions in mesocosms: 11%, 24%, 41%, and 46%. Three controls were also set up: one undiluted, one completely replaced with undiluted bay water, and one with the bottom covered by plastic to remove benthic influence.

A cold front passed through the area a few days after the mesocosm experiment was started. This lowered water temperatures by 10°C over a 3 day period. The strong winds associated with this front may have had a great effect on the planktonic organisms in the mesocosms, making it difficult to separate the experimental results from those induced by weather and runoff alone. The cooling event during the first week affected temperature inside and outside the mesocosms to exactly the same degree. Hydrographic measurements in and near mesocosms indicate changes in the bay occurred over the course of the experiment. The salinity and temperature of the mesocosm site adjacent to Cayo del Grullo increased over time. The increase is most likely due to the normal summer heating and evaporation processes. Salinity inside all of the mesocosms increased more than ambient bay waters, but temperature was identical inside and outside the mesocosms. The initial salinity concentrations differed because of the experimental design and those differences were maintained throughout the entire period. The maintenance of the salinity differences indicates the mesocosms were "sealed" in the sediments and no leakage or tidal pumping of water occurred through the sediment layer. Overall, the general physical condition inside mesocosms was similar to Baffin Bay outside the mesocosms.

Ambient concentrations of nitrate, nitrite and ammonium nutrients were very low most of the time. The nitrogen availability was very small for the biomass of phytoplankton present in mesocosms. Nutrient concentrations fluctuated. The infrequent increases in nitrogen nutrients appears to be related to increased nitrification and/or denitrification processes as indicated by the presence of nitrite. The sum of the dissolved inorganic nitrogen (DIN) was initially large due to additions of agricultural runoff water, whereas controls (without additions) remained low. The nutrients in mesocosms declined to control levels after 10 days. Later increases of DIN were likely to be related to sediment fluxes, because mesocosm 7 (with the plastic bottom) and the ambient samples did not increase.

Silicate and phosphate nutrients did not display behavior that would indicate influences on phytoplankton utilization. The initial phosphate concentrations were slightly increased due to the agricultural runoff water. Silicate was unaffected biologically, and was diluted conservatively by physical means.

Chlorophyll concentrations in mesocosms started at the level characteristic of brown tide bloom concentrations, ranging from 20 - 40 μ g l⁻¹. Chlorophyll concentrations increased in mesocosms 4 and 5 (with the most runoff) to 142 and 197 μ g l⁻¹ respectively over the first two weeks. Rates of primary production were also high, increasing to about 13 g C m⁻³ d⁻¹. These data demonstrate that agricultural runoff water had a stimulatory effect on brown tide populations after the initial dilution.

Nutrients (N, P, N+P, and biologically required trace metals) were added to mesocosm water on days 2, 10 and 15 to determine if any nutrients were limiting. Initial amendments were performed in freshly diluted, low salinity water where nutrient concentrations were already high. By the time the last dilution occurred, nutrients were declining in mesocosms. Amendments of mesocosm water on day 2 or day 10, when runoff nutrients were still relatively high in mesocosms, did not stimulate phytoplankton biomass compared to the control. The control, with no runoff addition, had the greatest response to everything except P amendment. Amendments of mesocosm water initiated on day 15, when nutrients were beginning to be depleted, had effects in in all mesocosms except the one with the highest runoff addition. Additions of N, N+P, and trace metals produced significant responses while P additional nutrients do not stimulate phytoplankton productivity, but amendments do stimulate growth when nutrients are low. When nutrients are high, trace metals may be limiting, but when nutrients are low, nitrogen appears to be limiting, and P does not appear to be limiting.

Populations of ciliates, which are potential grazers on brown tide, were generally low in the ambient waters surrounding the mesocosm tanks. In contrast, in the control mesocosm tank, there was an enormous increase in ciliate concentration 5-9 days after the mesocosms were established, and were more than 10 times higher than in surrounding waters. In all the mesocosms, there was stimulation of growth of microzooplankton following frontal passage, although in all cases the density decreases back to pre-front levels about one week later. In several of the mesocosms this temporary increase in grazers corresponded to a decrease in brown tide concentrations a few days later. In grazing experiments, levels of brown tide were uniformly low in the mesocosms. There was no evidence of enhanced brown tide growth in any of the treated mesocosms, so the general conclusion is that runoff waters from the King Ranch exhibited no enhancement of brown tide growth. There were no changes to the zooplankton populations that could not be interpreted as due simply to the changes in salinity.

There was no evidence that runoff has any effect on macrofauna in the mesocosm experiments. In contrast, meiofauna populations may be negatively impacted by roughly an order of magnitude. No information exists on the relative roles of meiofauna and macrofauna in Baffin Bay. In general, it is thought that meiofauna and macrofauna have different functions in shallow marine ecosystems. Meiofauna have direct benthic development, in contrast, macrofauna have pelagic larvae. Meiofauna also have much shorter generation times that macrofauna. The ability to reproduce directly into sediments, the short generation time, and predator exclusion are plausible explanations for the meiofaunal bloom in mesocosms. Meiofauna are thought to be more closely linked to nutrient cycling than macrofauna because they are smaller and feed primarily on bacteria, diatoms, and protozoa. However, the nutrient stimulation introduced by runoff dilutions did not enhance meiofauna. In contrast, meiofauna declined relative to undiluted mesocosms. It is not known what could have caused the relative decline, but mortality due to pesticides or simple salinity changes are a plausible explanations. Feeding guilds of nematodes, the dominant meiofaunal organisms, changed in the dilution series and over time from a deposit feeding community to a non-deposit feeding community. The change could be due to a lack of deposition of fresh material derived from tides or wind-driven water movement. The change from deposit feeders was less in the higher runoff dilutions, most likely due to higher particle loads being placed in mesocosms. Overall, an effect on meiofauna was observed in mesocosms.

The bay tracer study did not exhibit clear evidence that agricultural nitrogen enters the food web of the Baffin Bay region. The filter feeders (hydrozoa and barnacles) in Baffin Bay were not significantly depleted in ¹⁵N during post-flood conditions relative to higher ¹⁵N content of fauna during dry periods. The δ ¹⁵N values associated with synthetic fertilizers are low (ranging from - 3 to +3 ‰ for nitrate from synthetic fertilizers) due to the conversion of atmospheric N₂ during manufacturing. Fertilizer application increases soil denitrification resulting in increased δ ¹⁵N values in nitrate pools. The overall range of δ ¹⁵N values for algal producers and secondary consumers in Baffin Bay (7 to 13 ‰) are lower than those reported for the Guadalupe Estuary (10 to 17 ‰), but nearly match values for Lavaca and Corpus Christi Bays.

Dilution of Baffin Bay water by agricultural runoff collected from drainage ditches had only a slight effect on brown tide and phytoplankton production. This was due to the already high levels of nutrients in bay waters. Chlorophyll biomass and rates of primary production increased in mesocosms with runoff additions compared to controls with no additions during the first 7 - 10 days. No effects were found on microzooplankton, or macrobenthos, but effects were noticed on meiofauna. Effects of runoff are confounded with salinity effects (caused by the dilution) and temperature effects (caused by frontal passage just after the mesocosms were set up). Stable isotope tracers in the bay did not respond as if influenced by a large addition of fertilizer laced runoff. Results from the tracer and experimental studies indicate that effects of runoff due to the three-inch rainfall event that was studied must have been very small to unmeasurable. Biological responses to experimental additions of runoff were not linear, or a function of the dilution performed. In spite of lack of a clear runoff signal in the mesocosm experiments, some positive results were noted. When microzooplankton increased, brown tide decreased, indicating grazing control exists. When nutrients were added to depleted mesocosm water, phytoplankton was stimulated. Only nitrogen and trace metals were responsible for phytoplankton stimulation. Meiofauna densities were higher in control than in mesocosms with runoff added, and the trophic structure of nematodes changed from a deposit feeding community to an epigrowth (i.e., grazing on epibionts) feeding community. Zooplankton in the bay did exhibit lower nitrogen isotope values after the runoff event, as predicted if nitrogen from fertilizers was being incorporated into the food chain. Overall, the experimental design could be improved to detect the weak biological response in the mesocosms. Distilled water controls could be used to distinguish effects due to salinity versus runoff water. Specific dilutions should be replicated because there is no clear functional relationship between dilution and response. The dilution series was realistic, because strong storms are know to lower salinities in Baffin Bay to as low as 20 psu.

1. INTRODUCTION

1.1. Background

A phytoplankton bloom, such as the brown tide alga *Aureoumbra lagunesis*, is the result of a balance between growth of the algal population and losses due to dispersion, sinking, grazing and disease. Agricultural runoff water often contains dissolved substances that can affect this balance. Unused nutrients can remain in soil after fertilizer application. Unused nutrients may enter marine systems via runoff and stimulate phytoplankton growth. Alternately, residues from pesticide and/or herbicide applications may have toxic effects on both phytoplankton and their grazers.

Estuarine phytoplankton assemblages are a complex association of species that can respond rapidly to freshwater inputs and nutrient loadings. The seasonal and inter-annual variations in freshwater flow can influence both phytoplankton production and taxonomic distributions through three mechanisms: (1) changing nutrient input patterns, (2) changing dilution or advection rates of algal populations, and (3) altering light availability by stratification and turbidity fluxes. These mechanisms account for phytoplankton abundance and production that has been correlated to freshwater nutrient inputs, or mean concentrations, in several estuarine systems (Boynton *et al.* 1982; Cadee 1986). The linkage between nutrient input and phytoplankton production provides an explanation of recent phytoplankton blooms associated with agricultural runoff.

The brown tide bloom began in January 1990 in Baffin Bay and still persists seasonally as of this writing. The onset of the bloom appears to have been related to the confluence of natural climatic events. The long-term maintenance of the bloom is more difficult to explain. Anthropogenic activity may play a role in maintaining the bloom. Land use in the watershed feeding Baffin Bay and its three tertiary bays (Cayo del Gullo, Alazan, and Laguna Salada) is primarily agricultural, not municipal. This leads to the question of what role agricultural nonpoint sources may have in maintaining the brown tide bloom.

1.2. Objectives

The purpose of the research was to assess the direct effect of cropland runoff on growth and maintenance of brown tide in the waters of Baffin Bay and its associated tertiary bays. The experimental approach was to add runoff collected from drainage ditches to mesocosms containing bay water in various mixtures and compare it to an untreated mesocosm and ambient bay water. The research coupled a cropland runoff study performed on King Ranch by the Texas Agricultural Experimental Station (TAES) with in bay studies of cropland runoff effects on the bay community by the Marine Science Institute. The hypothesis was that agricultural runoff could contain nutrient that might influence biomass and primary productivity of brown tide organism or pesticides (or herbicides) that might influence phytoplankton, zooplankton and benthic organisms. Indirect, food chain effects on zooplankton and benthic organisms could occur if phytoplankton populations increased or decreased. There were five specific objectives to achieve the goal of the study.

