A COMPARISON OF METHODS FOR MONITORING EPIPHYTES ON EELGRASS *(ZOSTERA MARINA* L.*)*

ASSATEAGUE ISLAND NATIONAL SEASHORE MARYLAND AND VIRGINIA

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EXECUTIVE SUMMARY

The efficacy and efficiency of several methods of monitoring of epiphytes on eelgrass (*Zostera marina* L.), a submerged aquatic vascular plant, were tested in the Maryland and Virginia Coastal Bays to support the development of long term monitoring program for this indicator of estuarine health. Both direct methods (removal of epiphytes from eelgrass leaves and measuring either dry weight or chlorophyll a concentration) and indirect methods (epiphyte light attenuation properties through artificial eelgrass leaves ("mimics")) of measurement of epiphyte abundance were tested. Statistical power, timing of monitoring, biological relevance, and logistical considerations are noted and discussed.

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INTRODUCTION

The role of submerged aquatic vegetation (SAV) as an important component of estuarine ecosystems, serving as a food source and nursery for a variety of organisms, contributing to water quality, and indicating ecosystem health has been well recognized (Orth and Moore 1984; Batiuk et al. 1992: Bohlen et al. 1997). The Maryland and Virginia Coastal Bays contain large and apparently healthy beds of SAV, which have increased in area from 2,134 hectares in 1986 (Orth et al. 1987) to 5,598 hectares in 1997 (Orth et al. 1998). Within the Maryland part of the Coastal Bays, over 90% of this area occurs within Assateague Island National Seashore; this significant natural resource is regarded to be crucial to the maintenance of regional biological diversity and ecosystem health (National Park Service 1994)

The decline of SAV due to attenuation of light in the water column has been observed in many estuaries worldwide, with anthropogenically induced increases in suspended solids and/or phytoplankton responding to nutrient enrichment often implicated (Orth and Moore 1983; Tomasko et al. 1996). More recently, the role of organisms that are epiphytic on SAV leaves and that increase in abundance in response to increased water column nutrients has been implicated in SAV decline (Twilley et al. 1985; Short and Burdick 1996).

There is a need for long term monitoring of SAV in order to detect and/or avert loss from anthropogenic impacts (National Park Service 1994). The National Park Service (NPS) has monitored estuarine water column parameters, including those most likely to affect SAV distribution and abundance (Batiuk et al. 1992) in the Coastal Bays since 1987 (National Park Service 1991; Sturgis 2001). The boundaries and densities of SAV beds in the Coastal Bays have been delineated and mapped from aerial photographs annually by the Virginia Institute of Marine Science since 1986 (e.g., Orth et al. 1998). This annual census serves as a very useful monitoring system to assess SAV abundance in the bays. However, it is possible that monitoring through remote sensing may detect declines in SAV bed size or density only well after stresses causing declines have begun to operate. A monitoring program which serves as an earlier warning and possibly shows correlations between abundance as measured from remote sensing and plant condition and stress levels is necessary to protect this important resource.

The monitoring of SAV epiphytes as an indicator of SAV and estuarine condition is a logical strategy from two perspectives if a model of epiphyte increases with water column nutrient increases and of shading of SAV at the leaf surface by epiphytes is accepted. First, epiphytes may be regarded as a stress on SAV, with high abundances generally regarded as a potential threat to SAV health and abundance. Second, epiphytes, which often increase rapidly and opportunistically in response to water column nutrient enrichment, may be regarded as biological indicators of water column trophic status and, consequently, of level of degradation.

From 1998 through 2000, the NPS conducted a comparison of several methods and schedules of monitoring SAV epiphytes, in order to determine the efficacy of long-term monitoring of health and

condition of SAV in the Maryland and Virginia Coastal Bays (Chincoteague, Sinepuxent, Newport, Isle of Wight, and Assawoman Bays). The results are presented here as discussion items for subject-matter experts seeking to assess ecologically and statistically valid and efficient methods of monitoring epiphytes.

METHODS

Direct measures of epiphyte abundance.

In 1998, six fixed SAV monitoring plots (stations) were established in SAV beds dominated by eelgrass (*Zostera marina* L.) (Table 1) (Figure 1). The beds were selected as representing different combinations of geographic position, depth, and water column nutrient loading that occur in the Maryland and Virginia Coastal Bays. Plots were circular with a radius of 10 meters. The position of the center of each plot was

Table 1. Monitoring stations for investigations of parameters establishing SAV habitat requirements in Maryland-Virginia Coastal Bays, 1998-2000.

Station name and letter	Location of Station, with Easting (E) and Northing (N) in UTM meters (NAD-83)	Station Depth Mean (95% Conf. Int.) (n)	Years Epiphytes Monitored	Years Mimics Monitored
Channel Marker 25 (A)	Sinepuxent Bay E 485495 N 4231120	0.97 (0.94-1.01) (57)	1998-2000	1999-2000
Rum Point (B)	Sinepuxent Bay E 485495 N 4231120	0.62 (0.59-0.65) (57)	1998-2000	1999-2000
Tingles Island Shallow (Ds)	Chincoteague Bay E 481944 N 4223786	0.86 (0.81-0.92) (34)	1998-1999	1999
Tingles Island Deep (Dd)	Chincoteague Bay E 481803 N 4223681	1.19 (1.14-1.25) (36)	1999-2000	1999-2000
Coards Marsh Shallow (Es)	Chincoteague Bay E 471490 N 4206256	0.89 (0.82-0.96) (34)	1998-1999	1999
Coards Marsh Deep (Ed)	Chincoteague Bay E 471265 N 4205943	1.29 (1.25-1.34) (37)	1999-2000	1999-2000
Spence Cove (G)	Newport Bay E 482755 N 4231111	0.98 (0.93-1.03) (49)	1998-2000	1999-2000
Route 90 (Z)	Isle of Wight Bay E 493465 N 4248543	0.89 (0.85-0.93) (48)	1998-2000	1999-2000

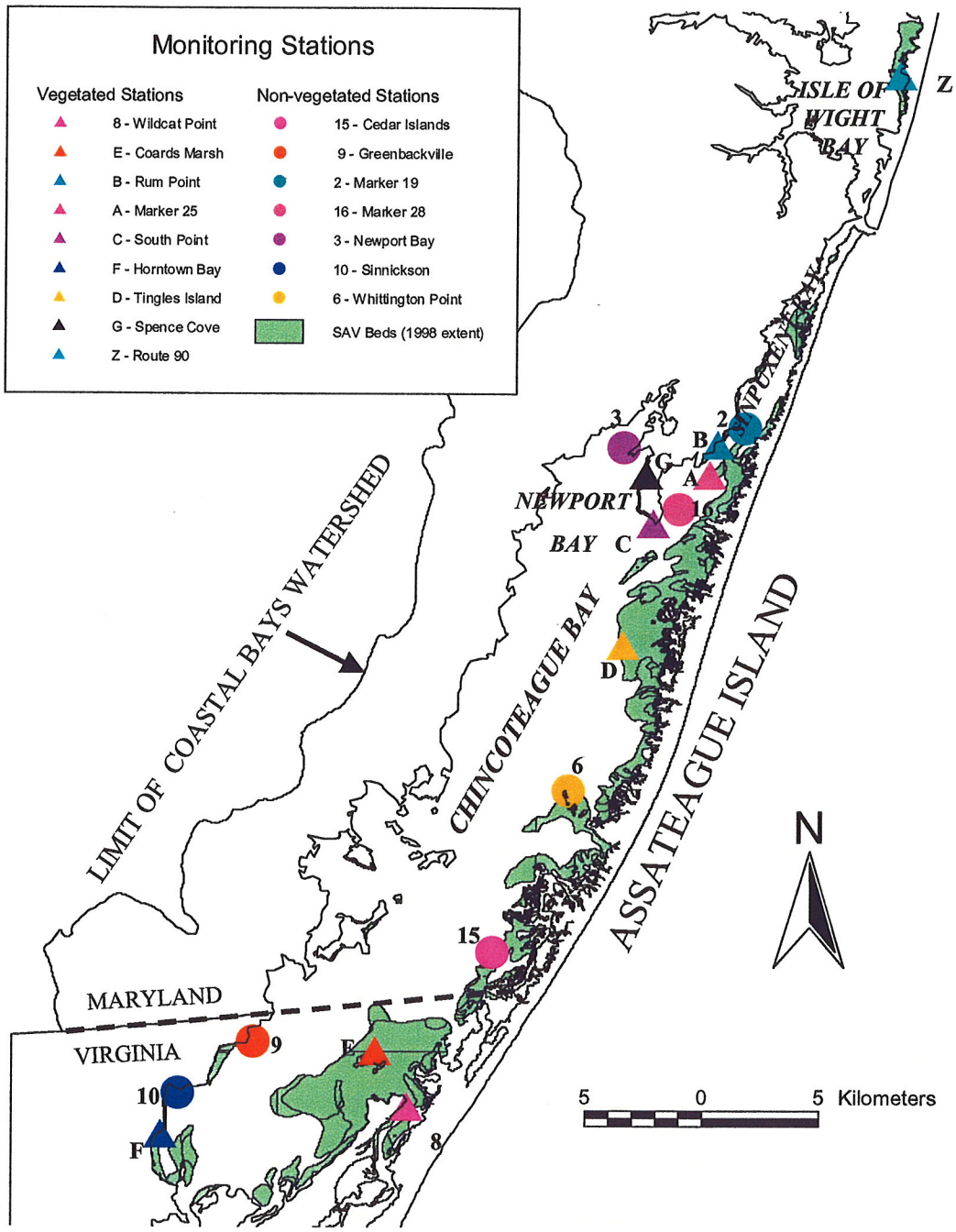


Figure 1. SAV habitat requirement water quality monitoring stations, Maryland-Virginia Coastal Bays, 1998-2000. Pairs of stations used for comparing vegetated and non-vegetated sites are of like color (stations G and Z were not used for paired comparions).

marked by a polyvinyl chloride (PVC) stake driven into the sediment and was recorded by Global Positioning System (GPS). In 1999 an additional station of a different depth from those established in 1998 was established near each of the Tingles Island and Coards Marsh stations to create paired stations at each of those areas that were assumed to differ from each other only by water depth.

Each station was visited by boat, in sequence, at approximately one-month intervals. At a station, 10 replicate sample units of eelgrass leaves were collected, by selecting a point within 10 meters of the station center post determined by a random azimuth and a distance in meters equal to the square root of a random number from 0 to 100. If no eelgrass was present at the point designated for collection of a sample unit, an alternate random point was visited until ten sample units were visited. In 1998, from ten to twelve shoots (ramets) of eelgrass were collected at each sample unit point, to be split between epiphyte biomass (= dry weight) measurement and epiphyte chlorophyll measurement. In 1999 and in 2000, from ten to twelve eelgrass shoots were collected for epiphyte biomass measurement and from five to seven different shoots were collected for epiphyte chlorophyll analysis. All sample unit collections were enclosed individually in plastic bags filled with water collected at the station. Bags were placed on top of ice in a cooler. All collected material was transported to the laboratory and placed in a refrigerator until processing, which usually occurred the following day and always within two days of collection.

Monitoring station depths were measured at the plot center at each monitoring session and during other visits to the station by holding a PVC pole marked at 5 cm increments vertically with the bottom resting on the sediment at the bottom of the water column. The mean depths calculated for the station are listed in Table 1. Water column concentrations of dissolved inorganic phosphorus (DIP) (= PO_4^{2}) and of dissolved inorganic nitrogen (DIN) (= sum of concentration of NO_2^{-} - NO_3^{2-} and of NH_4^{+}, $^{-}$) were measured at similar intervals at stations as described by Lea et al. (2003).

In the laboratory, all leaves from each sample unit were removed at their bases from the shoot of each plant (the lowermost part of the plant shoot was not included for the analysis). The length of each leaf was measured by ruler to the nearest 5 mm, and the width was measured by ocular micrometer in a 10x dissecting microscope to the nearest 0.5 mm. The product of the leaf length times the leaf width times two was calculated as the total surface area of the leaf (from which epiphytes were to be removed). Individual surface areas of all leaves from all plants collected for a sample unit were summed to yield the total leaf surface area for the sample unit. For each of the ten replicate sample units made for chlorophyll a measurement and for each of the ten replicate collections made for epiphyte biomass measurement, epiphytic material from all sample unit plants was scraped from both sides of each leaf into a container of filtered sea water (i.e., the scrapings of all plants collected for a single sample unit pooled into a single sample unit). For each of the ten sample unit collections, the scrapings/sea water slurry was filtered through a 0.7 μm fiberglass filter using a vacuum pump. In many cases, it was necessary to first filter the slurry

through one or more 1.5 μm fiberglass filters to accommodate the volume of epiphytic material; in such cases, the pooled material on all filters represented the quantity of epiphytes for the sample unit. It was assumed that the filters captured all epiphyte material (i.e., epiphyte material that was small enough to pass through 0.7 μm filters contributed negligible amounts to the total loadings on the SAV leaves).

In 1998, the epiphyte material collected for each sample unit was split for separate measurements of epiphyte biomass and epiphyte chlorophyll by cutting all filters holding captured epiphyte material in half by hand using a clean razor blade, and assigning one half of each filter to either biomass or chlorophyll a analysis for the sample unit. In 1999 and 2000, the separately collected epiphyte biomass and epiphyte chlorophyll sample units were filtered separately.

For the each of the ten sample units collected from a single station during a single session for measurement of chlorophyll a density, the filter and material collected on it were enclosed in an aluminum foil wrapper and frozen at -15° C until transported to the Horn Point Laboratory in Cambridge, Maryland. At Horn Point, each filter was cut, placed into a grinding tube with 90% acetone, and ground until macerated. The acetone slurry was filtered by vacuum pump through a 1.5 μm fiberglass filter into a 15 ml calibrated test tube. The extraction volume was measured and the chlorophyll a concentration was measured by Turner Designs TD-700 fluorometer (Arar and Collins 1992), using the non acidification method (Welschmeyer 1994); the concentration (in μg/L) was multiplied by the volume of the filtrate/extraction volume to derive the concentration of chlorophyll a in μg/L for the sample unit.

For the each of the ten sample units collected from a single station during a single session for measurement of epiphyte biomass (dry weight), a pre-weighed filter was used. The filter and material collected on it were dried at 40° C. in an oven for at least 24 hours and subsequently weighed. The difference between the weight of the dried filter plus scraped material and that of the pre-weighed filter were recorded as the epiphyte dry weight for the sample unit. The dry weight for a sample unit was divided by the sample unit leaf area to yield the epiphyte [dry weight] biomass for the sample unit. Inorganic material scraped from leaves was observed generally to be negligible and was assumed to contribute no weight.

For each sampling session at each station, the mean and standard deviation of the sample was calculated, for both the epiphyte chlorophyll a density and the epiphyte biomass density samples. A power analysis was conducted for each individual sample, at three levels of Type I and Type II error rates by calculating the minimal detectable difference for epiphyte chlorophyll a density for each ten-sample unit sample, using the following equation, modified from Elzinga et al. (1998):

$$MDD = [(s)*(Z_\alpha + Z_\beta)]/ \sqrt{n}$$

Where:

MDD = the minimum detectable difference from a threshold value

s = the standard deviation of the 10 sample [original] sample unit

Z_α = Z (standard normal deviate) value for specified false-change (Type I) error rate

Z_β = Z (standard normal deviate) value for specified missed-change (Type II) error rate

n = sample size to be used for future sampling (to detect difference from original sample) (10 used)

or, $MDD = (Z_\alpha + Z_\beta)*SE$

since $s/\sqrt{n}$ is equal to the standard error of the sample mean (SE).