1.2.1. To characterize water quality at freshwater discharge points in the King Ranch runoff channels above Cayo del Grullo to determine concentrations of nutrients and other chemical constituents.

1.2.2. To measure response of brown tide algae and natural phytoplankton assemblages to freshwater runoff coming from the King Ranch cropland drainage channels.

1.2.3. To measure zooplankton grazing rates on brown tide in experimental mesocosms that have recently received runoff waters from the King Ranch cropland drainage.

1.2.4. To measure benthic (meiofauna and macrofauna) response in experimental mesocosms that have recently received runoff water from the King Ranch cropland drainage.

1.2.5. To measure stable nitrogen and carbon isotope ratios of nitrate, ammonium and particulate organic matter in freshwater discharged into upper Alazan and Cayo de Grullo Embayments to estimate carbon and nitrogen incorporation into seagrasses, macroalgae and the pelagic-benthic food webs in the Baffin Bay complex.

1.3. Approach

The effects of surface water runoff of agricultural chemicals on ecosystem processes was studied in a tertiary bay of Baffin Bay. Cayo del Grullo, the study site, receives the greatest proportions of runoff from cultivated fields on King Ranch. The public has access from parks on the west side of the bay, but the King Ranch shoreline on the east side is completely undeveloped except for agriculture. Freshwater inflows to Cayo del Grullo are received from San Fernando and Santa Gertrudis Creeks, which join just prior to entering the upper bay. Although freshwater runoff into Baffin Bay is sporadic, a rich phytoplankton assemblage exits within these shallow waters including diatoms, dinoflagellates and blue-green algae (Cornelius 1984), many of which are associated with the upper surface of the sediment. To assess effects from runoff, two experimental approaches were used: experimental introduction of runoff water into mesocosms and stable isotope tracers of nitrogen in bay organisms.

Open bottom mesocosms were used along the shoreline of Cayo del Grullo to surround parcels of bay water containing brown tide (Fig. 1). A series of mesocosms were filled with incremental amounts of runoff water taken from King Ranch runoff channels. Two mesocosms were used as non-addition controls. This approach focuses on direct short-term effects of cropland runoff on planktonic and benthic processes over the period of a few days to weeks. This provides information on direct and immediate effects resulting from freshwater and its dissolved constituents. Mesocosms emplacement location and runoff water sampling sites were established after an exploratory trip to the King Ranch and consultation with TAES investigators.



Figure 1. Study area in Baffin Bay region. The runoff water sampling sites (\blacktriangle), mesocosm deployment site (\bigcirc), and isotope sampling locations (\blacksquare).

Stable isotopes are used to integrate effects over longer time periods and can resolve partitioning between new and regenerated productivity. The uptake and remineralization processes selectively utilize lighter isotope atoms and results in relatively "lighter" molecules of regenerated substances and relatively "heavier" molecules of the material remaining. Stable nitrogen isotope (δ^{15} N) values of estuarine organisms are characterized by large inputs of terrestrially derived nitrogen from agricultural sources. Two nitrogen isotope δ^{15} N ranges have been defined for nitrate from different sources. Fry et al. (1987) and Dunton (unpub. data) found δ^{15} N values for marine plants generally range from +3 to +6, which reflects nitrate δ^{15} N of estuarine waters, +2 to +5 (Owens, 1987). In contrast, Kreitler (1975) and Kreitler and Jones (1975) found that the δ^{15} N values of animal-waste nitrogen ranged from +10 to +20. In South Texas, the δ^{15} N values of estuarine plants, herbivorous invertebrates, and fish in the Guadalupe Estuary (San Antonio Bay) provide direct evidence for animal-waste as a source of nitrogen in this system. The δ^{15} N values of estuarine macrophytes and herbivorous fauna range from +12 to +15, compared to +3 to +6 for the same species in Corpus Christi Bay (Dunton, unpub. data). Application of fertilizers (which have $\delta^{15}N$ values ranging from -3 to +2; Kreitler, 1975) to promote plant growth and biomass will enhance production of animal-wastes that become enriched with $\delta^{15}N$ as the "lighter" nitrogen ($\delta^{14}N$) is released into the atmosphere via denitrification. Finally, there is a considerable body of evidence that clearly relates nitrate contamination of ground water to agricultural practices in Texas using $\delta^{15}N$ as a tracer (Kreitler et al., 1978).

2. MATERIALS AND METHODS

2.1. Project Organization

The analysis of agricultural nonpoint source runoff and its possible effect on the brown tide in Baffin Bay was undertaken by scientists from The University of Texas at Austin, Marine Science Institute with funding from the Corpus Chrisit Bay National Estuary Program. The study was coordinated the TAES study to assess surface runoff water for sediment, nutrients and chemicals from the King Ranch croplands. The collaboration with Bobby Eddleman (TAES) included participating in decisions concerning the startup and execution of the experimental plan, e.g., choosing the study period and locations to be studied. Dr. Terry Whitledge, Project Director, completed characterization of hydrographic and nutrient conditions. Dr. Dean Stockwell measured phytoplankton biomass and productivity. Dr. Ed Buskey measured brown tide cell densities, zooplankton densities, and zooplankton grazing rates in mesocosms. Dr. Paul Montagna measured meiobenthos and macrobenthos community responses (i.e., density and diversity) in mesocosms. Dr. Ken Dunton measured carbon and nitrogen isotope values of biota in the bay.

2.2. Mesocosm Experiment on Agricultural Runoff

2.2.1. Study Site

A small embayment located between Sandy Hook and Kleberg Point bordering Cayo del Grullo was chosen as the site for the mesocosm experiments (Fig. 1). The location was chosen because

it is near a King Ranch road (approximately 0.5 mile), brown tide is present in Cayo del Grullo, it was sheltered, and had a flat bottom of sand covered by 6-12 inches of mud. The sheltered embayment was a vital requirement, because the mesocosms could be upset during high tides or a storm. In fact, a strong storm and high tides were experienced shortly after the experiments were initiated.

2.2.2. Timing of Study and Collection of Agricultural Runoff Water

The climate of South Texas is variable and unpredictable. Although the study began in FY96, weather conditions were not suitable for the experiments. During summer 1996, while waiting for rainfall, preliminary experiments were run in the cooling water pond of the Barney Davis CPL power plant. These preliminary experiments demonstrated the need for mesocosms with and without sediment bottoms. Finally, sufficient rain fell in Spring 1997 to fill ditches and collect water to create the planned experiments.

Rainfall of 3.0 and 2.57 inches was measured on 2 and 3 April 1997 at the King Ranch weather station. This produced a significant runoff event that flooded roads surrounding cultivated fields on King Ranch. On 4 April, about 2400 liters of runoff water was collected using a gasoline powered pump at TAES monitoring site #3. The runoff water was stored in clean 100-gallon polyethylene tanks for 3 days at which time the flooded dirt roads and pastureland were passable by a 4-wheel drive truck.

2.2.3. Mesocosm Experimental Setup

The mesocosms are tanks consisting of a fiberglass ring with a diameter of about 1.3 meters and a height of about 1.6 meters (Fig. 2). The ring was pushed into the sediment using the weight of several people and was anchored with line tied to 8-foot steel fence posts that were driven into the sediment. The mesocosms were aligned along the bay bottom to maintain a relatively constant depth of about 1 meter. The seven mesocosms were located about 50 meters from the shoreline (Fig. 2).

The day after the mesocosms were installed in Sandy Lagoon, the experiment was initiated by introducing runoff water. The mesocosm experiment was started on 8 April 1997, which is referred to as day 0. A gasoline powered pump was floated to the mesocosms installed in the embayment and salt water was pumped out to allow space for addition of runoff water. Runoff was added to five of the seven mesocosms. It was estimated that the mesocosms contained about 1000 liters of water based on the depth of the water column enclosed. A series was set up at an estimated 0%, 10%, 20%, 40% and 50% dilution. These estimates closely resembled the actual final dilutions in mesocosms (Table 1).





Figure 2. Photographs of mesocosms.

Mesocosm 1 was maintained with ambient bay water to act as a control and had a salinity of 37 practical salinity units (psu). About 10% of mesocosm 2 was pumped out and 100 liters of runoff water was pumped in through a garden hose from the shoreline. Assuming a conservation of salt in the mesocosms, the dilution was 11% because the final salinity was 32.8 psu. About 200 liters of runoff water was pumped into mesocosm 3, with a final salinity of 28.2 psu and dilution of 24%. About 400 liters of water was pumped into mesocosm 4 making a final salinity of 21.7 psu and dilution of 41%. About 500 liter of water was pumped into mesocosm 5 making a final salinity of 20.0 psu and dilution of 46%. All of the salt water was pumped out of mesocosm 6 to test for leakage. After standing empty for about 4 hours, the tank was refilled with ambient bay water, the salinity had increased 0.5 psu during this time. Mesocosm 7 was installed during the next day with ambient bay water but a plastic sheet covering bottom sediment was added to eliminate effects of benthic processes on the mesocosm water column.

Mesocosm	Runoff Volume Added (liters)	Salinity After Addition (psu)	Calculated Dilution (%)
1	0	37.0	0%
2	100	32.8	11%
3	200	28.2	24%
4	400	21.7	41%
5	500	20.0	46%
6	0	37.5	0%, Replaced water
7	0	37.0	0%, Bottom covered
Outside	-	37.0	Ambient conditions

Table 1. Setup of the runoff experiment in mesocosms. For making the dilution series, it was estimated that mesocosms contained approximately 1000 liters of sea water.

2.2.4. Analytical Measurements In Mesocosms

Mesocosms were sampled on days 0, 2, 3, 5, 6, 8, 9, 12, 15, 22, 30, 36, 37, 47, and 63 for nutrients, hydrography, chlorophyll and primary productivity. Additional nutrient ammendment studies were performed on samples taken from mesocosms on days 2, 10, and 15. Zooplankton samples were taken on days 1, 3, 5, 6, 8, 9, 10, and 15. Benthos were sampled on days 2 and 63.

2.2.4.1. Nutrients and Hydrography

Presence of a sustained brown tide phytoplankton bloom over a long period of time indicates sufficient biogenic nutrients are available in the water column to support high growth rates and biomass accumulation. The physical hydrographic conditions of temperature, salinity, and penetration of incident radiation into the water column were measured.

Nutrient samples were collected in labeled polyethylene bottles with polyethylene caps after three rinses with the sample water. Each bottle number was recorded on a field log sheet as the samples were collected. The samples were immediately placed on ice and in the dark for transportation to the laboratory. Concentrations of nitrate, nitrite, ammonium, orthophosphate and silicate were determined in according to published methods of USEPA (1983; Reference Numbers 365.3, 370.1, 353.2, 353.1, and 350) and Whitledge *et al.* (1981) using automated continuous flow Technicon Auto Analyzer II. Calibration of the automated nutrient channels occurs with each set of samples. A series of five concentrations for each analyte is analyzed prior to analysis of field samples to ascertain proper operation. All standards are prepared in the laboratory with ultra-pure grade deionized distilled water water or as standard additions to low nutrient seawater.

Physical hydrographic measurements were collected at the surface and near the bottom for each sampling site and recorded on the field log sheet. Conditions recorded during sampling include location, date, time, latitude, longitude, sample depth(s), Secchi depth, water depth, and weather conditions. A multiparameter YSI model 610 sonde was used for in situ measurements of temperature, salinity, dissolved oxygen, per cent oxygen saturation, pH, and depth values

2.2.4.4. Phytoplankton measurements

Phytoplankton primary production rates were measured using replicate ¹⁴C incubations under natural sunlight using the method of O'Reilly and Thomas (1983). Replicate samples are incubated in light and dark conditions. Radioactivity of ¹⁴C in samples was measured with a Beckman model LS5801 liquid scintillation counter, which employs self calibration with known sources and calculates counting efficiency.

Chlorophyll *a* is an index of phytoplankton biomass. Changes in Chlorophyll *a* were measured the method of Holm-Hansen *et al.* (1965). Chlorophyll and pigment samples were analyzed with a model 10-005RU Turner Designs fluorometer. Calibration of the in vitro chlorophyll analysis was made with pure chlorophyll obtained commercially and standardized with a spectrophotometer.

Bioassay techniques were employed in the field to evaluate the relative influence of nitrogen, phosphorus, or trace metal additions to changes in biomass (Chl a). These bottle assays were enrichment modifications of the productivity estimates and are useful to determine possible nutrient limitations. The bioassay amendment was performed in screw cap test tubes containing 50 ml of sample water. Initial samples were analyzed for chlorophyll content. Four replicates of each sample was amended with 10 umole/liter of ammonium, 10 umole/liter of phosphate, 10 umole/liter ammonium plus 10 umole/liter phosphate, or 100 uliter of f/2 trace metal stock. Four replicates of a control sample with no additions were also utilized. After the additions, caps were tightened and in vivo fluorescence readings were taken on samples. The amended samples were taken daily for 4 days. The mean of the four in vivo fluorescence samples was used to represent the effect of the amendment additions. No readings were discarded. When the incubations were terminated the samples were analyzed for extracted (in vitro) chlorophyll content.

Brown tide cell densities were measured using a polyclonal antibody assay specific to Aureoumbra lagunensis, the Texas brown tide alga (Lopez-Barreiro et al., 1998). Whole water samples were collected and preserved in 3% formaldehyde for the assay. Between 100 and 400 µl of sample was added to a test tube containing 1 ml of 3 % goat serum (Sigma Immuno Chemicals G-9023). The sample was gently agitated and incubated at room temperature for 30 min. The contents were poured into a filter funnel with a 5-µm porosity nitrocellulose backing filter under a 2.0 µm black polycarbonate filter. The tube was rinsed with 10 mM phosphate buffered saline (PBS), pH 7.4 (Sigma P-3813) and the rinse was also added to the filtration funnel. The sample was filtered to <2 ml volume without letting the filter dry, and rinsed two more times with 10-ml PBS. One ml of antiserum BTD-22 (R-5310 and R-5313, Charles River Pharmservices) at a concentration of 1:1000 (antibody:PBS) was added to the filtration funnel and incubated for 30 min. The filter was then rinsed three times with 10-ml PBS, 1 ml of secondary antiserum (Anti-Rabbit IgG FITC Conjugate; Sigma F-0382) was added and incubated for 20 min. The filter was again rinsed three times with 10-ml PBS, and the black polycarbonate filter was mounted on a glass slide, with one drop of glycerol-PBS (9:1) and topped with a cover slip. Slides were immediately frozen and stored in the dark until enumerated with an Olympus IMT-2 or BHS epifluorescence microscope at 1000X magnification using blue excitation.

2.2.4.3. Zooplankton measurements

Microzooplankton (zooplankton between 20 and 200 μ m in length) abundance was measured. Whole water samples were collected and preserved with Lugols iodine. Microzooplankton abundance was determined using settling chambers and an inverted microscope.

Microzooplankton grazing rates were measured in three mesocosms on three occasions. Grazing rates of microzooplankton (the dominant grazers on the brown tide algae) were measured using the dilution method as described by Landry and Hasett (1982). Each dilution series was incubated in the field for 24 hours, and chlorophyll and microzooplankton samples were returned to the laboratory for analysis.

2.2.4.4. Benthos

Sediment was sampled with core tubes held by hand to measure both meiofauna and macrofauna densities. Macrofauna were sampled with a 6.7-cm diameter tube, and sectioned at depth intervals of 0 - 3 cm and 3 - 10 cm. Meiofauna were sampled with a 1.8-cm diameter tube, and sectioned at depth intervals of 0-3 cm only. Samples were preserved with 5% buffered formalin. In the laboratory, meiofauna were sorted on 63 μ m sieves, and macrofauna were sorted on 0.5 mm sieves. Macrofauna were identified to the lowest taxonomic level possible, usually the species level, counted, and weighed to the nearest 0.01 mg for biomass. Meiofauna were identified to higher taxonomic levels, usually phylum, class or order, and counted. Nematode trophic guild was assigned according to Weiser (1953)..

Biomass of macrofauna was measured by combining individuals into higher taxa categories, i.e., Crustacea, Mollusca, Polychaeta, and others. Samples were dried for 24 h at 55 °C, and weighed. Mollusks were placed in 1 N HCl for 1 min to 8 h to dissolve carbonate shells, and washed before drying.

All meiofauna and macrofauna data was digitized, and proofread. For macrofauna, species diversity was calculated by replicate and by pooling all replicate cores for each site. Diversity is calculated using Hill's diversity number one (N1) (Hill, 1973). It is a measure of the effective number of species in a sample, and indicates the number of abundant species (Ludwig and Reynolds, 1988). It is calculated as the exponentiated form of the Shannon diversity index:

$$N1 = eH'$$

As diversity decreases N1 will tend toward 1. The Shannon index is the average uncertainty per species in an infinite community made up of species with known proportional abundances (Shannon and Weaver, 1949; Hutcheson, 1970). The Shannon index is calculated by:

$$H' = \sum_{i=1}^{S} \left[\left(\frac{n_i}{n} \right) \ln \left(\frac{n_i}{n} \right) \right]$$

Where n_i is the number of individuals belonging to the *i*th of *S* species in the sample and *n* is the total number of individuals in the sample. Hill's N1 was used in most analyses because it is easier to interpret than most diversity indices.

All statistical analyses were performed using SAS software (SAS 1991). All data were log transformed prior to analysis. A one-way ANOVA was used to test for differences in macrofauna abundance biomass, and diversity among mesocosms. Tukey multiple comparison procedures were used to find *a posteriori* differences among sample means.

Community structure of macrofauna a species and nematode feeding groups were analyzed by multivariate analysis. Principal components analysis (PCA) is a multivariate method to transform the data matrix to create new variables that are 1) mutually orthogonal, which means they are uncorrelated, and 2) extracted in order of decreasing variance. Principal components analysis is a variable reduction technique because of the decreasing variance property, which implies that much of the information (i.e., variance) of the original set of variables is concentrated in the first few principal components (PCs). The PCs can also be used as predictors in regression analysis because they are orthogonal and collinearity does not exist. All multivariate analyses were performed with the SAS FACTOR procedure (SAS, 1991) using the PC method on the covariance matrix. When performing PCA on the covariance matrix, the analysis does not treat all the variables as if they have the same variance. All count or measurement data was log transformed prior to multivariate analysis.

Results of the PCA are visualized in bivariate plots. Generally, only the first two PC factors (PC1 and PC2) are used in the plots. The results are visualized in two ways: as factor patterns and as loading scores. Each data set is simply a matrix, i.e., rows of observations versus columns of variables. The factor patterns are the PC coefficients for each variable or column. These vector patterns are used to interpret what PC1 and PC2 represent by plotting the column heading as the symbol for each point. Next, the loading scores for each observation are plotted using the site name as the symbol for each point. The plot of the loading scores allows us to visualize the relationships or correlation among the sites.

2.3. Tracing Agricultual Runoff in Bay

2.3.1. Collection Sites

Biota were collected from three sites: Alazon Bay, Baffin Bay Marker 36, Cayo del Grullo for stable isotopic composition analysis (Fig. 1). Collections were performed two times: in April 1996 (during a period of minimal freshwater inflow and precipitation), and again in April 1997 (following an extended period of precipitation and freshwater inflow). In addition, water samples from several freshwater tributaries of Baffin Bay were collected during dry periods and following runoff events.

2.3.2. Stable Carbon and Nitrogen Measurements

Particulate organic matter (POM), benthos, and zooplankton were collected. All biological isotopic samples were frozen or dried for later analysis.

Water samples were filtered using pre-combusted GF/F filters and then frozen. Water samples were analyzed to determine the stable nitrogen isotopic composition of the dissolved inorganic-N pool (DIN; $NO^{-2} + NO^{-3} + NH^{4+}$).

Measurements of δ^{15} N and δ^{13} C were determineded following standard methods (Fry *et al.*, 1987). Samples were analyzed at the University of Texas, Marine Science Institute, Stable Isotope Laboratory using a Finnegan MAT continuous flow isotope ration mass spectrometer. All samples were weighed and loaded into tin capsules and combusted in an elemental analyzer; gases were then separated and purified before introduction into the mass spectrometer. Precision of replicate analyses is ± 0.2 ‰. Stable isotope concentrations are expressed in δ notation according to the following:

$$\delta X = [(R_{sample}/R_{standard})-1) \times 1000]$$

where $X = {}^{13}C$ or ${}^{15}N$ and R is the corresponding ratio ${}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. R_{standard} for ${}^{13}C$ and ${}^{15}N$ are the PDB standard and atmospheric N₂ (AIR), respectively. Replicate values are accurate to $\pm 0.2\%$ for both isotopes.