This analysis provides an estimate of the level of change in the parameters that may be detected, at a given probability of detection, with a similar sample size, if it is assumed that the present level of within-sample variability will not be exceeded.

METHODS

Investigation of response of epiphytes to nitrogen concentration (applicability of epiphyte abundance as a trophic status indicator).

If epiphytes increase in abundance in response to increases in nutrient concentrations, then they may be used as an indicator of nutrient status in an estuary. Compared to direct measurement of nutrients, this approach has the disadvantage in that other factors (light, temperature, grazing) may also influence epiphyte abundance and are often sources of greater variation in epiphyte abundance. However, epiphyte abundance may show less temporal variability than do concentrations of nutrients, especially dissolved inorganic nitrogen, if epiphytes take up nutrients and show a delayed, cumulative response in abundance to nutrient concentrations.

Concentrations of DIN were measured at SAV monitoring stations at approximately 4 week intervals in 1998 and at approximately 2 week intervals in 1999 and 2000 (Lea et al. 2003). Estimates of daily concentrations were made by linear interpolation between the measured concentrations of the DIN measurement immediately preceding the date and the DIN measurement immediately following the date. Daily DIN concentrations for the station were averaged over periods ranging from 1 to 45 days preceding each epiphyte measurement session to obtain cumulative DIN concentrations for the preceding 1 to 45 days.

For each of the years of the study, *r*, the Pearson product-moment correlation coefficient (Zar 1996) between the mean values of epiphyte biomass concentrations for all sampling sessions that year and the DIN concentrations for the immediately preceding day was calculated. The value of *r* between the epiphyte biomass concentration and the preceding 2-day average was then calculated, and the process repeated for

up to the preceding 45-day interval. These 45 values of *r* were plotted against the number of days over which the DIN concentration was averaged. The process was repeated, substituting epiphyte chlorophyll a concentrations for epiphyte biomass concentrations. Finally, the analysis was also performed by calculating progressive values of *r* for the six individual stations.

METHODS

Use of artificial substrates (SAV mimics).

Methodology for this experiment follows procedures developed by staff of the University of Maryland Chesapeake Biological Laboratory (Stankelis et al. 2003), with some modifications. 508mm x 25.4 mm (20" x 1") x 1 mm thick Mylar™ strips were used as SAV mimics (Figure 2). On each strip, three equal 5" x 1" (127 x 25.4 mm) sections (top, center, bottom) were marked on the strip, with the remainder of the length of the strip divided into two short handling/anchoring sections, each with a hole punched in them, at either end. At the beginning of the eelgrass growing season in 1999 and in 2000, a square polyvinyl chloride (PVC) frame, weighted with sand, was deployed on the bottom of the bay at each epiphyte monitoring station (Figure 2). Mimics were attached to each frame by plastic cable ties through the anchoring hole at one end and attached to another cable tie on the frame. A small foam float was attached to the top of each mimic using plastic cable ties. The mimics were thus anchored near the bay bottom and suspended vertically by the float in the water column. Four mimics were attached to a frame to form an array and were left *in situ* to become fouled with epiphytes. These "interval monitoring" mimics were recovered at a more or less constant intervals of approximately 15 days and placed in a capped [opaque] PVC tube filled with seawater. Fresh mimics were deployed, and the fouled mimics were transported, stored in a cooler with ice, to the laboratory. In the laboratory, the tubes were immediately placed in a refrigerator (3-5 ° C.) for no longer than two days before light attenuation data collection. Because of the potential of loss or unacceptable disturbance to the arrays from storms or human activities (e.g., vandalism or accidental strikes by boats or personal watercraft), two replicate arrays were deployed at each station in 2000; this usually allowed a sample of 8 mimics to be collected every 15 days.

In 1999 only, in order to investigate the effects of longer periods of mimic deployment, three additional arrays, each equipped with 14 mimics, were deployed at the beginning of the growing season. At each retrieval of the "interval monitoring" mimics, three of these "seasonal monitoring" mimics (one from each array) were retrieved (so that subsequent retrievals were of mimics that progressed from shorter to cumulatively longer exposure, rather than of mimics of a constant time of exposure) until all were recovered by the season's end. Seasonal monitoring mimics were deployed for a period of from 15 days to 181 days.

In the laboratory, the mimics were submerged in a box (Light Attenuation Measurement Apparatus or LAMA) (Stankelis et al. 2003) (Figures 3 and 4) that was filled with 25-30 ppt seawater (either water

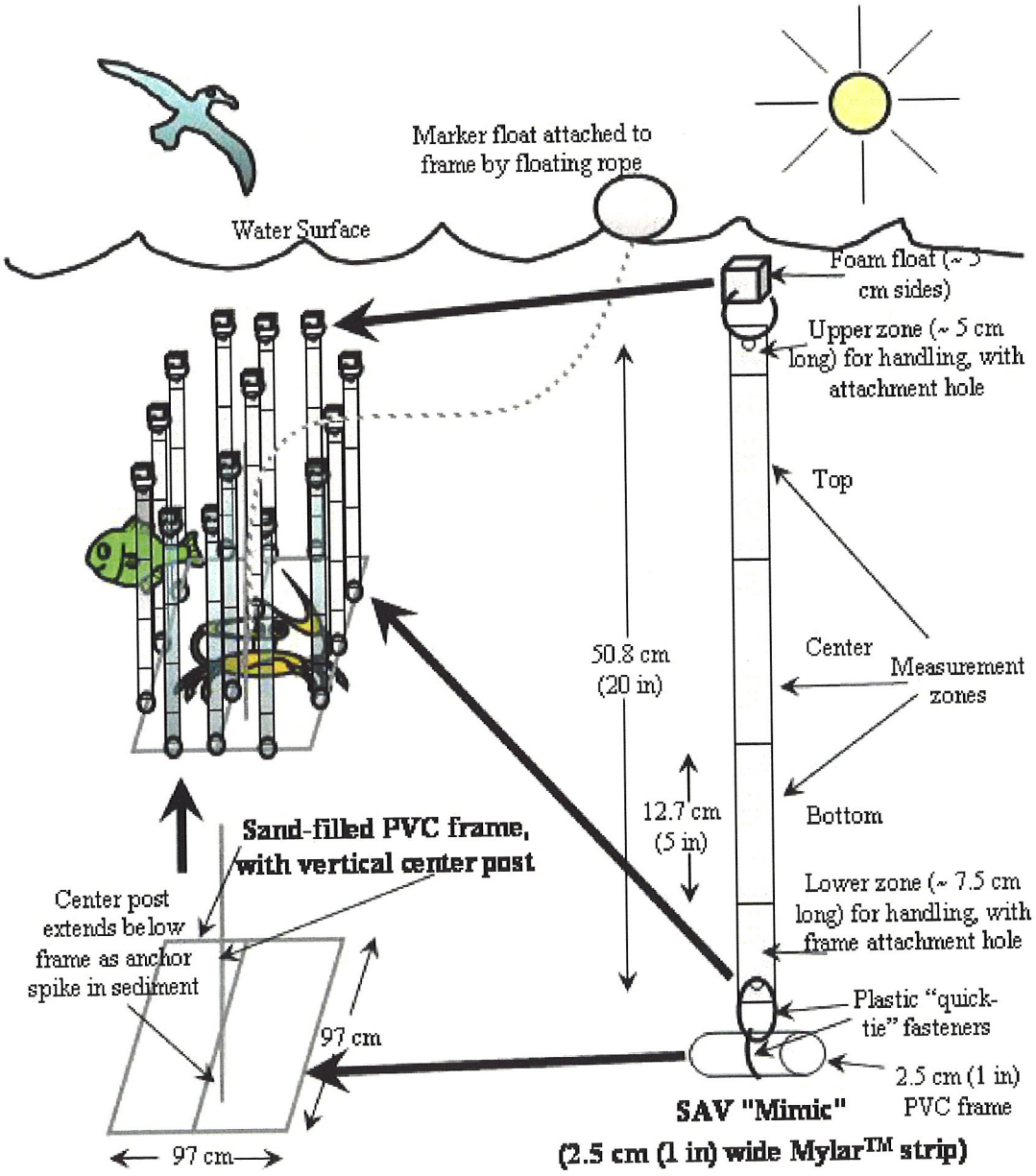


Figure 2. Diagram of SAV Mimic Array (adapted from Stankelis, 1999)

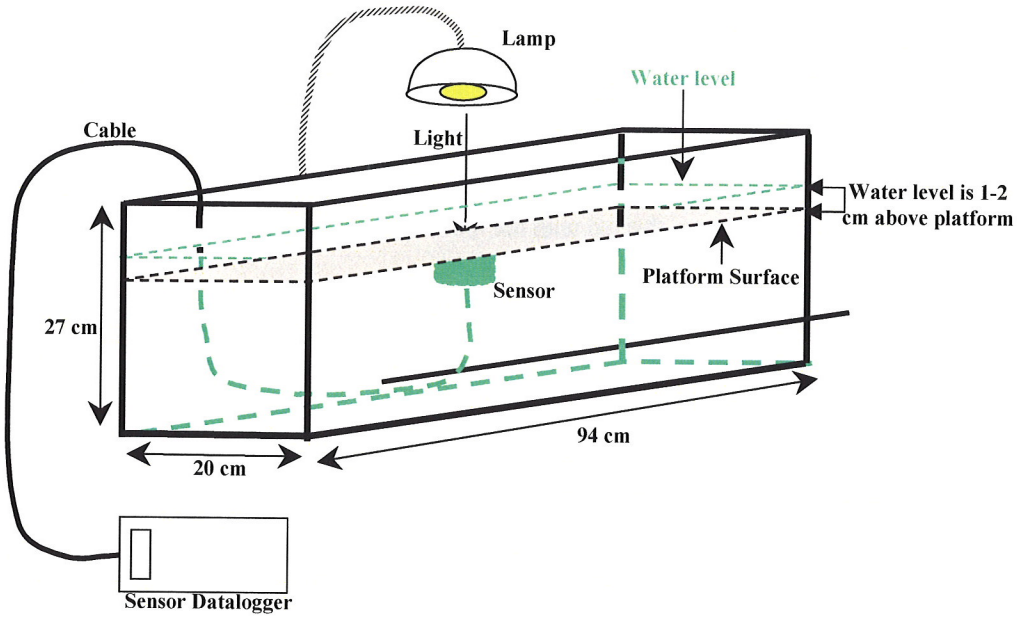


Figure 3. Side View of Light Attenuation Monitoring Apparatus (LAMA), showing objects inside box (dash lines). Objects underwater are delineated in green. (Modified from Stankelis, 1999)

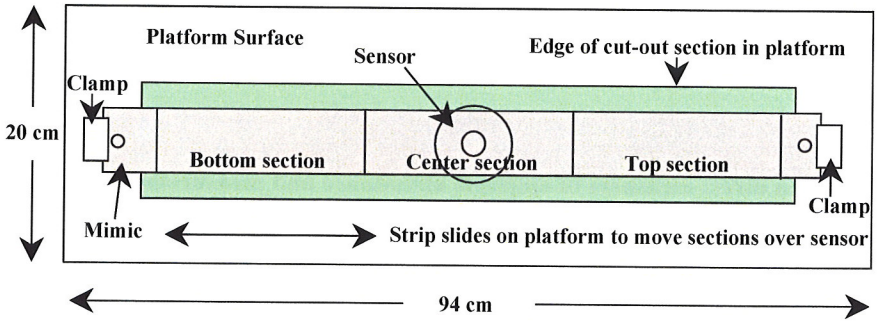


Figure 4. Top View of Light Attenuation Monitoring Apparatus (LAMA). (Lamp omitted). (Modified from Stankelis, 1999)

collected from the bay and filtered or aquarium salt mixtures to 30 ppt), by placing them over a slot cut in a supporting platform (board) within the box and anchoring their ends. A Li-Cor® LI-192SA underwater quantum sensor (which measures 400-700 nm wavelength radiation – approximately equivalent to the range of photosynthetically active radiation (PAR)) was anchored in the water below the mimic holding board. A desk lamp with a 100-watt bulb was positioned above the water level in the LAMA directly above the slot, so that light shone through the mimic and the slot to the sensor. PAR reaching the sensor through the mimic was recorded, as averaged over 15 seconds, for each of the three marked sections (top, center, bottom) of the mimic. PAR data were collected with the room darkened to reduce potential variability from changing ambient light conditions.

The fouled mimic was then removed, and replaced by a clean (control) mimic. The amount of PAR reaching the sensor through the clean strip was recorded, as for the fouled mimic. The ratio:

PAR recorded through fouled mimic / PAR recorded through clean mimic

represented the light attenuation (expressed as a percentage from 0-100) due to material on the fouled mimic (K_e). For "interval monitoring" mimics, this percentage was standardized to a percentage of light attenuated per day by dividing by the number of days of exposure. Arcsine transformation (Zar 1996) was not performed; with percentage data, this may improve the fit of the sample distribution data to that of a normal distribution and may be warranted for future analysis.

Means and standard errors for all replicates of individual sections of the mimics for each sampling session at a station were calculated. Although an analysis of variance detected significant differences between strip sections (top, center, or bottom), the amount of variance was small compared to station and session differences; thus, strip sections of different levels were pooled for additional graphic presentation and for a power analysis.

METHODS

Relationship between direct measures of epiphyte abundance and measurements of light attenuation through mimics.

It is reasonable to question how well the parameter of light attenuation through mimics can serve as a metric for epiphyte abundance and productivity. To measure this relationship, the chlorophyll a abundance on 223 replicate mimic sections was measured for the "seasonal monitoring" deployment experiment. Seasonal deployment mimics were used for this comparison to make the data set more representative of a wider range of trophic conditions than presently exist in the Coastal Bays; the longer period of deployment compensated for the relatively low fouling rates in the Coastal Bays. This allowed for a number of sample

units to accumulate epiphyte loads of 10 $\mu g/cm^2$ mimic surface or more, which are frequently reached in 7-10 days in the Patuxent River (R. Stankelis, pers. comm.).

After measuring light attenuation, as described above, through each of the 223 mimic sections used for this comparison, the sections were enclosed in aluminum foil and transported to the Horn Point Laboratory. To extract fouling material from mimic sections, the sections were cut in half and placed in a 45 ml centrifuge tube, to which 40 ml of 90% acetone was added. The tube was capped and centrifuged to loose fouling material from the mimic, then placed in a freezer overnight. The tube was then centrifuged for 10 minutes. 5 ml of supernatant was removed by pipette. The chlorophyll a concentration of the supernatant was measured by Turner Designs TD-700 fluorometer (Arar and Collins 1992), using the non acidification method (Welschmeyer 1994). This concentration (in $\mu g/L$) was multiplied by the volume of the acetone (40 ml) and divided by the areas of mimic section surface to derive the concentration of chlorophyll a in $\mu g/cm^2$ of mimic surface. Logarithmic regression was used to employed to assess the relationship between chlorophyll a concentration and light attenuation for each of the 223 sample units.

METHODS

Determination of SAV growing season limits.