3. RESULTS

3.1. Mescosom Experiment

Nutrients were conservatively mixed during set up of the mesocosm experiment (Fig. 3). Conservative mixing assumes that nutrients are diluted approximately 1:1 with salt in sea water. Values below the conservative mixing line indicate losses or sinks, and values above the line indicate sources of nutrients. Nitrogen may have already been slightly and instantaneously consumed in the highest dilution. The dotted line in Fig. 3 is the regression without the 46% dilution, and the 46% mesocosm has DIN considerably below the conservative mixing line. This is not true for either phosphate or silicate. The two treated controls with 0% dilution (covered

bottom and water replacement) also had values off the conservative mixing line. In all cases, the values were above the line indicating nutrients were added during the setup of these treatments. The sediment covered mesocosm had the highest nutrient addition, not withstanding the dilution series. Placement of the plastic cover could have been associated with squeezing or pumping pore water from sediments and subsequent addition of nutrients. The complete water replacement mesocosm also had higher than expected values indicating the pump intake may have been too close to the bottom. In any event, these two controls start with higher than ambient nutrient concentrations.

A strong cold front passed through the area a few days after the mesocosm experiment was started. This lowered water temperatures by 10°C over a 3 day period. This combined with the strong winds associated with this front may have had a great effect on the planktonic organisms in the mesocosms, making it difficult to separate the experimental results from those induced by weather.

3.1.1. Hydrography

3.1.1.1. Salinity

The experimental setup was based on the salinity of water from Cayo del Grullo that was diluted with varying amounts of agricultural runoff water from the King Ranch. The initial salinity of ambient bay water was 33 psu. Bay water salinity (outside mesocosms) increased to 43 psu over 63 days. Control mesocosms (1, 6 and 7) had the largest final salinity increases ranging from 48 to 56 psu (Fig. 4). It is likely that high temperatures and strong sea breezes contributed to strong evaporation rates during the latter half of the experimental period and these are responsible for the high salinities. Mesocosms had markedly increased salinity compared to the ambient bay water.

3.1.1.2. Temperature

Water temperature was initially 22 °C in the bay water and mesocosms, but abruptly decreased to about 14 °C during a storm event that persisted for four days after the mesocosms were implaced (Fig. 5). Water temperature increased steadily through May then was nearly invarient during June. Final water temperature measured in June was < 1 °C higher than ambient bay water.

3.1.1.3. Dissolved Oxygen

Dissolved oxygen (DO) concentrations varied widely during the experimental period. Initial concentrations were $4.5 - 6.6 \text{ mg } l^{-1}$ for ambient bay water and in mesocosms 1 through 4. Mesocoms 5 and 6 had the majority of bay water pumped out before runoff or bay water was added. After day 5, all mesocosms and ambient bay water contained more than 5 mg l^{-1} (Fig. 6). The lowest dissolved oxygen concentration occurred in tank 3 on day 12 and the highest DO was observed in tank 5 on day 12. These observations indicate that mesocosms generally contained lower concentrations of DO than the ambient bay waters, which may be due to reduced water circulation.

3.1.1.4. pH

Initially, pH in ambient bay water and in mesocosms 1 - 3, and 7 was between 8.4 - 8.6. Mesocosms 4, 5, and 6 started with lower pH values probably due to the reduced buffering caused by the introduction of freshwater runoff and hydrogen sulfide release from the disturbed sediments. Subsequent pH measurements displayed no trend in mesocosms 1 - 6 (Fig. 7). Mesocosm 7, with no sediment surface, had higher pH during the latter part of the experimental period.

3.1.1.5. Secchi Depth

Secchi disk depth, measured in meters, was quite low due to the density of phytoplankton populations. Initial Secchi depths in control mesocosms were small because they contained only bay water. Mesocosms 4 and 5, containing the largest amount of freshwater runoff, had relatively large Secchi depths. Later, ambient water of the bay had increased Secchi depths, while the mesocosms decreased depths (Fig. 8). The smallest Secchi depth, 5 cm, was equivalent to that measured during the most dense brown tide bloom in Laguna Madre.

3.1.2. Nutrients

3.1.2.1. Nitrate

Nitrate concentrations were low in ambient bay water and control mesocosms, but the concentration increased to 22 - 31 umol 1^{-1} in runoff additions. Nitrate added from runoff water was reduced to undetectable levels within a week (Fig. 9). On day-37, mesocosms 4 and 5 contained increased nitrate, which may be related to nitrification processes.

Ammonium (20 umol l^{-1}) was added to all mesocosms on day-36, so rapid appearance of nitrate indicates that nitrification occurred within the time frame of a few hours. After the initial nitrate from runoff addition, nitrate concentrations were very low and could limit phytoplankton production.

3.1.2.2. Nitrite

Nitrite concentrations were initially low in ambient bay waters, but mesocosms with runoff water added contained more than 3 umol l⁻¹. Subsequent samples contained low concentrations of nitrite until day-60 when concentrations in bay water and mesocosms increased markedly (Fig. 10). Nitrite is an intermediate product in nitrification/denitrification processes, so during the last 40-days of the experimental period those processes were active. The increase of nitite in mesocosm 4 at day-37 coincides with possible nitrification discussed above.

3.1.2.3. Ammonium

Ammonium concentrations were generally low except for 3 values in mesocosms 4 and 6. Subsequent concentrations remained relatively low, but there was a small increase at days-85 and -107 that could coincide with the nitrification processes (Fig.11). The ambient bay waters contained about 0.6 umol l^{-1} initially, while added runoff water was about 3 umol l^{-1} in mesocosms 4 and 5.

3.1.2.4. Dissolved Inorganic Nitrogen

Dissolved inorganic nitrogen (DIN) is the sum of nitrate, nitrite and ammonium available for autotrophic processes. Mesocosms 4, 5 and 6 contained enhanced DIN concentrations during the first 12 days. Low DIN concentrations prevailed except in mesocosm 4 and 5 at day-37 (Fig. 12). During the last 20 days of the period, DIN concentrations increased in ambient bay water and mesocosms due to increased microbial processes, probably associated with nitrification/denitrification.

3.1.2.5. Silicate

Silicate concentrations were initially about 90 umol l⁻¹ in ambient bay water. Mesocosms with runoff water were initially large as 160 umol l⁻¹. Later samples were nearly constant, or decreased slowly over about 40 days (Fig. 13). Near the end of the experimental period, silicate increases were observed in most mesocosms. The smallest silicate values were observed in mesocosm 7 where the sediment surface was covered by polyethylene.

3.1.2.6. Phosphate

Phosphate concentrations were initially low (1.1 umol l⁻¹) in ambient bay water, but higher in mesocosms with runoff water (7.9 umol l⁻¹). Later samples from mesocosms had reduced phosphate concentrations that were similar to ambient bay water concentrations (Fig. 14). Phosphate concentrations near the end of the experimental period were large, and possibly related to increased nitrogen reminerialization that also occurred at that time.

- 3.1.3. Phytoplankton
- 3.1.3.1. Chlorophyll

Chlorophyll concentrations were initially high $(35 - 40 \text{ ug } l^{-1})$ in ambient bay water and control mesocosms due to the brown tide bloom at that time. Mesocosms with freshwater runoff additions contained smaller (20-25 ug l⁻¹) chlorophyll concentrations due to dilution effects. The brown tide bloom declined markedly in ambient bay waters during the experimental period (Fig. 15), but concentrations in mesocosms greatly increased to 300 - 550 ug l⁻¹ after day-80. Only ambient bay water and mesocosm 7 (with covered sediments) contained relatively low chlorophyll concentrations after 80 days.

3.1.3.2. Primary Production

Initial primary production rates were larger in ambient bay waters $(3 - 6 \text{ g C m}^{-3} \text{ d}^{-1})$ than in control mesocosms and mesocosms with runoff water additions $(0.2 - 0.25 \text{ g C m}^{-3} \text{ d}^{-1})$. After 7 days, mesocosms 4 and 5, with freshwater additions, had extremely large primary production rates $(8 - 13 \text{ g C m}^{-3} \text{ d}^{-1})$, while controls remained at 3 - 6 g C m⁻³ d⁻¹ (Fig. 16). The large primary

production rates in mesocosms 4 and 5 appeared to be directly proportional to the amount of added freshwater runoff. Later primary production rates were similar to initial values except for mesocosm 3, which experienced large rates (13 g C m⁻³ d⁻¹).



Figure 3. Mixing model of nutrients in mesocosms at startup. Day 0 is 8 April 1997. Solid lines are regressions through ambient and dilution mesocosms. Dashed line is regression without 46% dilution for DIN. Abbreviations: R-replaced with bay water, -B=bottom covered.



Figure 4. Salinity in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.

Temperature



Figure 5. Temperature in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 6. Dissolved oxygen in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 7. pH in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 8. Secchi depth mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 9. Nitrate in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.

Nitrite



Figure 10. Nitrite in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 11. Ammonium in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.


Figure 12. DIN in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 13. Silicate in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 14. Phosphate in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 15. Chlorophyll in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.



Figure 16. Primary production in mesocosms. Day 0 is 8 April 1997 and day 63 is 10 June 1997. Abbreviations: M=mesocosm, -R-replaced with bay water, -B=bottom covered.

3.2.3.3. Nutrient Amendment Studies

Nutrient amendments were performed to determine whether phytoplankton growth in mesocosms could be stimulated by addition of nitrogen, phosphorus or trace metal nutrients. Biomass of phytoplankton (as measured by chlorophyll) was used to indicate biological response to the ammendments. Three amendment were performed to examine nutrient limitations at 2, 10 and 15 days after the mesocosm experiments were initiated.

3.2.3.3.1. Amendment Series 1: 10 - 14 April 1997

Addition of nutrients to samples collected from mesocosms on day 2 (10 April) did not generate a large response in phytoplankton biomass as represented by in vitro chlorophyll measured on 14 April (Fig.17). Mesocosm 1 (no runoff control) had the largest response to nutrient additions compared to control tubes with no additions. Trace metal additions had the largest response followed by N+P and N additions. All were slightly larger than the control, but P was probably not significant. Mesocosm 2 (11% runoff addition) had the smallest chlorophyll biomass and the highest control value. There were no significant differences in the additions except trace metals, which was about 50% of N and P additions. Mesocosm 3 (24% runoff addition) had an intermediate response between mesocosms 1 and 2, and only trace metal additions had a response that was greater than control. Mesocosm 4 (41% runoff addition) had a response similar to mesocosm 1 including possible chlorophyll enhancements stimulated by N+P and trace metal additions compared to control tubes. Mesocosm 5 (46% runoff addition) had a somewhat smaller response to nutrient additions than mesocosm 4, but N, N+P and trace metal additions stimulated chlorophyll biomass compared to the control. The short-term temporal response of phytoplankton to additions is indicated by daily readings of in vivo chlorophyll fluorescence, and was used to monitor progress of amendments and determine when to terminate the experiment (Fig. 18).

3.2.3.3.2. Amendment Series 2: 18 - 21 April 1997

Addition of nutrients to samples collected from mesocosms on day 10 (18 April) did not generate a large response in phytoplankton biomass as indicated by in vitro chlorophyll measured on 21 April (Fig.19). Mesocosm 1 (no runoff control) had significant responses for N, N+P and trace metal additions compared to the control tubes with no additions. Trace metal additions had the largest response followed by N+P and N additions. All were slightly larger than the control, but P was probably not significant. Mesocosm 2 (11% runoff addition) had only a small response to N addition but not N+P, which would indicate the change is not significant. Mesocosm 3 (24% runoff addition) had a small response to N, N+P and trace metal additions. Mesocosm 4 (41% runoff addition) had a relatively large response to N+P additions and smaller responses to P and trace metals and total chlorophyll biomass was low. Mesocosm 5 (46% runoff addition) had no response to nutrient additions. Mesocosm 6 (control with complete bay replacement) had the highest biomass, but there was no response to nutrient additions. Mesocosm 7 (sediments covered with plastic) had some response to N and N+P additions and total chlorophyll biomass was low and similar to mesocosm 4. The chlorophyll temporal responses were used monitor progress of the amendment additions and determine when to terminate the experiment (Fig. 20).

3.2.3.3.3. Amendment Series 3: 23 - 27 April 1997

Addition of nutrients to samples collected from the mesocosms on day 15 (23 April) did not generate a large response in phytoplankton biomass as indicated by in vitro chlorophyll measured on 27 April (Fig. 21). Mesocosm 1 (control with no runoff additions) had significant responses to N and N+P additions compared to control tubes, while P and trace metals declined. Mesocosm 2 (11% runoff addition) had a significant response to N, N+P and trace metal additions. Mesocosm 3 (24% runoff addition) had a significant response to trace metal additions only. Mesocosm 4 (41% runoff addition) had a relatively large response to N, N+P and trace metal additions and no response to P. Mesocosm 5 (46% runoff addition) had a response to N and a negative response to trace metal additions. Mesocosm 7 (control with no runoff addition and sediments covered with plastic) had large responses to N, N+P and trace metal additions. Total chlorophyll biomass remained low probably due to isolation of sediments thereby eliminating nutrient regeneration from the sediments. Temporal chlorophyll responses were used to monitor the progress of amendment additions and determine when to terminate the experiment (Fig. 22).



Figure 17. Nutrient amendments 10 April 1997. Abbreviations: C = control (no addition), N = ammonium addition, P = phosphate addition, NP = ammonium and phosphate addition, and TM = f/2 trace metal addition.



Figure 18. Nutrient amendments 10 April time series. Mesocosm dilution abbreviations: M1 = 0%, M2 = 11%, M3 = 24%, M4 = 41%, and M5 = 46%.



Figure 19. Nutrient amendments 18 April 1997. Abbreviations: C = control (no addition), N = ammonium addition, P = phosphate addition, NP = ammonium and phosphate addition, and TM = f/2 trace metal addition.



Figure 20. Nutrient amendments 18 April 1997 time series. Mesocosm dilution abbreviations: M1 = 0%, M2 = 11%, M3 = 24%, M4 = 41%, and M5 = 46%.



Figure 21. Nutrient amendments 23 April 1997. Abbreviations: C = control (no addition), N = ammonium addition, P = phosphate addition, NP = ammonium and phosphate addition, and TM = f/2 trace metal addition.



Figure 22. Nutirent amendments 23 April 1997 time series. Mesocosm dilution abbreviations: M1 = 0%, M2 = 11%, M3 = 24%, M4 = 41%, and M5 = 46%.

3.1.4. Brown Tide

In mesocosm 3, with 24% runoff water added, there was a dramatic decrease in brown tide concentration after the frontal passage from about 0.8 to 1.5 million cells l^{-1} (Fig. 23). In in mesocosm 5, with 46% runoff water added, brown tide concentrations were lower throughout the experiment in these low salinity (12 - 20 psu) waters ranging from 0.05 to 0.3 million celles l^{-1} (Fig. 24). In the replacement-control (mesocosm 6), frontal passage was accompanied by a temporary increase in brown tide concentration to a maximum of about 3 million celles l^{-1} (Fig. 25). The weather change after start of the experiment appeared to have little effect on brown tide densities in waters surrounding the mesocosms ranging from 0.4 to 1.2 million celles l^{-1} (Fig. 26)

3.1.5. Zooplankton

Populations of ciliates, which are potential grazers on brown tide, were generally low in the ambient waters surrounding the mesocosm tanks, except on the final day of sampling. In contrast, in the control mesocosm tank, there was an enormous increase in ciliate concentration 5-9 days after the mesocosms were established, and ciliate concentrations were more than 10 times higher than in surrounding waters. In mesocosm 3, an even greater increase in ciliate populations occurred on days 8 and 9 (20X higher than in surrounding waters), and this appears to have contributed to the rapid decline in brown tide cell densities. In mesocosm 5, there was less stimulation of ciliate populations. In all the mesocosms, the cold front appears to have stimulated the growth of the microzooplankton, although in all cases the number decrease back to pre-front levels about one week later. In several of the mesocosms this temporary increase in grazers corresponded to a decrease in brown tide concentrations a few days later.

Dilution experiments were carried out on days 1, 8, and 15 during the experiment in mesocosm 3 (24% dilution), mesocosm 5 (46% dilution), and mesocosm 6 (control). Grazing rates of the microzooplankton community (mainly ciliates) is estimated as the percent of standing stock of phytoplankton (estimated as chlorophyll a) that can be grazed per day. If grazing balances growth, no change in phytoplankton biomass would occur. If grazing exceeds growth, phytoplankton biomass should decline; similarly if growth exceeds grazing, phytoplankton biomass should increase. Because these experiments are bottle incubations and the benthic grazers are excluded, these estimates are less than total grazing by the planktonic and benthic community. On day 1 of the experiment, grazing rates declined with increasing dilution with runoff water, with 42%, 22%, and 12% of the phytoplankton standing stock grazed per day in mesocosms 6 (control), 3 (24% dilution) and 5 (46% dilution), respectively. This represents the sum of both physical dilution of planktonic grazers and negative impacts of the rapid salinity change on these single celled organisms. On day 8, grazing rates were high in all mesocosms, with 77%, 68%, and 81% of standing stock being grazed per day in mesocosms 6 (control), 3 (24% dilution) and 5 (46% dilution), respectively. This reflects the increase in phytoplankton productivity (Fig. 15) and standing stock (Fig. 14) and the increase in grazer (ciliate) populations (Figs. 23, 24, 25). On day 15, grazing rates were uniformly low, with 18%, 18%, and 15% of the phytoplankton standing stock grazed per day in mesocosms 6 (control), 3 (24% dilution), and 5 (46% dilution), respectively. These reflect the decline in ciliate populations by the second week of the experiment (Figs. 23, 24, 25).



Figure 23. Salinity, temperature, brown tide, and ciliate density in mesocosm 3 (24% dilution).



Figure 24. Salinity, temperature, brown tide, and ciliate density in mesocosm 5 (46% dilution).



Figure 25. Salinity, temperature, brown tide, and ciliate density in mesocosm 6 (46% dilution).



Ambient Bay Water (Outside Mesocosms)

Figure 26. Salinity, temperature, brown tide, and ciliate density outside of mesocosms.

3.1.6. Benthos

The additions of runoff to mesocosms concordant reductions in salinity (Table 1). Salinity increased slightly in the bay from April to June, but more in the mesocosms. Salinity in the two most dilute mesocosms was near ambient salinity in June on day 63 when benthic samples were taken. All mesocosms except for #4 experienced hypoxia ($DO < 2 \text{ mg } l^{-1}$) during the course of the experiment (Fig. 6). All mesocosms experienced low DO (< 3 mg l^{-1}) during the month May. The hypoxia was most intense in mesocosm #3, beginning as early as day 12 (20 April 1997).

3.1.6.1. Macroinfauna

There were no differences for macrofauna in either log-transformed biomass (P = 0.2771) or logtransformed density (P=0.2482) among mesocosm treatments and ambient measurements. Even though there was no significant difference among the sample means, the range was about an order of magnitude for both biomass and density (Table 2). It is tempting to infer that the two middle dilution treatments (24% and 41%) had enhanced productivity, because values for biomass and density were similar with beginning ambient conditions in April, but not the reduced ambient values found in June. The oddest result was that mesocosm 6 (0 dilution, water replaced) had the highest biomass and density, in spite of having the highest salinity.

Macrofauna diversity was similar in April and June ambient samples (Table 3), but for totally different reasons (Table 4). Dominance patterns shifted, as well as species occurrence. Statistically, the ambient samples were similar, clustering together in a PCA (Fig. 27). The PCA Factor 1 axis (PC1) explained 68% of the variance in the data set, and Factor 2 axis explained only an additional 15% of the variance. The dominant organisms were oligochaetes and were responsible for high PC axis 1 scores (Table 4 and Fig. 28). A diverse fauna of amphipods was found in ambient treatments as well as the 41% and 0%-water replacement dilution mesocosms (4 and 6 respectively). The first 0%, 11%, and 46% dilutions (mesocosms 1, 2, and 5 respectively) had similar community structure without amphipods (Table 4 and Figs. 27, 28). Overall, diversity declined and community structure was different relative to ambient in all treatments except the 41% dilution (mesocosm 4). The ambient community was composed of four annelid and four crustacean species, and all mesocosms were subsets of this community.

3.1.6.2. Meiofauna

The meiofauna community was composed of Nematoda, Copeoda, and seven other taxa. Nematodes comprised 53%, and copepods compirsed 45% of all organisms on average. The other taxa comprised only 2% of all other organisms found and included permanent meiofauna (Turbellaria and Kinorhyncha) and temporary meiofauna (Polychaeta, Oligochaeta, Gastropoda, Ostracoda, Amphipoda). The average total number of meiofauna ranged 500 fold, from 7 to 1,880 individuals per core, which is equivalent to 25,000 m⁻² to 6.6 million m⁻² (Table 5). In contrast to macrofauna, meiofauna densities exhibited differences among treatments (Tables 5 and 6). In particular, there was a large enhancement in all mesocosms over ambient levels, and the undiluted mesocosms had the highest density of total meiofauna and copepods. The undiluted mesocosms were significantly different from ambient and the 1 dilution for total meiofauna and copepods. Community analysis was performed on the meiofauna data set at the higher taxonomic level (Figs 29, 30). The PCA Factor 1 axis (PC1) explained 82% of the variance in the data set, and Factor 2 axis explained only an additional 11% of the variance. The 0%, 11%, and 46% dilutions clustered together with positive PC1 values, and the ambient and 24% and 41% dilutions clustered together with negative PC1 values. Positive values represented a community driven by the dominant organisms (copepods and nematodes) and oligochaetes and ostracods. The negative values were more diverse communities having the remaining other taxa.