Because epiphyte density is dependent on the rate of SAV growth, as well as the rate of epiphyte growth, establishing periods of differential rates of SAV growth may be important to establishing monitoring periods that are biologically relevant and that reduce unwanted variability in data. In Batiuk et al. (1992), Moore determined that eelgrass exhibited a bimodal pattern of increased above ground growth in a polyhaline salinity regime in the York River of Virginia, a comparable environment to the Maryland Coastal Bays. The highest rates of shoot and leaf growth occurred during the spring and fall of four temperature-defined spring and fall seasons, with lower growth rates during the summer and winter. The spring period of maximum above ground growth occurred when the [mean daytime] water temperature was between 9.2° C. and 22.7° C; the fall period of maximum above ground growth occurred when the [mean daytime] water temperature was between 25.0° C. and 13.2° C.

For purposes of scheduling monitoring, it is probably more practical to define growing season by calendar date than by temperature. Thus, establishing a function that closely approximates the relationship of date to water temperature in SAV beds in the Coastal Bays is desirable. To estimate growing seasons for eelgrass in the Maryland Coastal Bays for each of the three years, a 3 parameter Gaussian regression equation:

$$y=a*e^{(-0.5*((x-x_0)/b)2)}$$

was fitted by least squares methods to temperature data measured at individual monitoring stations (Lea et al. 2003), with y representing temperature and x representing the Julian date of temperature collection. The

variables a, b, and x_0 are constants individually calculated for each data set. The curve representing by the date/temperature function calculated for individual monitoring stations was plotted on scatter plots of epiphyte abundance data for individual stations. The date limits of the spring and fall growing seasons were estimated for each station each year by solving for the date (x) value that yielded the critical temperature limits to growing seasons (y), as specified by Moore in Batiuk et al. (1992). These estimated dates were plotted on scatter plots of epiphyte abundance depicting results for individual stations.

Temperature data for all stations were pooled by year to obtain provisional growing season limits (dates) for eelgrass in the Coastal Bays that may be used for guiding timing of future monitoring. These dates were depicted on scatter plots of epiphyte abundance data that depict data from multiple stations.

RESULTS AND DISCUSSION

Direct measures of epiphyte abundance.

Results of epiphyte abundance measures are depicted by season and by station (Figures 5-28, Appendix). Linear regression models (Figures 29-31, Appendix) showed an only mildly strong relationship between epiphyte biomass density and epiphyte chlorophyll a density, for each year of the study. In general, both epiphyte biomass density and epiphyte chlorophyll a density remain low throughout the spring growing season, remaining relatively stable to slightly increasing. Marked increases in abundance occurred at the end of the spring growing season (when water temperatures exceed 23° C.). While epiphyte growth would be expected to increase with increasing temperature, the often abrupt increases seen at this period may be due to the fact that growth of eelgrass leaves slows markedly with the onset of higher temperatures. The previously continuous leaf elongation which produces new uncolonized leaf surface may be decreased, so that the rate of leaf area added decreases, as epiphytes increase at a rate equal to or greater than during the spring growing season for eelgrass.

This pattern has significant implications for scheduling sampling, from both biological and statistical perspectives. Differences in growth may mean differences in biological demand for light by eelgrass between the spring and summer growth periods, so that tolerance of a given level of epiphyte abundance may be more critical in one period. Differences in absolute abundances of epiphytes are likely to yield differences in sample variability, affecting the statistical power of monitoring to detect differences between populations measured by samples. Figures 32 and 33 (Appendix) indicate that the absolute minimum detectable change in epiphyte biomass and in epiphyte chlorophyll a between samples is greater during the summer growing period. Figures 34 and 35 (Appendix) indicate that when minimum detectable change is expressed as a percentage of sample mean, that variability between the seasons is fairly similar between the seasons or perhaps slightly greater in the spring.

Generally, epiphyte biomass was a slightly less variable, and, therefore more statistically powerful, measure of epiphyte abundance than was epiphyte chlorophyll a. However, epiphyte biomass (dry weight) may include inorganic and nonphotosynthetic (e.g., zoological) components that may not be as sensitive to anthropogenic nutrient loading and/or may be less effective than autotrophic epiphytes in attenuating light. The choice of which parameter to measure should be made with the ecological and conservation considerations that epiphyte biomass may better represent the total contribution of shading on SAV (e.g., includes inorganic and non-photosynthetic organic (e.g., animal) material), but epiphyte chlorophyll a may be representative of the component of material on SAV leaves that may be most sensitive to water column nutrients and may most readily respond to a nutrient control program. The correlation between the two parameters was only moderately strong, with the coefficient of determination (r^2) obtained from the linear regression analysis ranging from 0.379 in 2000 to 0.433 in 1999 to 0.468 in 1998 (Figures 29-31). It was assumed that the technique of splitting samples collected and filtered as pooled sample units for both chlorophyll a and biomass measurement in 1998 effectively controlled for small scale spatial variability (at each sample unit collection point) and laboratory scraping methodology variability that would be present between the separately collected and filtered chlorophyll a and biomass sample units in 1999 and 2000. The fact that the coefficient of determination (r^2) is not much greater for 1998 than in 1999 or 2000 suggests that variability in the relationship between the two parameters is probably more attributable to ecological reasons and/or (possibly) to post-filtering methods (e.g., drying and weighing for biomass or chlorophyll extraction and measurement for chlorophyll a) than to small scale spatial variability in epiphyte loads on plants or to differences in effectiveness of scraping among investigators. The higher r^2 in 1998 might also be an artifact of the use of smaller amounts of epiphyte material used for measurements in 1998 (i.e., sample units pooled by year may not be homoscedastic).

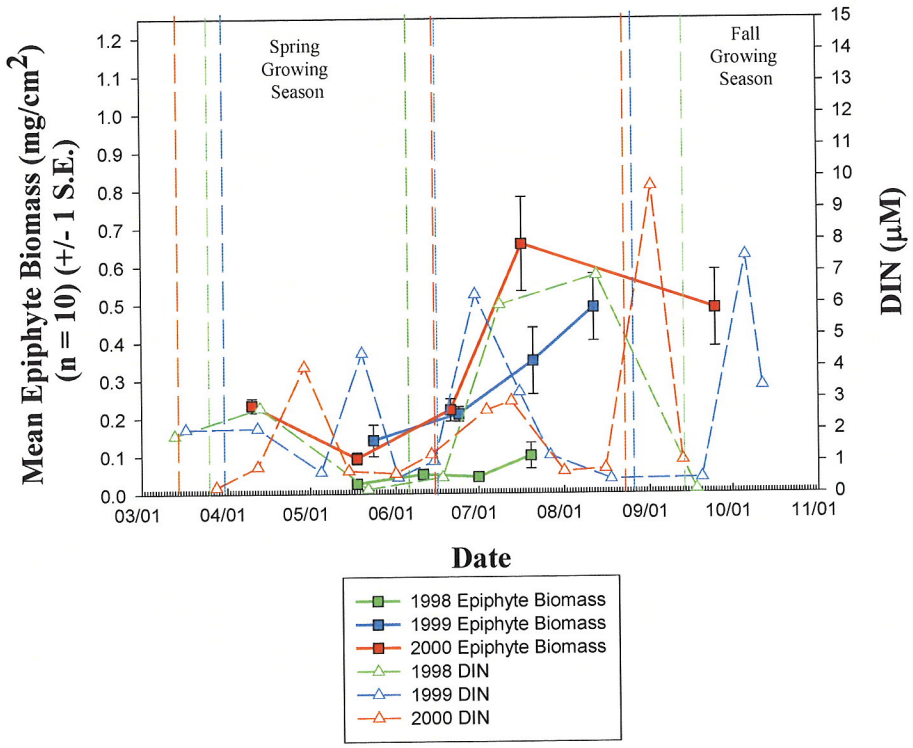


Figure 5: SAV epiphyte biomass and water column dissolved inorganic nitrogen, 1998-2000, Marker 25 (A).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

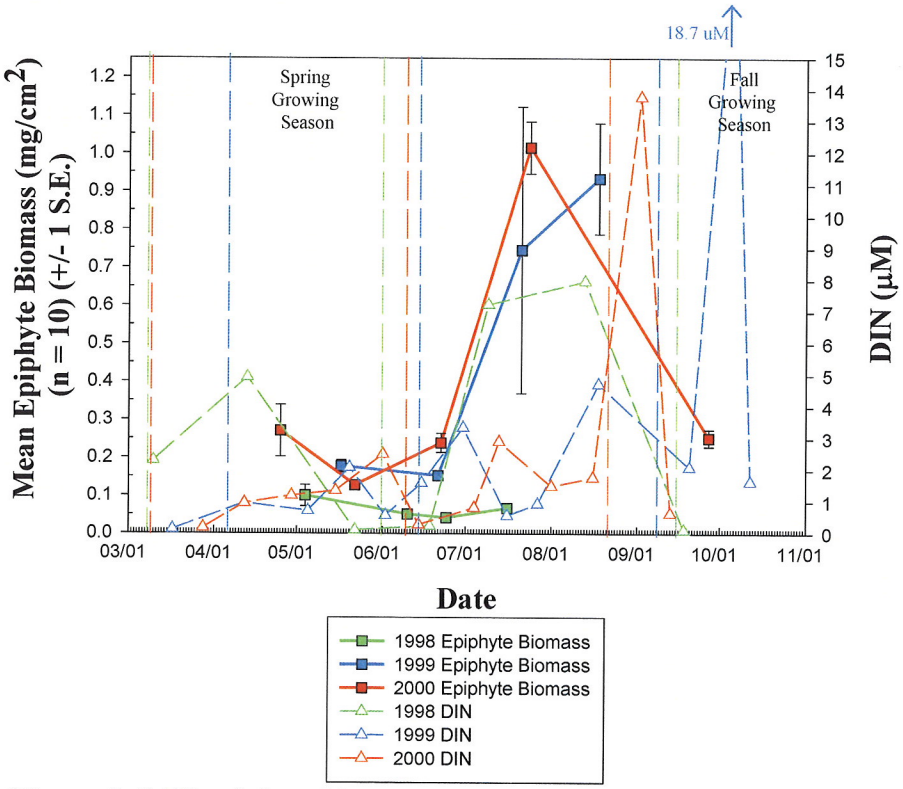


Figure 6: SAV epiphyte biomass and water column dissolved inorganic nitrogen, 1998-2000, Rum Point (B).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

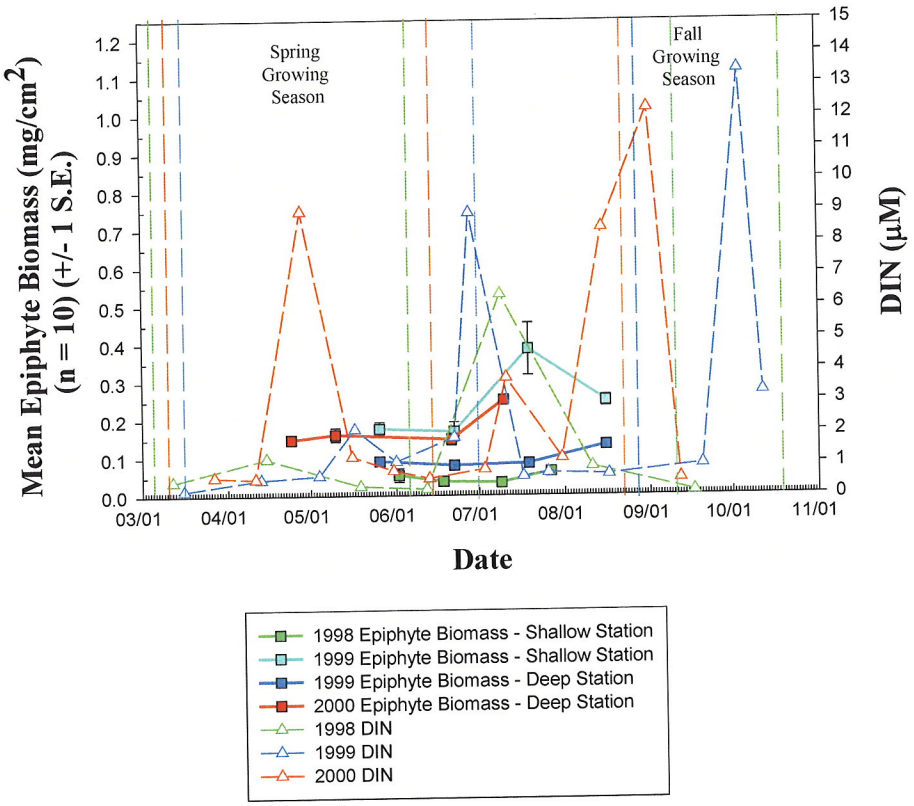


Figure 7: SAV epiphyte biomass and water column dissolved inorganic nitrogen, 1998-2000, Tingles Island (Ds and Dd).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

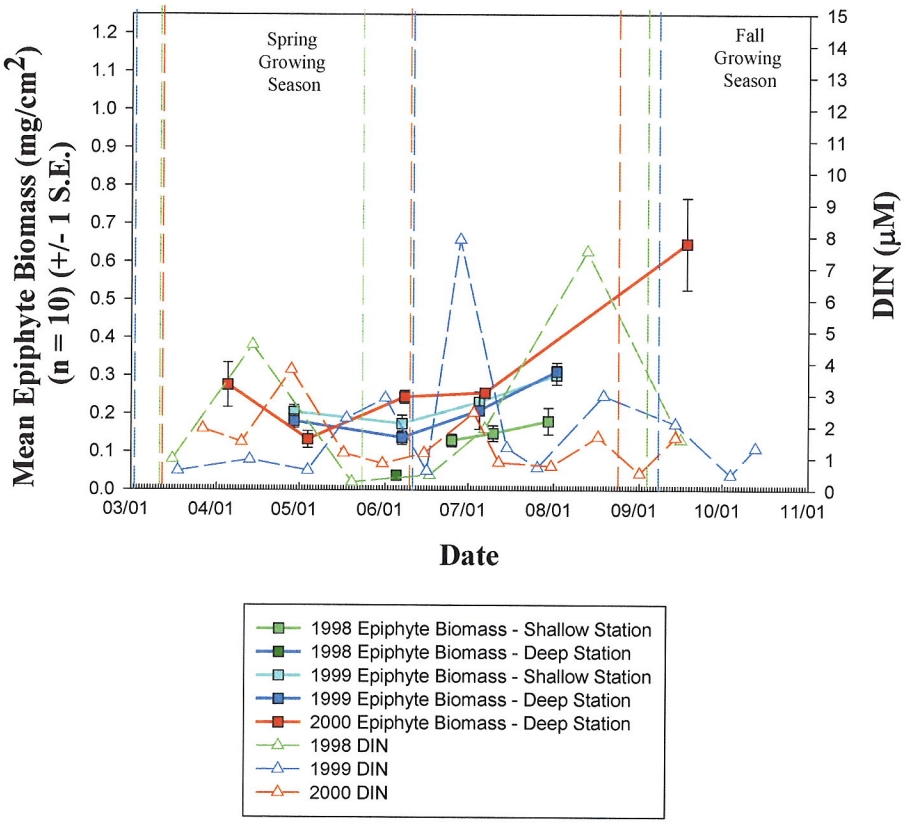


Figure 8: SAV epiphyte biomass and water column dissolved inorganic nitrogen, 1998-2000, Coards Marsh (Es and Ed).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

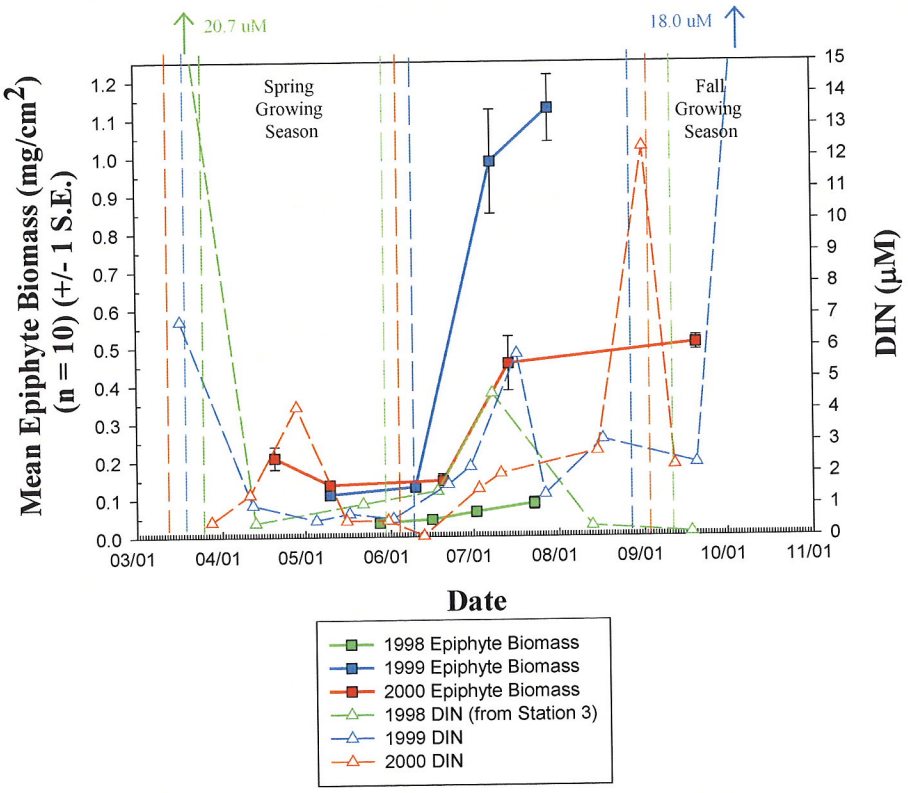


Figure 9: SAV epiphyte biomass and water column dissolved inorganic nitrogen, 1998-2000, Spence Cove (G).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

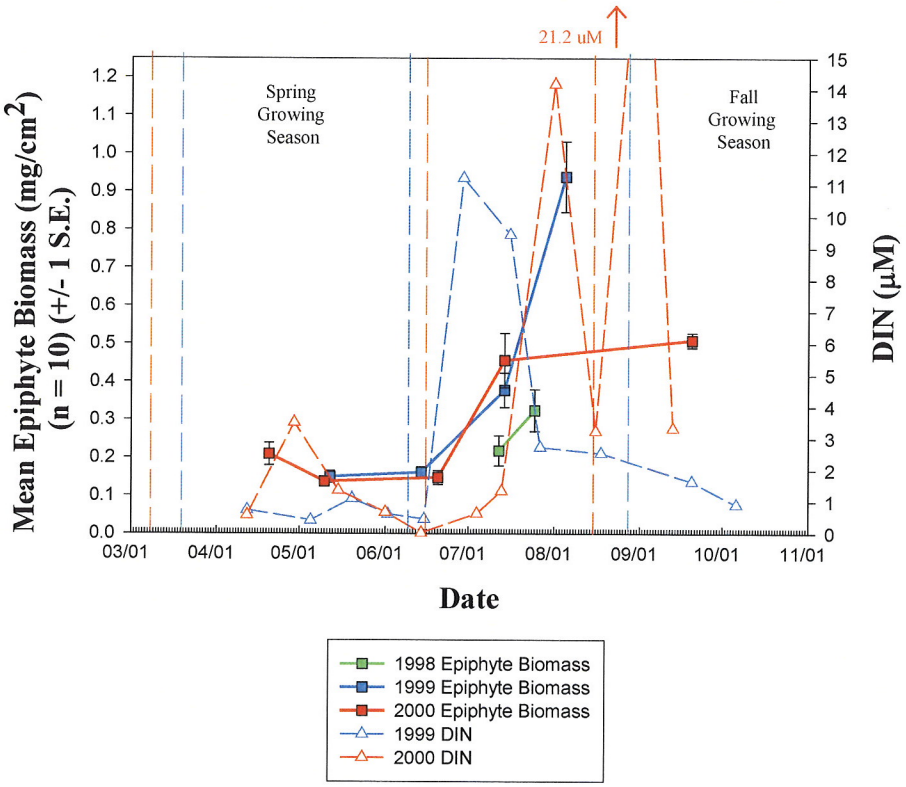


Figure 10: SAV epiphyte biomass and water column dissolved inorganic nitrogen, 1998-2000, Route 90 (Z). (1998 DIN missing).
(Vertical dash lines represent annual growing season limits: 1999 (blue), 2000 (red).

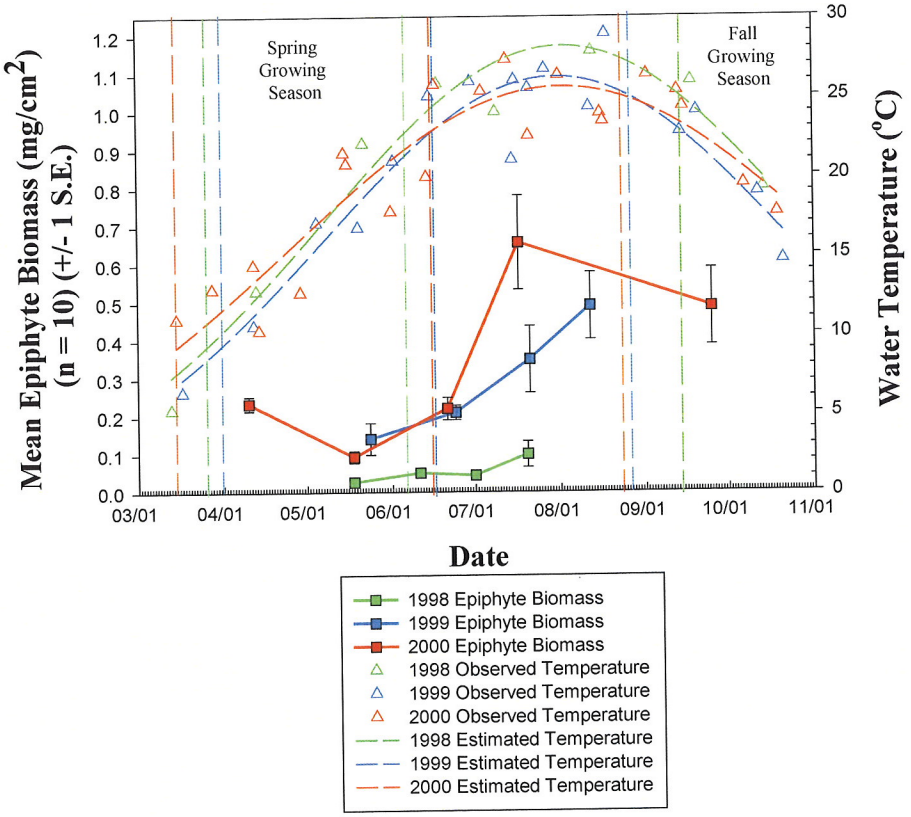


Figure 11: SAV epiphyte biomass and water column temperature, 1998-2000, Marker 25 (A).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

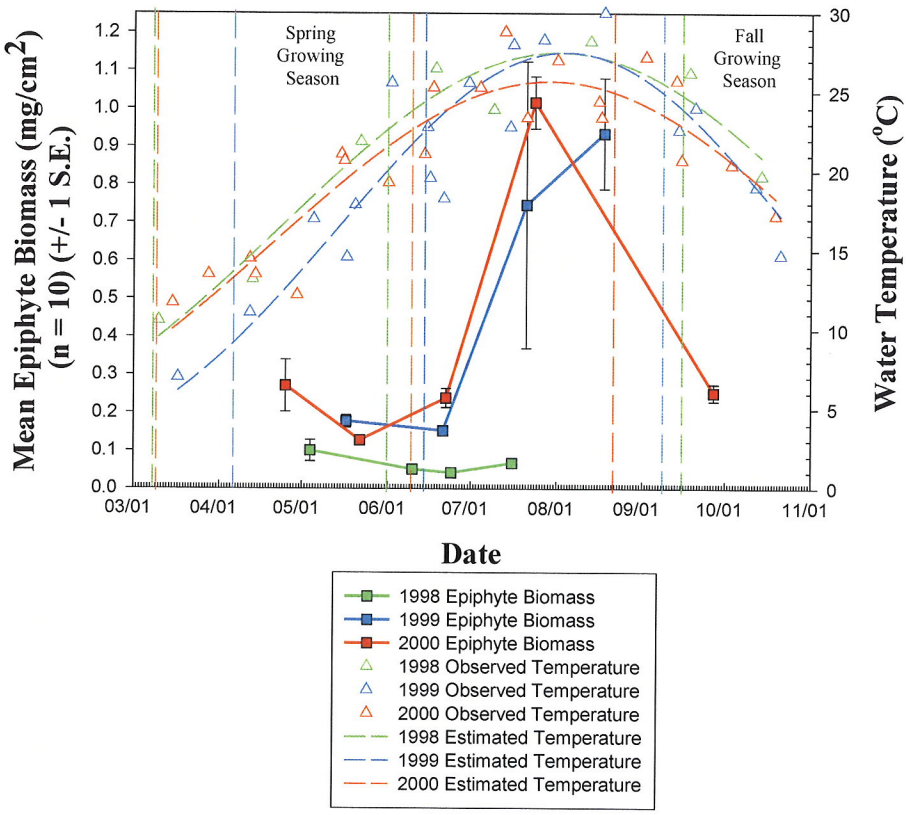


Figure 12: SAV epiphyte biomass and water column temperature, 1998-2000, Rum Point (B).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

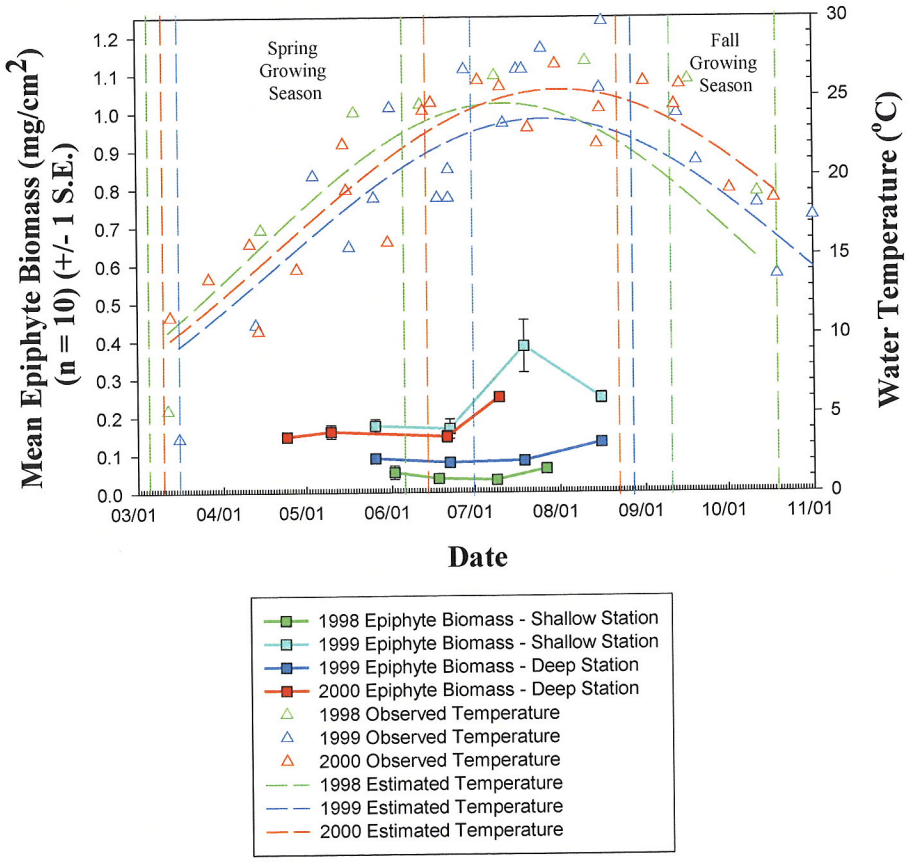


Figure 13: SAV epiphyte biomass and water column temperature, 1998-2000, Tingles Island (Ds and Dd).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

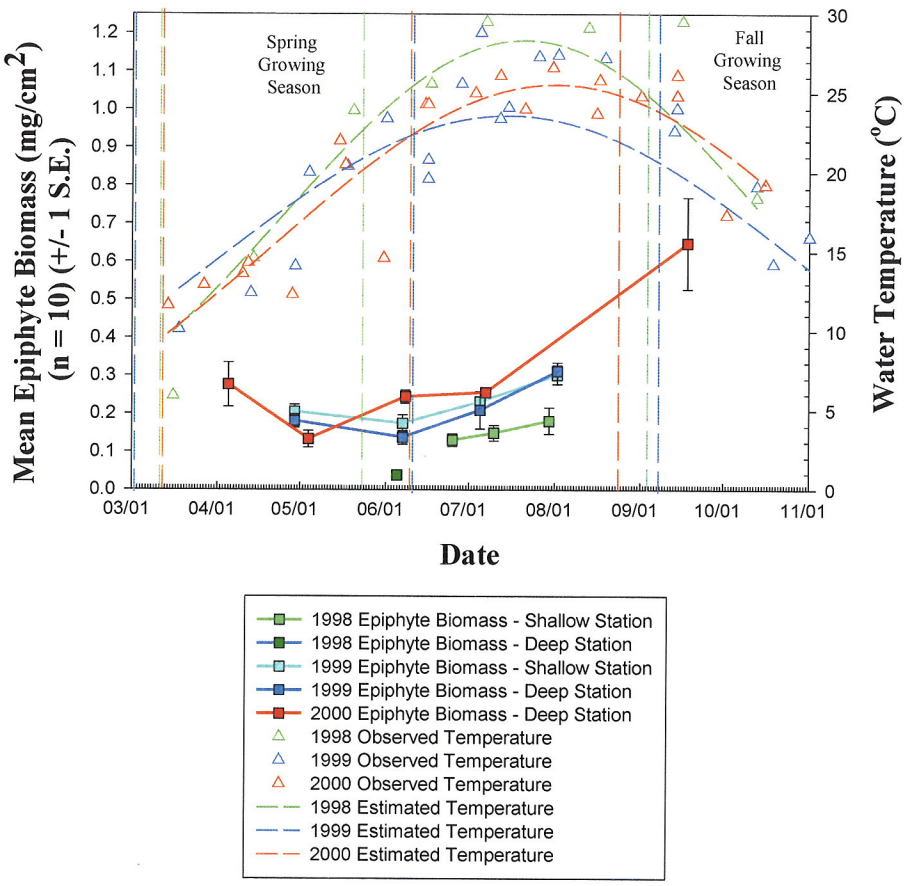


Figure 14: SAV epiphyte biomass and water column temperature, 1998-2000, Coards Marsh (Es and Ed).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