Nematode feeding groups were identified as another indication of meiofaunal community structure (Table 8). Non-selective deposit feeders (1A) dominated ambient samples at the beginning of the experiment in April, but other feeding types, particularly epigrowth feeders (2A) dominated mesocosms at the end of sampling in July. The two zero dilution controls had similar feeding guild structure. Selective deposit feeders (1B) increased with increasing dilution, but crashed in the highest dilution. Omnivores and predators (2B) were virtually absent in the dilution series mesocosms.

Date	Mesocosm	Treatment	п	Biomass	Density
10Apr97		Ambient Begin	6	1.53 (2.17)	15,100 (16,600)
10Jun97		Ambient End	6	0.52 (0.33)	1750 (1210)
10Jun97	1	0% Dilution	2	0.27 (0.32)	3,000 (3,400)
10Jun97	2	11% Dilution	2	0.10 (0.10)	1,600 (1,000)
10Jun97	3	24% Dilution	2	1.15 (1.44)	16,700 (22,100)
10Jun97	4	41% Dilution	2	2.06 (1.71)	28,600 (33,300)
10Jun97	5	46% Dilution	2	0.13 (0.14)	1,800 (600)
10Jun97	6	0%-Replaced bay water	2	4.57 (0.87)	32,800 (20,700)

Table 2. Macrofauna biomass (g m⁻²) and density (n m⁻²). Mean and standard deviation in parentheses. n = number of replicates. There were no statistically significant differences among biomass or density sample means.

			ĕ		
Date	Treatment	n	Species No.	H'	N1
10Apr97	Ambient Begin	6	6	1.2	3.3
10Jun97	Ambient End	6	5	1.3	3.7
10Jun97	0% Dilution	2	1	0	1
10Jun97	11% Dilution	2	1	0	1
10Jun97	24% Dilution	2	3	0.6	1.7
10Jun97	41% Dilution	2	5	0.5	1.7
10Jun97	46% Dilution	2	2	0.4	1.5
10Jun97	0%-Replaced	2	4	0.8	2.3

Table 3. Macrofauna diversity. Based on pooled averages over all replicate cores (n).

Table 4. Macrofauna species list. Overall mean at each station (n m⁻²).

	Dilution Treatment								
Species	Begin	End	0	0.5	1	2	2.5	0	
Polydora ligni	47	0	0	0	0	0	0	0	
Streblospio benedicti	3309	47	0	0	0	0	0	1135	
Capitella capitata	0	236	0	0	851	0	0	9218	
Oligochaetes (unidentified)	6382	851	2978	1560	8084	284	1560	21557	
Ostracoda (unidentified)	0	0	0	0	709	567	284	851	
Gammarus mucronatus	95	236	0	0	0	24535	0	0	
Corophium louisianum	331	378	0	0	0	425	0	0	
Grandidierella bonnieroides	5011	0	0	0	0	2836	0	0	

Table 5. Meiofauna density (n core⁻¹). Mean and standard deviation in parentheses. Multiply values by 3.527 to get n 10 cm⁻². n = number of replicates.

				Density				
Date	#	Treatment	n	Nematodes	Copepods	Others	Total	
10Apr97	В	Ambient Begin	6	28 (19)	6 (6)	3 (3)	37 (22)	
10Jun97	Е	Ambient End	6	2 (2)	4 (2)	1 (1)	7 (4)	
10Jun97	1	0% Dilution	2	39 (39)	449 (587)	11 (12)	498 (638)	
10Jun97	2	11% Dilution	2	47 (52)	95 (59)	3 (1)	144 (110)	
10Jun97	3	24%Dilution	2	29 (13)	10 (2)	3 (3)	41 (13)	
10Jun97	4	41% Dilution	2	59 (39)	15 (1)	9 (11)	82 (50)	
10Jun97	5	46% Dilution	2	186 (233)	77 (25)	4 (4)	267 (267)	
10Jun97	6	0%-Replaced	2	1,185 (553)	676 (298)	20 (6)	1,880 (924)	

Table 6. Meiofauna density differences among log transformed treatment means. There were statistically significant differences among sample means for nematodes (P=0.0007), copepods (P = 0.0001) and total meiofauna (P = 0.0002). Detransformed treatment means underlined are not different at the 0.05 level in a Tukey test.

Nematodes:								
Treatment:	0	46%	41%	11%	0	24%	В	Е
Mean:	1119	87	52	30	27	27	17	1
Copepods:								
Treatment:	0	0	11%	46%	41%	24%	В	E
Mean:	<u>642</u>	173	85	75	14	9	4	4
Total Meiofauna:								
Treatment:	0	0	46%	11%	42%	24%	В	Е
Mean:	1783	211	196	122	75	40	26	6

Table 7. Nematoda feeding groups. Based on pooled averages over all replicate cores. n = number of replicates.

			Deposit		Oth	ners
Date	Treatment	n	1A	1B	2A	2B
10Apr97	Ambient Begin	6	2±3	71±37	1±2	9±12
10Jun97	0% Dilution	2	0	28±11	44±18	28±30
10Jun97	11% Dilution	2	1±2	21±16	77±19	1±2
10Jun97	24% Dilution	2	9±13	50±24	41±11	0
10Jun97	41% Dilution	2	42±27	16±1	43±27	0
10Jun97	46% Dilution	2	0	19±1	81±1	0
10Jun97	0%-Replaced	2	5±5	34±27	56±30	5±2

Station Factor Scores Based on Macrofauna



Figure 27. Principal components analysis of macrofauna species data showing relationships among treatments. Abbreviations: B = beginning ambient, E = ending ambient, R replaced water mesocosm.



Macrofauna Species Factor Pattern

Figure 28. Principal components analysis of macrofauna species data showing relationships among species. Abbreviations: $Pl = Polydora \ ligni$, $Sb = Streblospio \ benedicti$, $Cc = Capitella \ capitata$, Ol = Oligochaetes (unidentified), Os = Ostracoda (unidentified), $Gm = Gammarus \ mucronatus$, $Cl = Corophium \ louisianum$, $Gb = Grandidierella \ bonnieroides$.



Station Factor Scores Based on Meiofauna

Figure 29. Principal components analysis of meiofauna taxa data showing relationships among treatments. Abbreviations: B = beginning ambient, E = ending ambient, R = replaced water mesocosm.



Meiofauna Taxa Factor Pattern

Figure 30. Principal components analysis of meiofauna taxa data showing relationships among taxa. Abbreviations: NEM = Nematodes, TUR = Turbellaria, POL = Polychaetes, OLI = Oligochaetes, GAS = Gastropods, OST = Ostracods, AMP = Amphipods, KIN = Kinorhynchs, COP = Copepods.

3.2. Tracers in Bay

Comparison of organism δ^{15} N values in 1996 revealed no significant differences in nitrogen isotopic ratios within species among the three sites (Table 8). Consequently, nitrogen isotopic data were pooled for each species from the three sites within each year. In 1996, four taxonomic groups of organisms were collected: a bivalves (the dwarf surf clam, *Mulinia lateralis*), hydroids and barnacles (*Balanus eburneus*), zooplankton (most likely *Acartia tonsa*), and fish (the anchovy, *Anchoa mitchilli*). Barnacles and hydroids are filter feeders, which persumable ingest phytoplankton and brown tide. *Mulinia lateralis* is known to eat brown tide in the laboratory (Montagna et al., 1993). Assuming a 3 ‰ enrichment per trophic level (Owens, 1987) these animals fell within the first two trophic levels of the food web (Fig. 31). Anchovies occupied the second trophic level , which reflects their diet of small zooplankton from the first trophic level, which in turn graze on particulate organic matter (POM). The low δ^{15} N values for bivalves may reflect their position in a benthic food web as deposit feeders that incorporate N from primary producers that are more depleted in ¹⁵N.

Comparison of the δ^{15} N values of organisms during a dry year (1996) and following a flood event (1997) revealed a 4‰ depletion in the mysid, *Mysidopsis bowmaniella*, which is an epibenthic species, relative to zooplankton. But less than a 1‰ ¹⁵N difference in hydrozoans or barnacles (Fig. 32). The drop in δ^{15} N values following a flooding event could reflect the addition of ¹⁵N depleted nitrate fertilizers to tributary waters flowing into Baffin Bay. However, this must be confirmed from isotopic analyses of the DIN pool of stream waters.

In 1997, the alga *Enteromorpha* sp., and amphipod *Grandidierella bonneroides* were also measured. The amphipod had the lightest ¹⁵N value measured. Amphipods graze algae and detritus in surface sediments.

Species	ID	Date	Site	$\delta^{15}N$	δ ¹³ C
Anchoa mitchilli	14A	4/22/96	Alazon Bay	13.12	-21.91
Anchoa mitchilli	14C	4/22/96	Alazon Bay	13.19	-21.56
Anchoa mitchilli	14B	4/22/96	Cayo del Grullo	12.91	-21.96
Anchoa mitchilli	14F	4/22/96	Cayo del Grullo	13.08	-21.66
Anchoa mitchilli	14E	4/22/96	Marker 36	12.13	-22.23
Anchoa mitchilli	14D	4/22/96	Marker 36	11.97	-22.61
Balanus eburneus	12C	4/22/96	Cayo del Grullo	10.21	-20.95
Balanus eburneus	12D	4/22/96	Cayo del Grullo	10.07	-20.24
Balanus eburneus	12E	4/22/96	Marker 36	8.69	-22.83
Balanus eburneus	12F	4/22/96	Marker 36	8.75	-22.16
Balanus eburneus	12G	4/17/97	Alazon Bay	9.60	-22.59
Balanus eburneus	12H	4/17/97	Alazon Bay	8.83	-22.27
Balanus eburneus	12A	4/17/97	Cayo del Grullo	8.84	-20.54
Balanus eburneus	12B	4/17/97	Cayo del Grullo	9.60	-18.75
bryozoa	11C	4/22/96	Marker 36	7.82	-23.62
bryozoa	11D	4/22/96	Marker 36	7.45	-23.88
copepod	22C	4/17/97	Alazon Bay	7.07	-25.81
copepod	22D	4/17/97	Alazon Bay	6.82	-25.64
copepod	22G	4/17/97	Cayo del Grullo	8.61	-25.27
copepod	22H	4/17/97	Cayo del Grullo	8.79	-25.32
copepod	22F	4/17/97	Marker 36	7.21	-25.98
copepod	22E	4/17/97	Marker 36	7.53	-25.66
copepod	22A	4/17/97	Marker 36	7.23	-25.33
copepod	22B	4/17/97	Marker 36	7.14	-25.24
Enteromorpha sp.	1C	4/22/96	Marker 36	7.26	N/A
Enteromorpha sp.	1D	4/22/96	Marker 36	6.97	N/A
Enteromorpha sp.	1A	4/17/97	Alazon Bay	8.83	-20.09
Enteromorpha sp.	1B	4/17/97	Alazon Bay	8.40	-20.73
Grandidierella bonneroides	19A	4/17/97	Cayo del Grullo	5.27	-20.78
Grandidierella bonneroides	19C	4/17/97	Marker 36	3.83	-19.86
Grandidierella bonneroides	19D	4/17/97	Marker 36	3.75	-19.85
hydrozoa	10E	4/22/96	Cayo del Grullo	8.22	-22.73
hydrozoa	10F	4/22/96	Cayo del Grullo	8.34	-22.67
hydrozoa	10A	4/17/97	Marker 36	7.13	-21.84
hydrozoa	10B	4/17/97	Marker 36	6.92	-21.54
Mulinia lateralis	18F	5/16/96	Alazon Bay	7.90	-22.33
Mulinia lateralis	18G	5/16/96	Alazon Bay	7.23	-22.27
Mulinia lateralis	18A	5/16/96	Marker 36	7.20	-21.80
Mulinia lateralis	18D	5/16/96	Marker 36	7.48	-21.94
Mysidopsis bowmaniella	5A	4/22/96	Alazon Bay	10.68	N/A
Mysidopsis bowmaniella	5B	4/22/96	Alazon Bay	11.02	-23.00
Mysidopsis bowmaniella	5C	4/22/96	Marker 36	9.60	-24.46
Mysidopsis bowmaniella	5D	4/22/96	Marker 36	10.19	-24.03

Table 8. $\delta^{15}N$ and $\delta^{15}C$ values of fauna collected in Baffin Bay in April 1996 and 1997.

Dry Conditions - 1996

⊢ **Fish** (Anchovy)

⊢ → Zooplankton (Mysid)

⊢ Hydroids & Barnacles

Here Bivalves (Surf Clam)





Figure 31. Nitrogen isotope values (δ^{15} N) for taxa in Baffin Bay based on samples collected at all sites under dry conditions (April 1966). Values are means ± SE (n = 4 - 6).



Figure 32. Comparison of nitrogen isotope(δ^{15} N) values for trophic groups during an extended dry period (April 1996) and following a runoff event (April 1997).

4. DISCUSSION

4.1. Mesocosm Experiment

4.1.1. Plankton Processes

Hydrographic measurements near mesocosms and inside each of them demonstrated that changes in Baffin Bay occurred after the runoff inflow events of 2 and 3 April 1997. The salinity and temperature of Cayo del Grullo, a tertiary bay of Baffin Bay, increased over time. The increase is most likely due to the normal summer heating and evaporation processes. Salinity inside all of the mesocosms increased more than ambient bay waters, but temperature was identical inside and outside the mesocosms (Fig. 4). The initial salinity concentrations differed because of the experimental design and those relative differences were maintained throughout the entire period. The maintenance of the salinity differences indicates the mesocosms were "sealed" in the sediments and no leakage or tidal pumping of water occurred through the sediment layer. The cooling event during the first week affected temperature inside and outside the mesocosms exactly the same (Fig. 5). The general physical condition inside mesocosms was similar to Cayo del Grullo outside the mesocosms.

Dissolved Oxygen (DO), pH and Secchi depth are variables related to reducing potential, buffering and transparency conditions of the water. While these measurements were variable, their ranges were typical for a sub-tropical lagoon ecosystems. The lowest DO occurred in mesocosm 5 with a 50% mixture of runoff water and bay water. The low DO could have bee due to osmotic shock of autotrophic populations, which then died and decayed leading to high oxygen demand by decomposer bacteria (Fig. 6). Secchi depths declined to 5 cm during the latter part of the period because of the very high phytoplankton populations in mesocosms (Fig. 8).

Nitrate, nitrite and ammonium nutrients are thought to be limiting phytoplankton growth in Laguna Madre and the Baffin Bay because of increased growth found in several nutrient addition studies (Whitledge, personal communication). Measurements of those nitrogen nutrients in the mesocosms indicate that most of the time very small ambient concentrations were present. The nitrogen availability was very small for the biomass of phytoplankton present in mesocosms. The infrequent increases in nitrogen nutrients appears to be related to increased nitrification and/or denitrification processes as indicated by the presence of nitrite (Fig. 10). The sum of the dissolved inorganic nitrogen (DIN) was initially large (Fig. 11) due to additions of agricultural runoff water, and controls (without additions) remained low. The mesocosms declined to control levels after 10 days. Later increases of DIN were likely to be related to sediment fluxes because mesocosm 7 (with the plastic bottom) and the ambient samples did not increase.

Silicate and phosphate nutrients did not display behavior that would indicate influences on phytoplankton utilization. The initial phosphate concentrations were slightly increased due to the agricultural runoff water (Fig. 14). Silicate was unaffected biologically, and was diluted conservatively by physical means (Fig. 15).

Chlorophyll concentrations in mesocosms started at the level of brown tide bloom concentrations, ranging from 20 - 40 μ g l⁻¹ (Fig. 15). Chlorophyll concentrations increased in mesocosms 4 and 5 to 142 and 197 μ g l⁻¹ respectively over the first two weeks. These data demonstrate that agricultural runoff water can have a stimulatory effect on phytoplankton after the initial dilution and salinity shock. In support of this finding, rates of primary production (Fig. 16) were generally high, ranging from 3 to 8 g C m⁻³ d⁻¹, but some values were very high (13 g C m⁻³ d⁻¹). Although phytoplankton in general increased, brown tide densities were not affected by the experimental runoff additions (Figs. 23 - 26). Brown tide did increase to bloom densities in the 24% and bay water replacement controls during the first eight days, but decreased afterward.

4.1.2. Nutrient Amendment Studies

Nutrient amendment studies were initiated on days 2, 10 and 15. Initial amendments were performed in freshly diluted, low salinity water where nutrient concentrations were already high. By the time the last dilution occurred, nutrients were declining in mesocosms. Amendments of mesocosm water started on day 2 (Fig. 17), when runoff nutrients were still relatively high in mesocosms 3, 4 and 5 did not appreciably stimulate phytoplankton biomass compared to the control. Only slight effects were observed for N and N+P additions while trace metal additions produced a somewhat greater increase. Mesocosm 1, the control with no runoff addition, had the greatest response to everything except P amendment. Amendments of mesocosm water started on day 10 (Fig. 19) did not exhibit responses in mesocosms with runoff additions, although there was a slight increase in mesocosm 4 with the N+P amendment. However mesocosm 1, with no runoff additions, had a significant response to all additions except P. Amendment of mesocosms water initiated on day 15 (Fig 21) had responses to nutrient additions in all mesocosms except mesocosm 5. Additions of N, N+P, and trace metals produced significant responses while P additions did not. Overall, the additions confirm results from prior research concerning nutrient effects on phytoplankton growth in the Baffin Bay region. When nutrients are high, additional nutrients do not stimulate phytoplankton productivity, but amendments do stimulate growth when nutrients are low. When nutrients are high, trace metals may be limiting, but when nutrients are low, nitrogen appears to be most limiting.

4.1.3. Benthic Processes

Estuarine benthic infauna are very susceptible to fluctuations in their environment because of limited mobility. Large changes in salinity or nutrient concentrations will affect distribution, abundance, and diversity of benthic infauna (Kalke and Montagna, 1991; Montagna and Kalke, 1992; 1995). Abundance and biomass of infauna may increase if nutrients from river input is transformed into food for benthic animals (Montagna and Yoon, 1991). This occurs when river derived nutrients stimulate primary production (Deegan et al., 1986; Nixon et al., 1986). Organic matter can be deposited, but it may also be advected and deposited further downstream, so increases in benthic productivity might occur away from the river mouth. This assumes salinity dilution or river borne xenobiotics, e.g., pesticides, do not have lethal or sublethal effects on benthos. Salinity stress on physiology (Finney, 1979), and hypoxia caused by algal blooms (Hull, 1987) could reduce benthic populations. The net effect of freshwater runoff is a function of the interaction between physical processes (i.e., sedimentation, resuspension, and advection),

chemical processes (nutrient enrichment), and biological processes (i.e., enhanced productivity, recruitment gains, and losses via low-salinity intolerance).

Benthic infauna are useful in environmental studies because they are relatively immobile and long-lived compared to plankton of similar sizes. Macrofauna and meiofauna could respond to runoff at different spatial and temporal scales. Macrofauna have planktonic larval dispersal, so would indicate effects over larger spatial scales and longer temporal scales. Meiofauna have direct benthic development, and generation times as short as one month, thus indicate effects over smaller smaller spatial scales and shorter temporal scales.

If freshwater runoff enhances benthic productivity then increased abundance and biomass should be found with greater sea water dilution by runoff water. In contrast, if agricultural runoff contains toxic compounds, abundance and biomass should decrease. Agricultural runoff was collected and used to dilute bay water in mesocosms. After two months, benthic response to the runoff in mesocosms was measured and compared to ambient conditions.

There were no obvious differences among the treatments for macrofauna. This is in spite of a 20 fold difference in macrofauna density in the lowest and highest treatments. Also, there was no agreement between the two 0 dilution replicate mesocosms. However, one control (mesocosm 6) had a complete replacement of water, and this could have affected recruitment of meroplankton larvae, which are characteristic of most macrofauna. Overall, the lack of trend for macrofauna was due to a very low diversity and abundant ambient community. The Baffin Bay community is typically low in diversity and abundance, but the ambient values recorded in June 1997 were among the lowest recorded since 1988. Changes in macrofauna community structure appears to be just a mesocosm effect. In general, high variability within mesocosms inhibited the ability to differentiate among mesocosms. Perhaps, with three or four replicate mesocosms, we could have observed statistically significant differences among treatments.

In contrast, meiofauna did respond to mesocosm treatments with enhanced productivity over ambient conditions (Table 5). In particular, the copepods had very high densitites. Typically, copepods make up 5 - 10% of a meiofaunal community. In this study area, copepods comprised 45% of the community. Copepod density was low in ambient treatments, about normal in the dilutions, but very high in the undiluted treatments. Feeding guilds of nematodes also changed in the dilution series and over time from a deposit feeding community to a non-deposit feeding community (Table 8). The change could be due to a lack of deposition of fresh material derived from tides or wind-driven water movement. The change from deposit feeders was less in the higher runoff dilutions, most likely due to higher particle loads being placed in mesocosms.