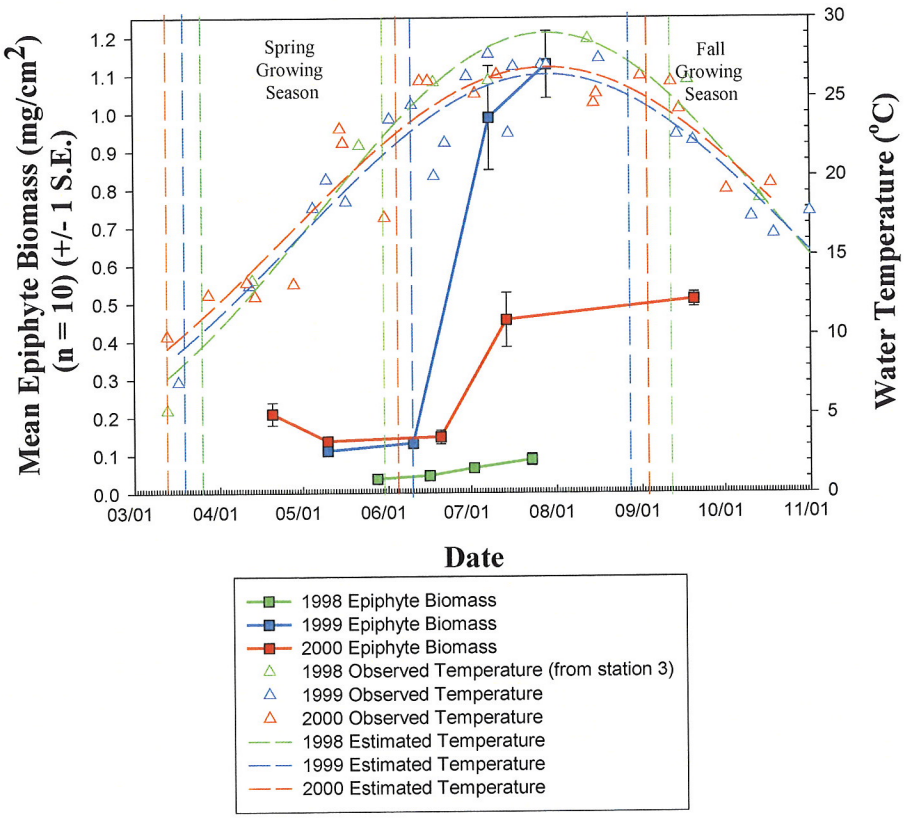


Figure 15: SAV epiphyte biomass and water column temperature, 1998-2000, Spence Cove (G).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

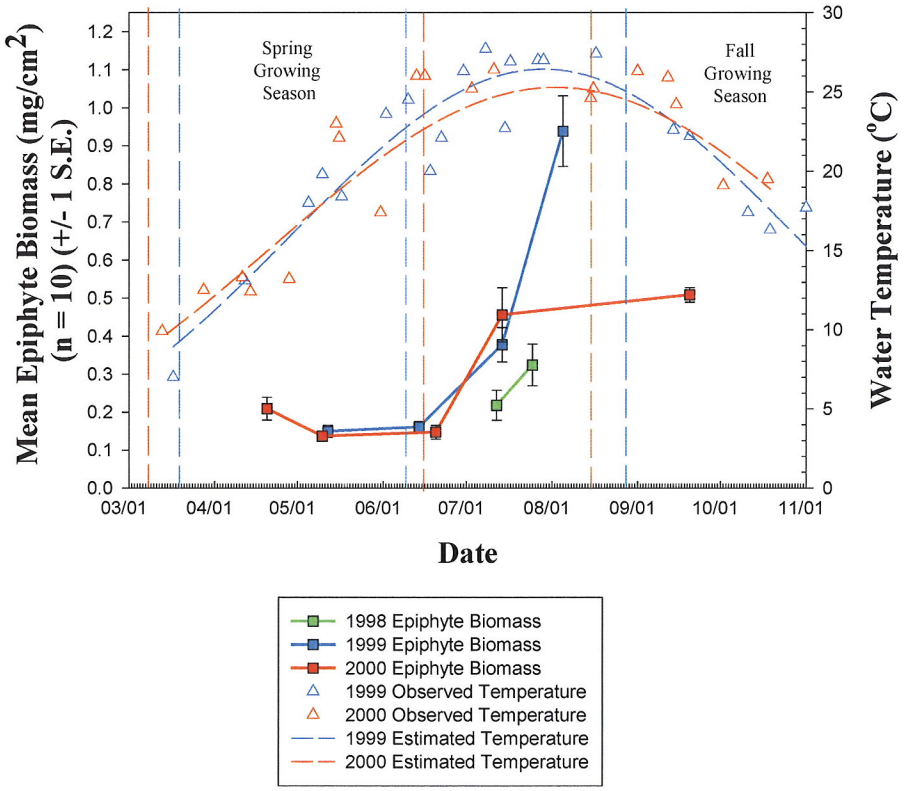


Figure 16: SAV epiphyte biomass and water column temperature, 1998-2000, Route 90 (Z).

(Vertical dash lines represent annual growing season limits: 1999 (blue), 2000 (red). (No temperature data for 1998. Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

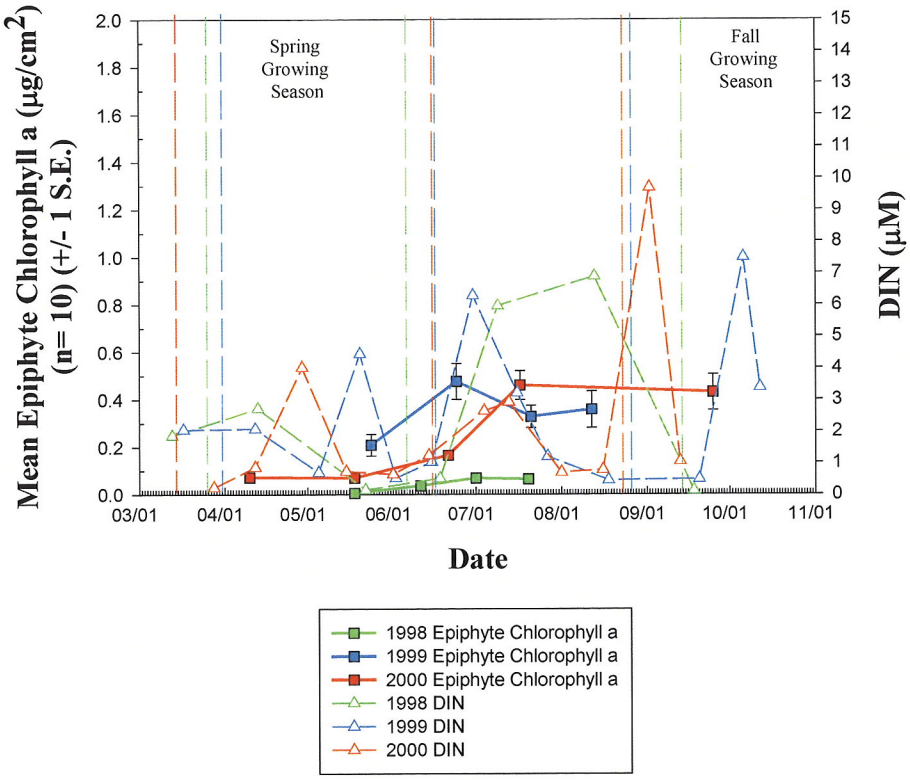


Figure 17: SAV epiphyte chlorophyll a and water column dissolved inorganic nitrogen, 1998-2000, Marker 25 (A).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

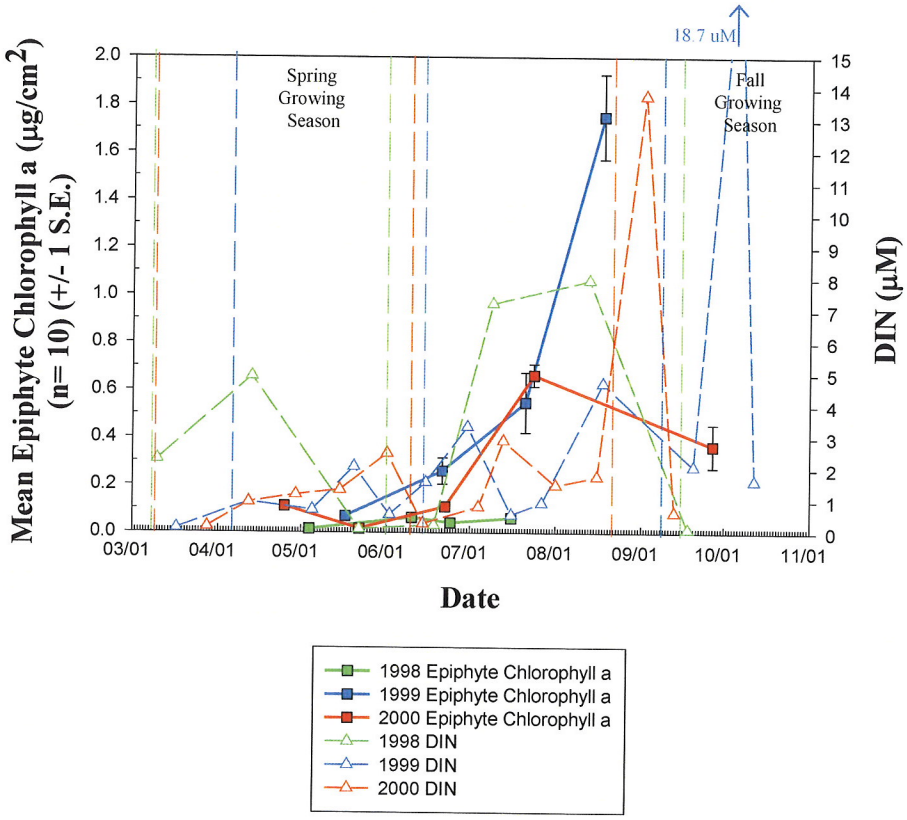


Figure 18: SAV epiphyte chlorophyll a and water column dissolved inorganic nitrogen, 1998-2000, Rum Point (B).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

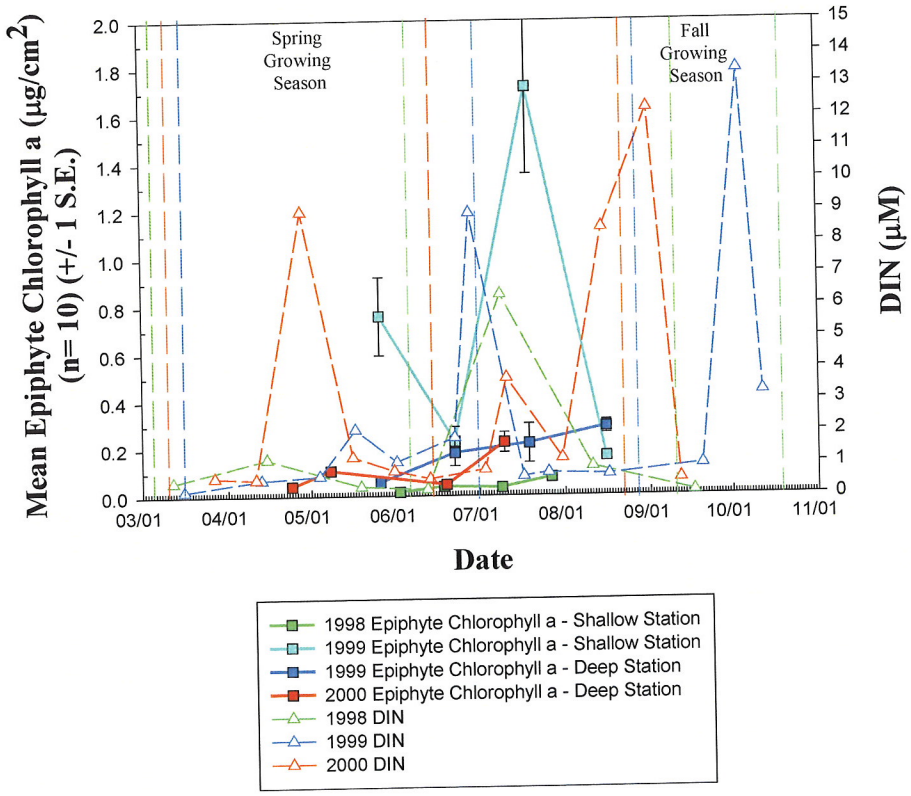


Figure 19: SAV epiphyte chlorophyll a and water column dissolved inorganic nitrogen, 1998-2000, Tingles Island (Ds and Dd).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

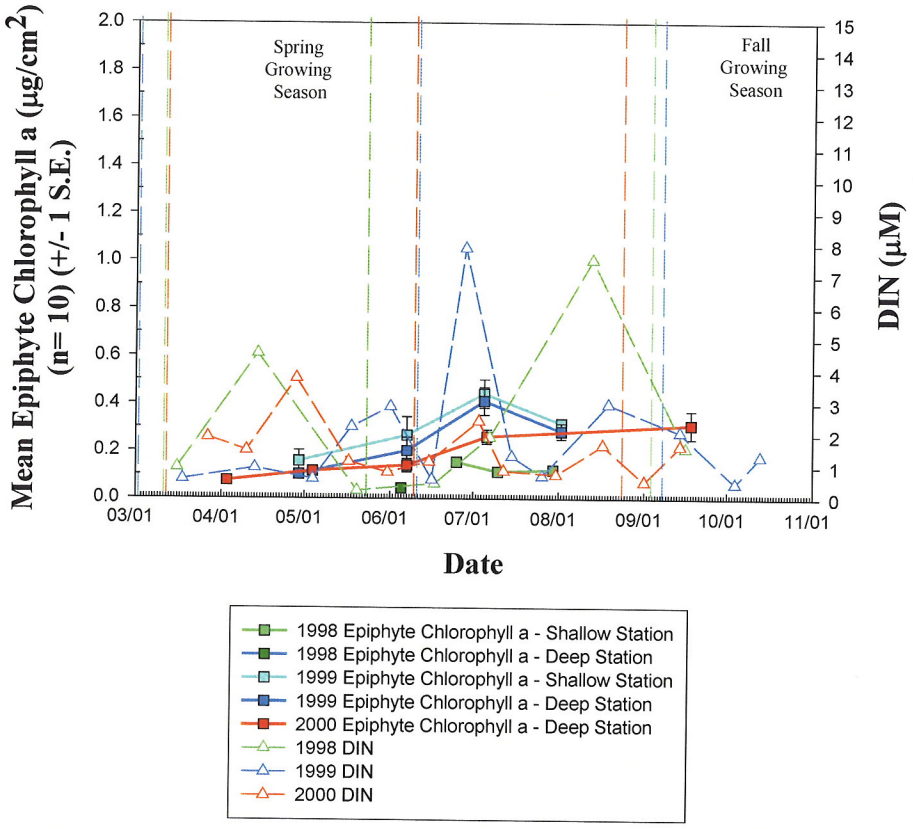


Figure 20: SAV epiphyte chlorophyll a and water column dissolved inorganic nitrogen, 1998-2000, Coards Marsh (Es and Ed).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

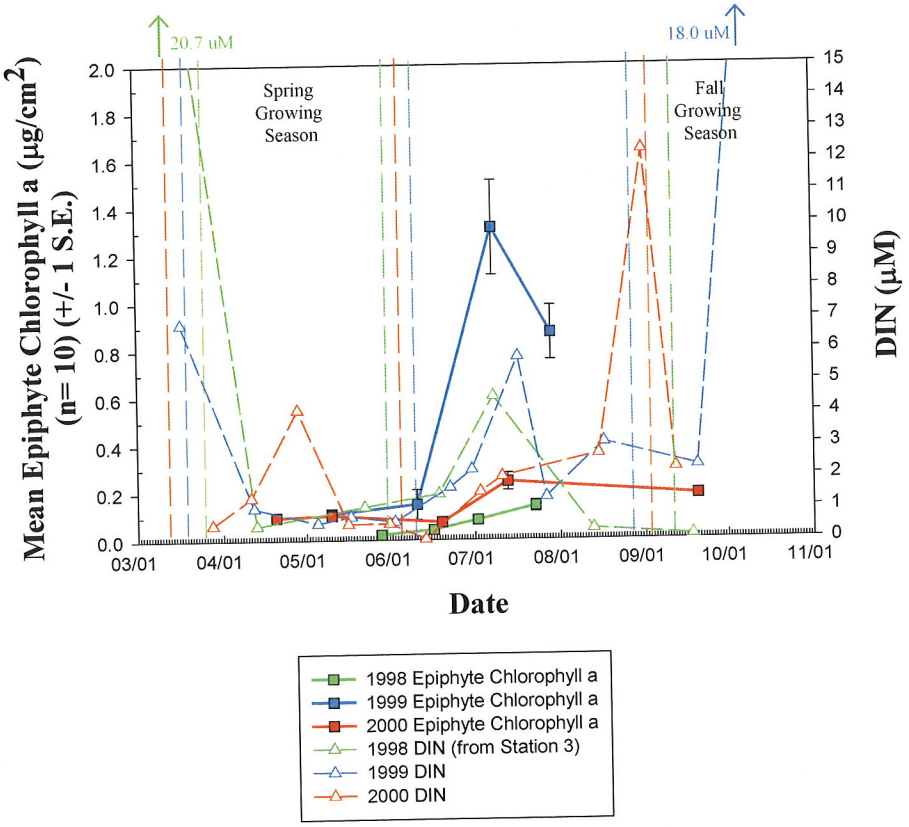


Figure 21: SAV epiphyte chlorophyll a and water column dissolved inorganic nitrogen, 1998-2000, Spence Cove (G).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red).