For meiofauna, the two undiluted treatments generally agreed with one another. Meiofauna, with direct benthic development, may not have been affected by the complete water replacement in mesocosm 6. The undiluted treatments act as a control for the dilution treatments. There are actually several effects in a mesocosm: predator exclusion, lack of mixing or resuspension, increased evaporation, and runoff addition. All but the latter are also occurring in the undiluted mesocosms. Three contrasts exist: ambient versus mesocosm, dilutions versus undiluted controls, and ambient versus controls. In addition, the study design contains a gradient of dilutions. The meiofauna response is an average 6200% increase in controls over ambient

conditions, but only a 680% increase in runoff over ambient conditions. That is, there was a 930% increase in undiluted versus diluted treatments. Therefore, the dilution treatments appear to have had a negative impact on meiofauna, because diluted treatment did not respond as well as undiluted treatments..

In summary, there is no evidence that runoff has any effect on macrofauna. In contrast, meiofauna populations may be negatively impacted by roughly an order of magnitude. No information exists on the relative roles of meiofauna and macrofauna in Baffin Bay. In general, it is thought that meiofauna and macrofauna have different functions in shallow marine ecosystems. Meiofauna have direct benthic development, in contrast, macrofauna have pelagic larvae. Meiofauna also have much shorter generation times that macrofauna. The ability to reproduce directly into sediments, the short generation time, and predator exclusion are plausible explanations for the meiofaunal bloom in mesocosms. Meiofauna are thought to be more closely linked to nutrient cycling than macrofauna because they are smaller and feed primarily on bacteria, diatoms, and protozoa. However, the nutrient stimulation introduced by runoff dilutions did not enhance meiofauna. In contrast, meiofauna declined relative to undiluted mesocosms. It is not known what could have caused the relative decline, but pesticides are a plausible explanation.

4.2. Tracers in Bay

The major sources of inorganic nitrogen (wastewater, fertilizer, and atmospheric deposition) to watersheds can be identified using stable isotope ratios (Kreitler et al., 1978; Gormly and Spalding, 1979; Kreitler, 1979). Consequently, stable nitrogen isotope ratios can be used to track sources of anthropogenic nitrogen into an estuarine food web. These major sources of anthropogenic nitrogen can be identified by their distinct isotopic signatures; e.g. δ ¹⁵N values of -3 to +3 ‰ for nitrate from synthetic fertilizers, 2 to 8 ‰ for groundwater only influenced by atmospheric deposition, and 10 to 20 ‰ for nitrate derived from human and animal wastes.

In Baffin Bay, seasonality of freshwater inflow and input of agriculturally derived nitrogen presents an excellent opportunity to examine the nitrogen stable isotope rations of the biota as a function of N loading from a defined source. In estuarine waters, the δ^{15} N values of the biota range form 3-6 ‰ (Fry et al., 1987; Dunton, unpublished data), which reflects the δ^{15} N value (2-5 ‰) of the dissolved inorganic pool (Owens, 1987). In contrast to the low δ^{15} N signatures of estuarine waters, Kreitler (1979) and Kreitler and Jones (1975) found that the δ^{15} N values of groundwater nitrogen on fertilized and grazed uncultivated fields ranged from 10-20 ‰. This is attributed to the volatilization of ¹⁴N -rich ammonia during the early stages of nitrogenous waste degradation, as well as microbial processes (Macko and Ostrom, 1994). The low δ^{15} N values associated with synthetic fertilizers are due to the conversion of atmospheric N₂ during manufacturing (Freyer and Aly 1974; Gormly and Spalding, 1979).

Based on these observations, anthropogenic inputs of inorganic N can be detected readily using natural abundance stable isotope techniques. In Texas watersheds, other studies have found that the application of fertilizers (which have δ ¹⁵N values that range from -3 to +3 ‰; Kreitler, 1975) promote plant growth and hence the production of animal wastes that are characterized by high δ

¹⁵N values. Application of fertilizers also increase soil denitrification, with resulting increases in the δ^{15} N of nitrate pools, as detected in nitrate contaminated ground waters.

The overall range of δ^{15} N values for algal producers and secondary consumers in Baffin Bay (7 to 13 ‰) is higher than values reported elsewhere for estuarine systems. In Waquiot Bay, MA δ^{15} N values range from 3 to 9 ‰ (McClelland et al., 1997), compared to 2 to 10 ‰ in a Georgia salt marsh estuaries (Peterson and Howarth, 1987). The range of values listed for Baffin Bay are lower than those reported for the Guadalupe Estuary (10 to 17 ‰; Dunton, unpub. data), but nearly match values for Lavaca and Corpus Christi Bays (5 to 13 ‰; Dunton, unpub. data). Higher δ^{15} N values for the Guadalupe Estuary are suspected to reflect addition of ¹⁵N enriched groundwater or river water derived from human and/or animal wastes.

In conclusion, based on tracers, there is no clear evidence that agricultural nitrogen enters the food web of the Baffin Bay region. The completion of isotopic analyses on water samples collected from major tributaries would provide critical data on the δ^{15} N value of the DIN pool that is utilized by primary producers. This should help explain the ¹⁵N depleted zooplankton collected during post-flood conditions as well as the higher ¹⁵N content of the fauna during dry periods.

4.3. Analysis of Effects of Cropland and Nonpoint Runoff on the Brown Tide Phytoplankton Bloom in Baffin Bay

There is a strong theoretical basis to predict that non-point agricultural runoff has a stimulating affect on phytoplankton populations. There are two main components, which act in the same way: fertilizers and pesticides. The fertilizers can supply limiting nutrients while the pesticides can limit grazers. The net effect would be blooms of phytoplankton.

Eutrophication from nutrient loading in coastal and estuarine systems is rapidly becoming a major problem as human population and development continues to soar in coastal areas (Valiela et al., 1992; Short and Burdick, 1996). Both urbanization and agricultural practices result in the release of nitrate enriched groundwater (Valiela et al., 1992) which are flushed into estuaries through run-off or riverine inputs (Stevenson et al., 1993). It is well recognized however, that although the magnitude of non-point source nutrient loading to nearshore systems is largely unknown for most of the Nation's estuaries, continued increases in N loading will lead to long-term or irreversible damage to estuarine living resources (Dennison, et al., 1993; Burkholder et al., 1995).

Nutrient loading has led to the increased abundance of both toxic (Burkholder et al., 1995) and nuisance algal blooms which reduce the oxygen concentration of the water column, resulting in losses of shellfish and finfish populations and changing the structure and function of valuable nearshore community food webs (Valiela et al, 1992). The effects of nutrient pollution on seagrass distribution have been clearly demonstrated by Short and Burdick (1996). Descriptive field studies have found that epiphytic algae appeared to inhibit or eliminate seagrasses entirely (Dennison et al., 1993) and experimental work has demonstrated that nutrient loading can reduce seagrass productivity and health by stimulating algal competition (Short et al., 1995) and by direct nitrate toxicity (Burkholder et al., 1994).

Dilution of Baffin Bay water by agricultural runoff collected from drainage ditches had only a slight effect on brown tide and phytoplankton production. This was due to the already high levels of nutrients in bay waters. Chlorophyll biomass and rates of primary production increased in mesocosms with runoff additions compared to controls with no additions during the first 7 - 10 days. No effects were found on microzooplankton, or macrobenthos, but effects were noticed on meiofauna. Effects of runoff are confounded with salinity effects (caused by the dilution) and temperature effects (caused by frontal passage just after the mesocosms were set up).

Stable isotope tracers in the bay did not respond as if influenced by a large addition of fertilizer laced runoff. Results from the tracer and experimental studies indicate that effects of runoff due to the three-inch rainfall event that was studied must have been very small to unmeasurable.

Biological responses to experimental additions of runoff were not linear, or a function of the dilution performed. In spite of lack of a clear runoff signal in the mesocosm experiments, some positive results were noted. When microzooplankton increased, brown tide decreased, indicating grazing control exists. When nutrients were added to depleted mesocosm water, phytoplankton was stimulated. Only nitrogen and trace metals were responsible for th stimulation. Meiofauna densities were higher in control than in mesocosms with runoff added, and the trophic structure of nematodes changed from a deposit feeding community to an epigrowth feeding community. Zooplankton in the bay did exhibit lower nitrogen isotope values after the runoff event, as predicted if nitrogen from fertilizers was being incorporated into the food chain.

5. CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

The responses of brown tide observed in mesocosms with mixtures of agricultural runoff water indicate that stimulation of growth occurs. There were observable increases in chlorophyll biomass in mesocosms with runoff additions compared to controls with no additions. The rates of primary production in mesocosms with runoff water during the first 7-10 days of the period were very large compared to controls. The biomass of phytoplankton in mesocosms with runoff more than doubled during the initial week. Increases of chlorophyll to extremely high concentrations in mesocosms with runoff point to potential long-term effects that may occur in ambient waters of Baffin Bay. Runoff coupled with the unique sluggish hydrography of Baffin Bay (due to long residence times of water within the region and microtidal ranges) could lead to high, but localized nutrient loading.

Nitrogen is the most likely element that limits growth of brown tide in the Baffin Bay ecosystem. Trace metals and phosphorus combined with nitrogen often produce an additional stimulation to phytoplankton growth. The role of sediments cannot be neglected when considering the nutrient environment of Baffin Bay. Mesocosm 7, with an enclosed bottom of plastic, did not behave like the mesocosms with bottoms open to the sediments. Late in the experiment, nutrients in mesocosms increased even though ambient waters and the sediment control did not, indicating high rates of regenerated nitrogen.
The experimental design of the study could be improved to increase power to detect the biological responses to runoff in the mesocosms. Distilled water controls could be used to distinguish effects due to salinity versus runoff water. Specific dilutions should be replicated because there is no clear functional relationship between dilution and response. The dilution series was realistic, because strong storms are know to lower salinities in Baffin Bay to as low as 20 psu.

This research does not conclude that agricultural runoff of nutrients is the sole reason brown tide appeared in the Baffin Bay complex in 1990, nor does it conclude that agricultural runoff is responsible for maintaining the bloom since that time. It does conclude that nutrients in the runoff can stimulate growth of phytoplankton. The increase of nitrogen concentrations (by about 20 umol 1⁻¹ or 0.28 mg 1⁻¹) was correlated with about a doubling of rates of primary production and biomass of phytoplankton. Other nitrogen inputs from atmospheric, groundwater, or sediment sources could add to the agricultural inputs to produce the brown tide bloom that has been observed in Baffin Bay over the past few years. A mass-balance of nitrogen derived from agricultural runoff, the atmosphere, groundwater, and sediment is needed to make a final assessment of the role that runoff derived from croplands has in promoting or maintaining algal blooms.

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