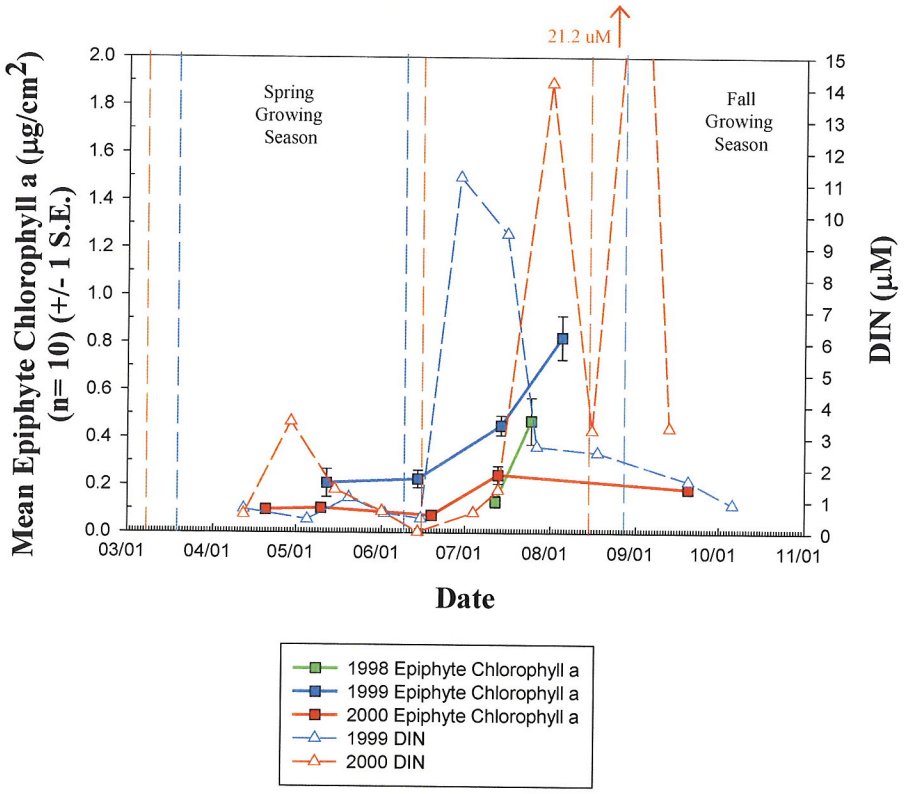


Figure 22: SAV epiphyte chlorophyll a and water column dissolved inorganic nitrogen, 1998-2000, Route 90 (Z).

(Vertical dash lines represent annual growing season limits: 1999 (blue), 2000 (red).

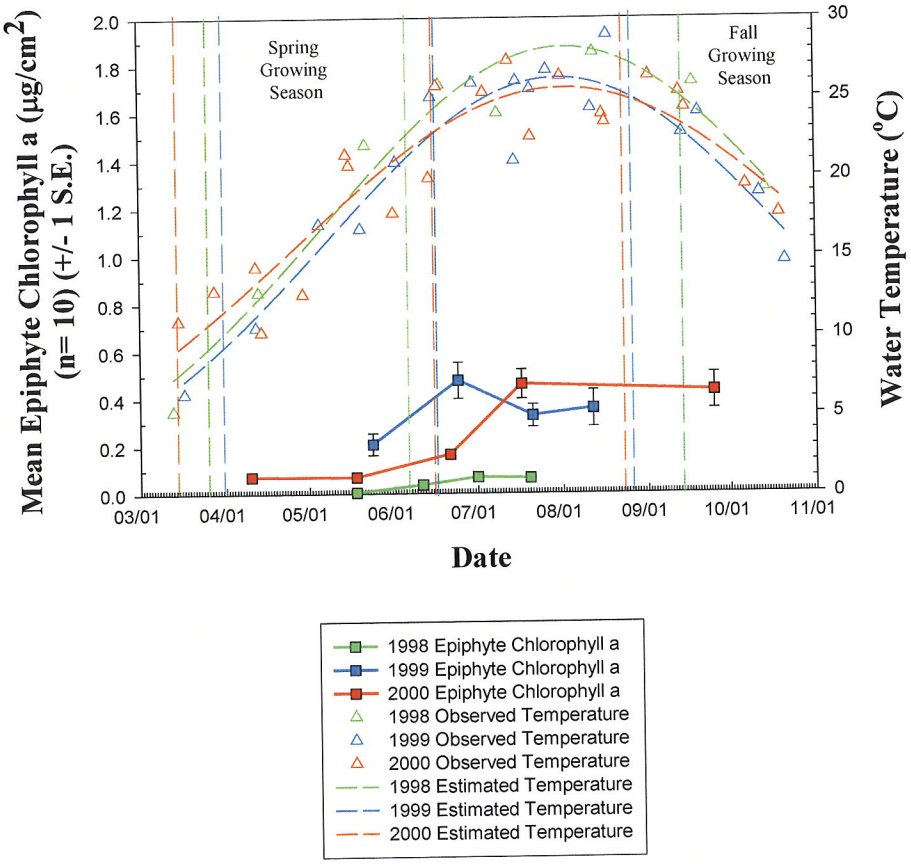


Figure 23: SAV epiphyte chlorophyll a and water column temperature, 1998-2000, Marker 25 (A).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

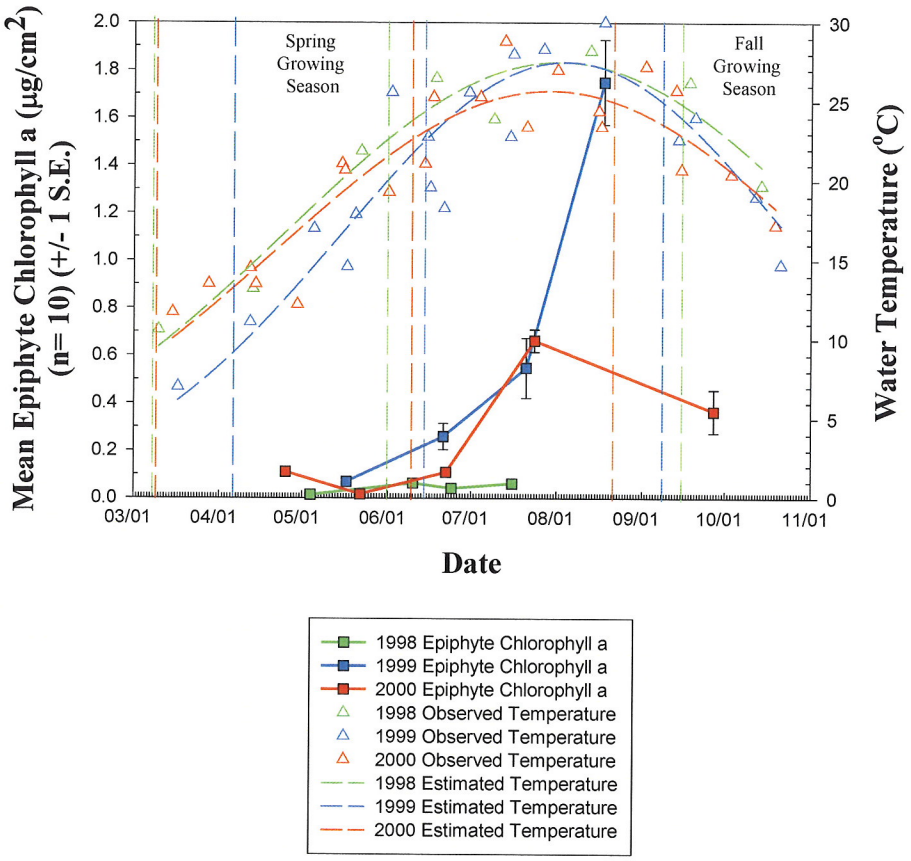


Figure 24: SAV epiphyte chlorophyll a and water column temperature, 1998-2000, Rum Point (B).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

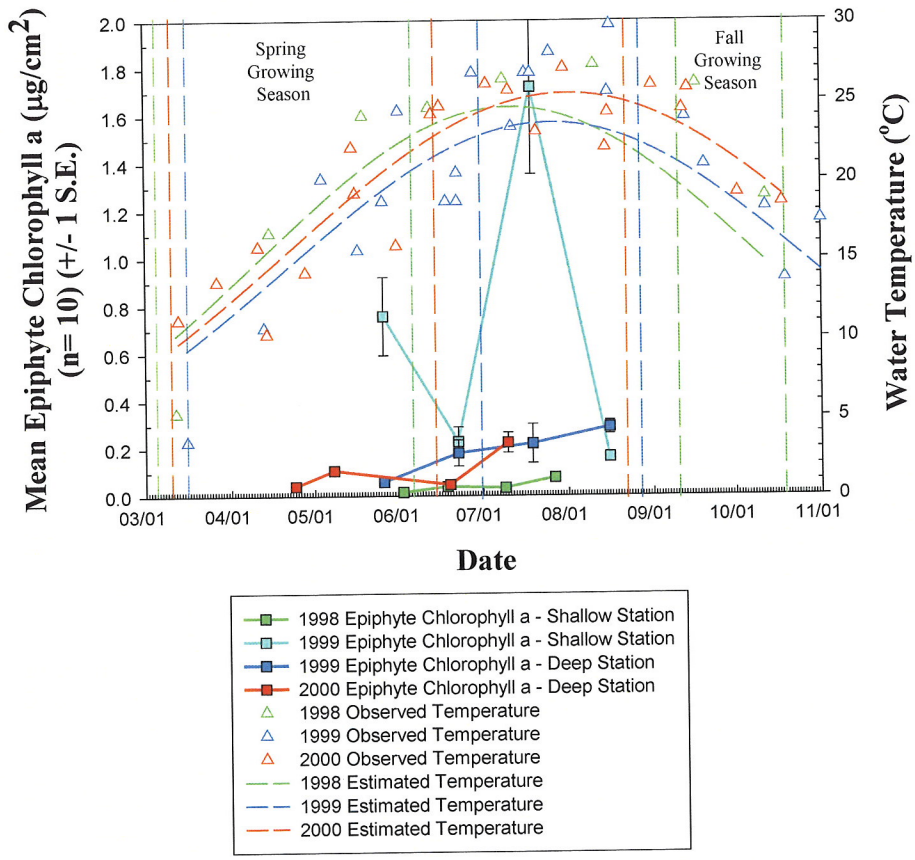


Figure 25: SAV epiphyte chlorophyll a and water column temperature, 1998-2000, Tingles Island (Ds and Dd).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

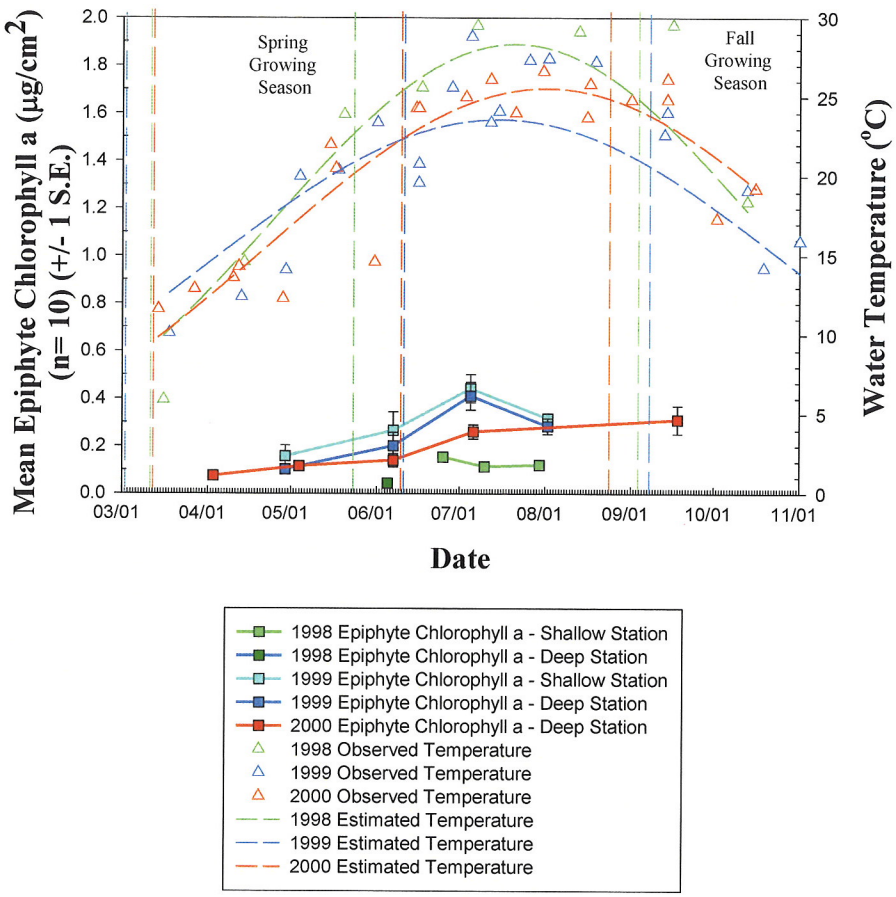


Figure 26: SAV epiphyte chlorophyll a and water column temperature, 1998-2000, Coards Marsh (Es and Ed).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

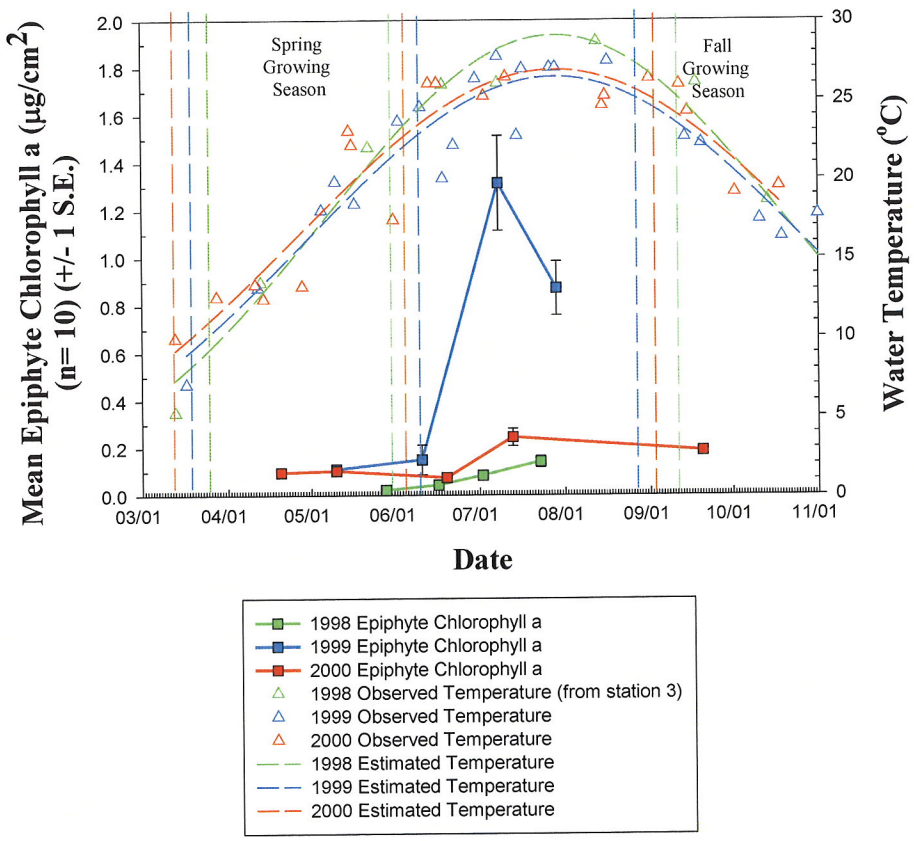


Figure 27: SAV epiphyte chlorophyll a and water column temperature, 1998-2000, Spence Cove (G).

(Vertical dash lines represent annual growing season limits: 1998 (green), 1999 (blue), 2000 (red). Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

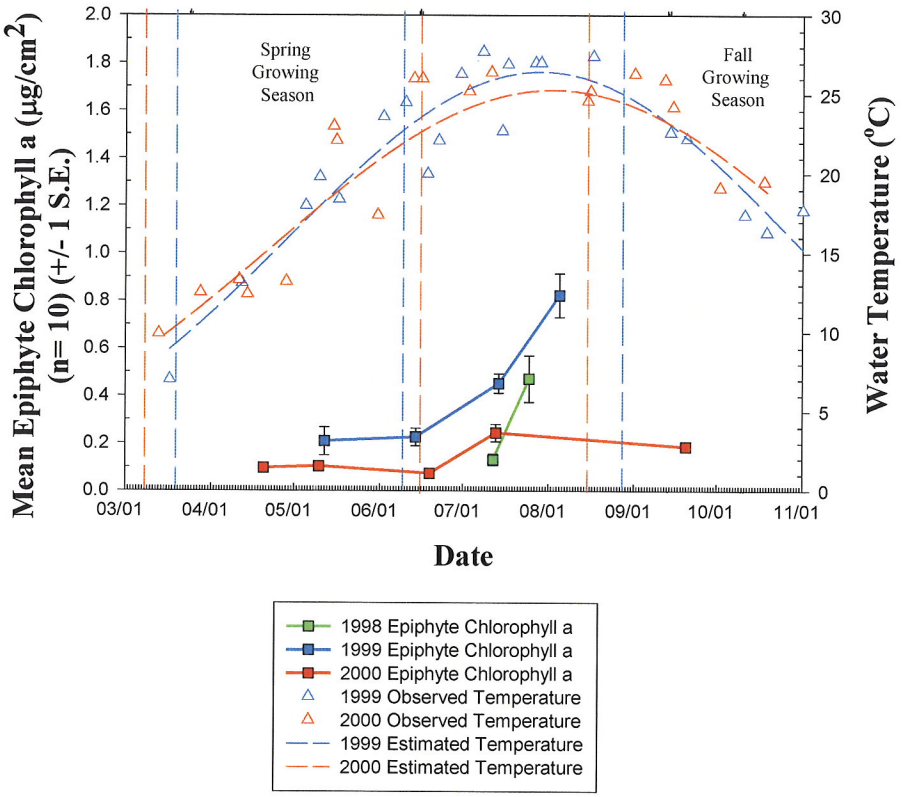


Figure 28: SAV epiphyte chlorophyll a and water column temperature, 1998-2000, Route 90 (Z).

(Vertical dash lines represent annual growing season limits: 1999 (blue), 2000 (red). (No temperature data for 1998. Temperature estimated from 3 parameter Gaussian regression using observed temperature data).

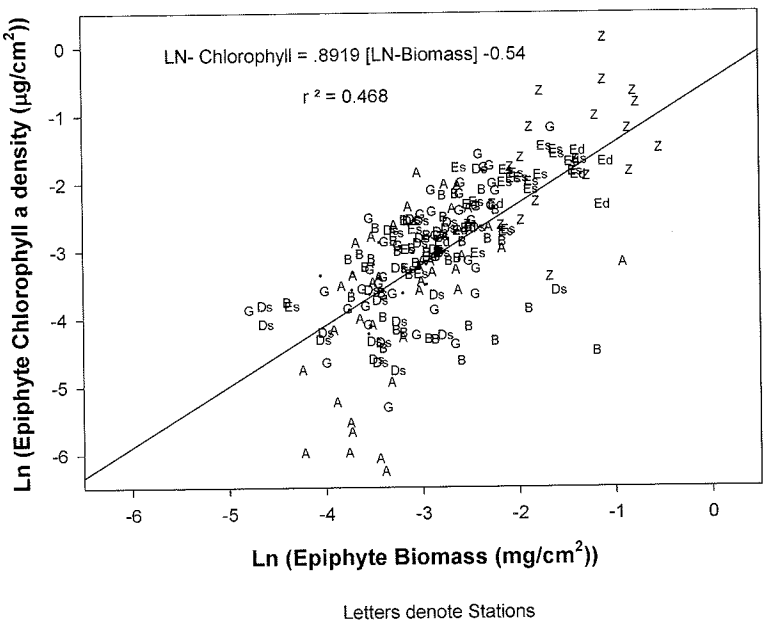


Figure 29: 1998 Epiphyte Chlorophyll a Density vs. Epiphyte Biomass Density

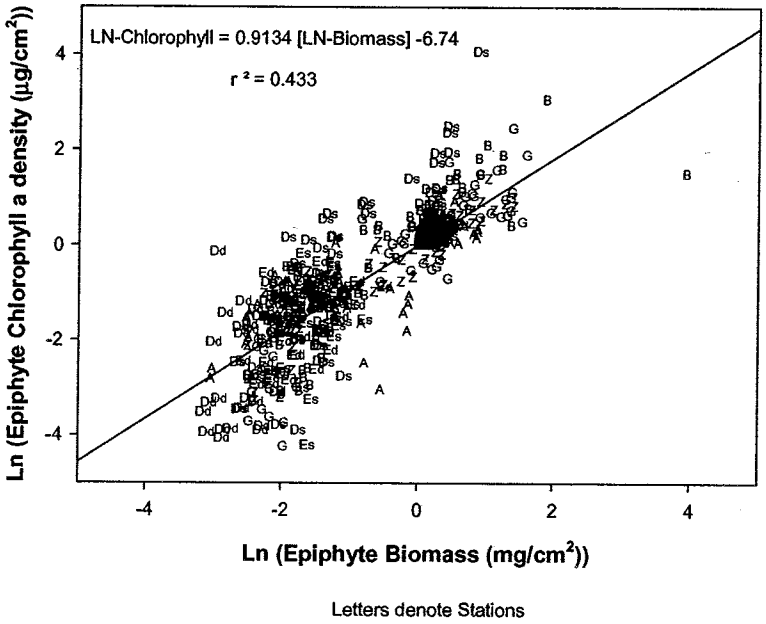
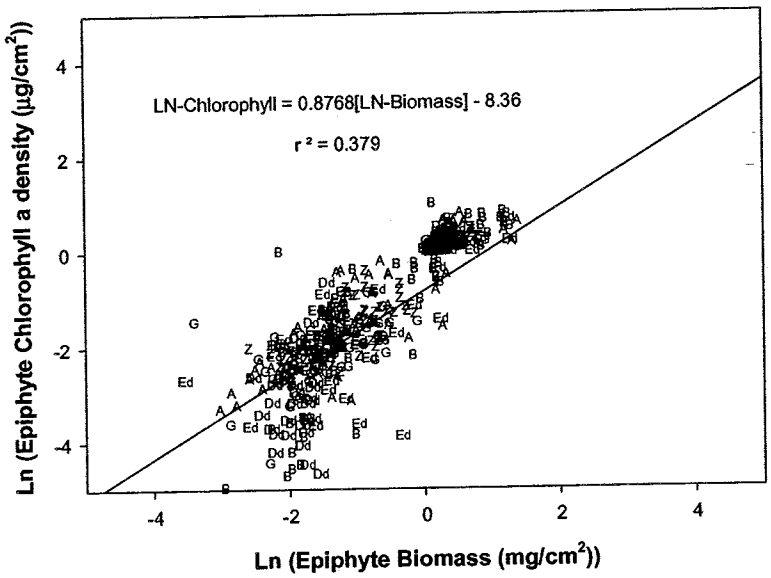


Figure 30: 1999 Epiphyte Chlorophyll a Density vs. Epiphyte Biomass Density



Letters denote Stations

Figure 31: 2000 Epiphyte Chlorophyll a Density vs. Epiphyte Biomass Density

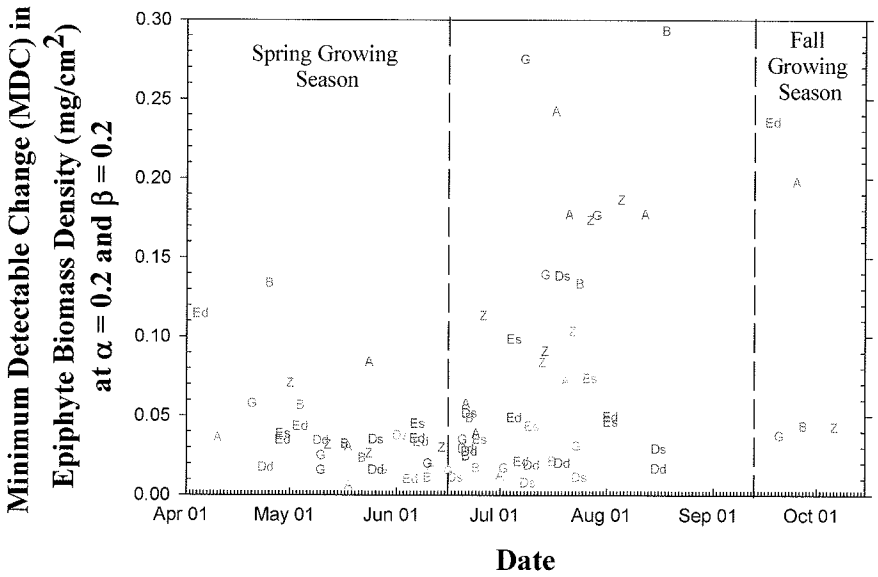


Figure 32: Epiphyte biomass density minimum detectable change for sample against date, with 10 sample units. Letters denote stations. Green letters denote 1998 data; blue letters denote 1999 data; red letters denote 2000 data.

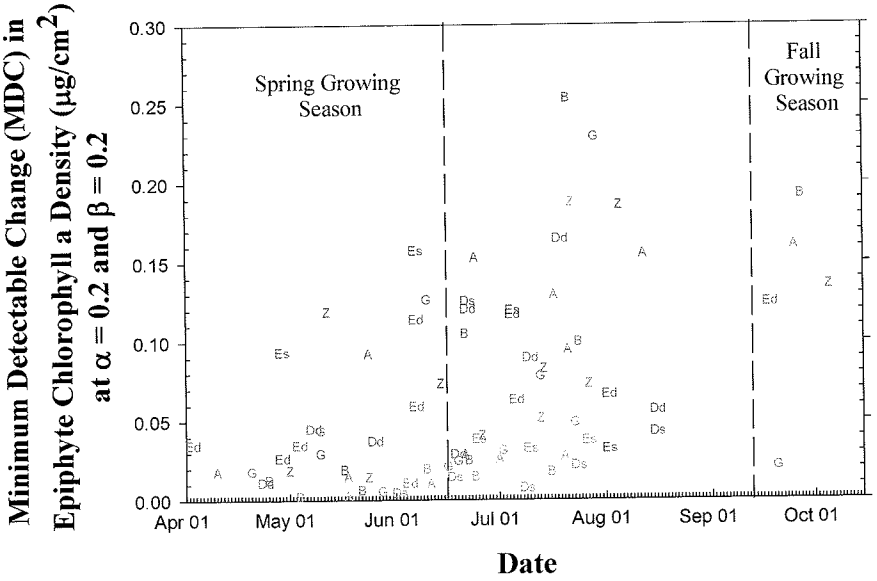


Figure 33: Epiphyte chlorophyll a density minimum detectable change for sample against date, with 10 sample units. Letters denote stations. Green letters denote 1998 data; blue letters denote 1999 data; red letters denote 2000 data.

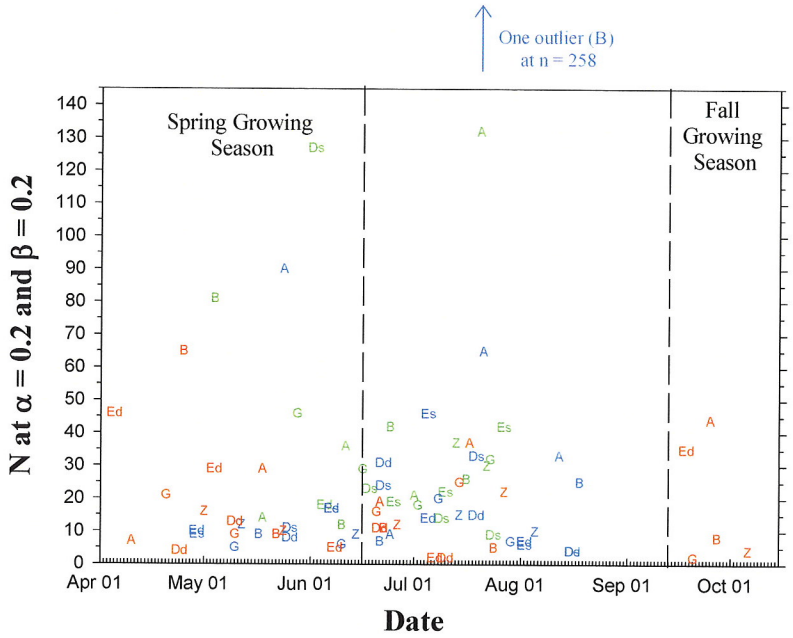


Figure 34: Minimum numbers of epiphyte biomass density sample units to detect change of 20% of sample mean, against date. Letters denote stations. Green letters denote 1998 data; blue letters denote 1999 data; red letters denote 2000 data.

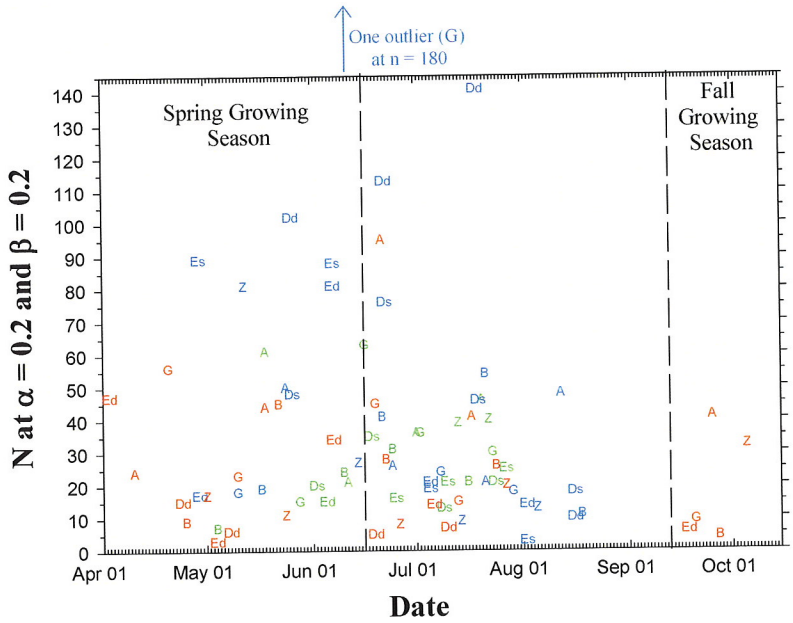


Figure 35: Minimum numbers of epiphyte chlorophyll a density sample units to detect change of 20% of sample mean, against date. Letters denote stations. Green letters denote 1998 data; blue letters denote 1999 data; red letters denote 2000 data.

RESULTS AND DISCUSSION

Investigation of response of epiphytes to nitrogen concentration (applicability of epiphyte abundance as a trophic status indicator).

Both among stations and among years, there is variability in the time scale of the [estimated] DIN concentration that produces the highest positive correlation with epiphyte abundance. In very few cases is this maximum within the several days immediately preceding the measure of epiphyte abundance and it often occurs from a 15 to 40 day period before epiphyte collection (Figures 36-39).

The values of *r* are more often positive than negative, suggesting a positive relationship between DIN loading and epiphyte abundance.

When the analyses are segregated by station (Figures 38-39, Appendix), values of *r* are generally higher between DIN and epiphyte chlorophyll a than between DIN and epiphyte biomass, suggesting that chlorophyll a is a more sensitive indicator of DIN loading.

This analysis has some drawbacks that should be considered. Linear interpolations of DIN concentrations may not represent well the relationship of DIN to time over daily time scales. Secondly, because of the limited number of DIN measurements that could be made, the individual values of *r* between epiphyte abundances and DIN concentrations as averaged over progressively longer time periods are not independent from one another. A study recording daily measurements of DIN [perhaps experimentally controlled] concentrations and epiphyte responses would likely shed more insight on the situation, but that approach was beyond the capability of this study. However, this analysis does appear to be unbiased, and the balance of the evidence suggests that epiphyte response to DIN loading persists for some time after the loading has occurred. Controlled experiments using eelgrass (e.g., Coleman and Burkholder 1994) also suggest that the response of epiphytes to nutrient loading is delayed and cumulative.

It is important to consider that investigators have debated the utility of using the abundances of epiphytes as an indicator of changing estuarine trophic status. A number of studies have found autotrophic epiphytes to be generally nutrient limited (e.g., Coleman and Burkholder 1994; Madden and Kemp 1996; Wear et al. 1999). Light and epiphyte grazer activity are other factors that are often limiting for autotrophic epiphytes; in a microcosm study of eelgrass systems from the York River (Chesapeake Bay system), Neckles et al. (1994) found different groups of epiphytes to be limited by different factors. In the San Juan Islands (Washington), Nelson and Waaland (1997) found eelgrass epiphyte biomass to be slightly positively correlated with ammonium concentration, but negatively correlated with nitrate concentration. Lin et al. (1996) considered epiphyte biomass to be a poor indicator of nutrient loading or eutrophication in shallow lagoons. Stankelis (unpublished data) found strong light limitation of epiphytes in the Patuxent River (Chesapeake Bay system). As a general trend in the literature on the subject, it appears that positive

correlations between nutrient loading and epiphyte abundance are more consistent for laboratory investigations than for *in situ* studies, a conclusion reached by Williams and Ruckelshaus (1993). It may be that grazer population effects and other factors limiting epiphytes may be difficult to duplicate *ex situ*. It seems reasonable to assume that, if other sources causing variability in epiphyte abundance can be accounted for, temporal changes in epiphyte abundance may be a useful indicator of trophic status in a spatially limited monitoring area. Since other factors can create great variation in epiphyte abundance, attention to adequate statistical power in such a monitoring program is imperative.

Evaluation of these findings for applicability to a monitoring program must consider the goals and the conceptual models of the program. If epiphytes are to be considered primarily or solely an indicator of trophic status, then factors that are confounding or that present greater sources of variation can be problematic. However, if epiphyte abundance is considered to be a potential stress to SAV, and an objective of the monitoring program is to evaluate status, then understanding the source of variability is not a critical need for the program, and cause can be determined by subsequent research, if needed. For example, if epiphyte abundance is observed to increase, it may be interpreted as a stress on SAV requiring some concern, regardless of whether it is caused by nutrient loading, depressed grazer populations, or other factors or combinations of factors.

The great variability of DIN concentrations in the Coastal Bays between even two-week sampling sessions (in 1999 and 2000) is evident in Figures 5-11 and 17-22 (Appendix). Many "peaks" or "lows" concentrations that would not be detected by the monthly sampling currently done by the present water quality monitoring program for Assateague Island National Seashore (National Park Service 1991; Sturgis 2001). Even in the absence of a formal analysis (of variance), it is clear that temporal variability (on this scale) is a greater source of variation than is spatial variability among sampling stations. This suggests that improvement in detecting trends and parameter levels through the National Seashore's current long-term water quality monitoring should be realized by increasing frequency of sampling sessions. Although DIN is perhaps the most temporally variable parameter examined by the program, it is expected that many other parameters will exhibit a greater source of variation over short time scales (days to weeks) than over the spatial extent of the current long-term water quality monitoring program. Any expansion of the program should concentrate on increasing temporal effort. If costs (salaries, laboratory analysis) of the program are to remain fixed, consideration should be given to decreasing the number of stations sampled in favor of increasing the frequency of sampling. An analysis of variance of the parameters currently measured should be able to confirm or refute this hypothesis.

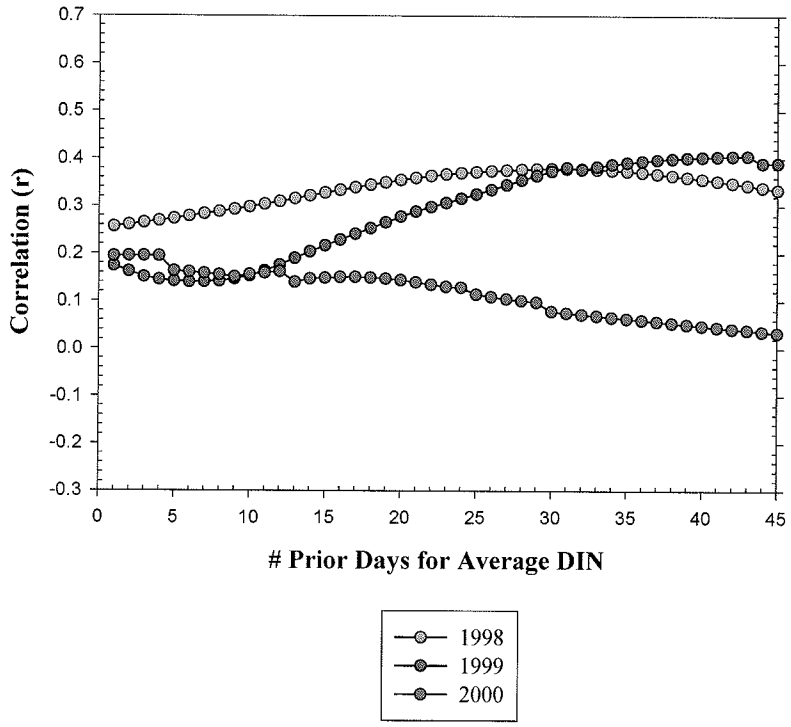


Figure 36. Relation of correlation of epiphyte biomass density with DIN as averaged over a prior number of days, by year.

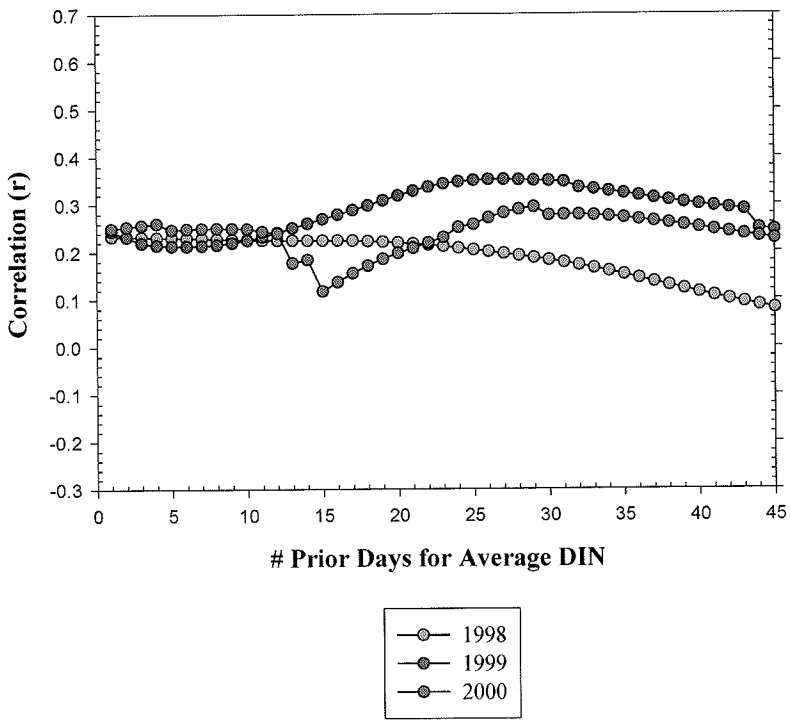


Figure 37. Relation of correlation of epiphyte chlorophyll a density with DIN as averaged over a prior number of days, by year.

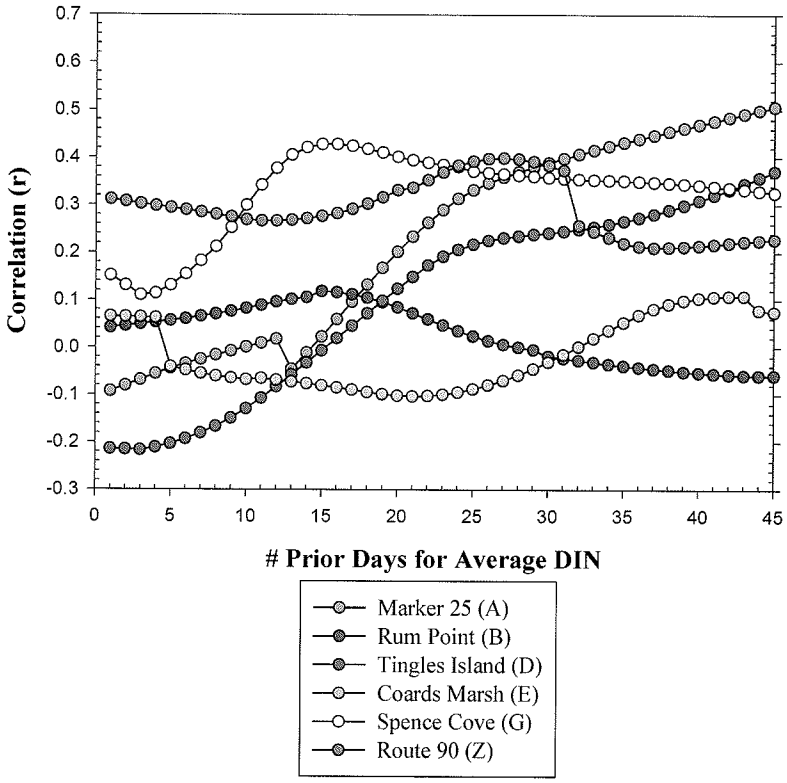


Figure 38. Relation of correlation of epiphyte biomass density with DIN as averaged over a prior number of days, by station.

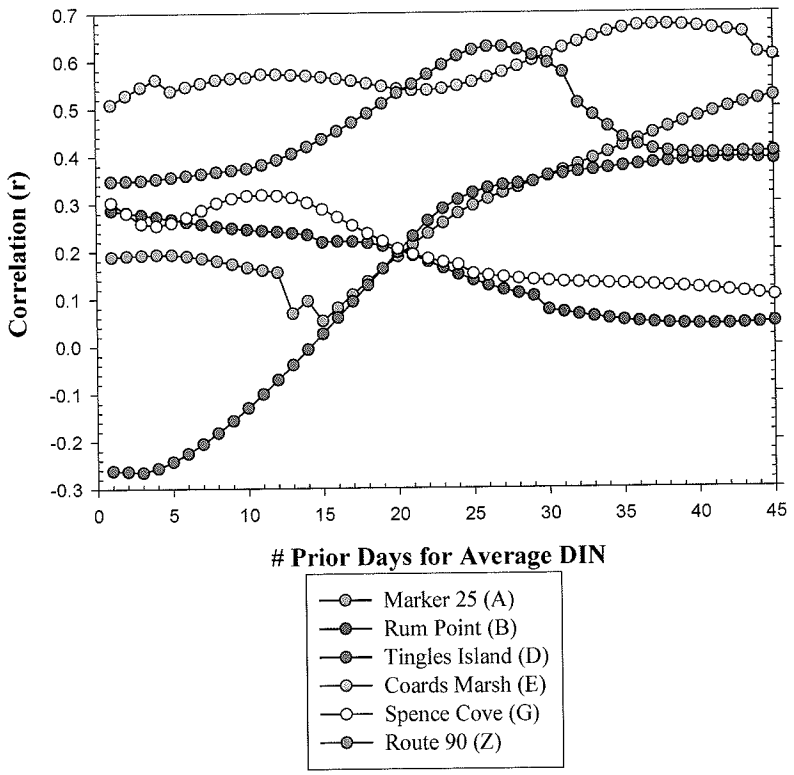


Figure 39. Relation of correlation of epiphyte chlorophyll a density with DIN as averaged over a prior number of days, by station.

RESULTS AND DISCUSSION

Use of artificial substrates (SAV mimics).

Results of interval monitoring of light attenuation through SAV mimics (K_e) are depicted in Figures 40-51 (Appendix). Although a general trend of increasing attenuation from spring to summer occurs (likely due to increased temperatures and/or light), the sharp increases across the spring/summer growing season "boundary" often present for the direct measures of epiphyte abundance are not evident. If such patterns do exist in the mimic sample data, they may possibly obscured by greater temporal variability in an equal size per sample of mimic observations.

A power analysis for each mimic sample (by station, by date) is depicted in Figures 52 and 53. The number of sample units to detect a given level of change, expressed as a fraction of the sample mean appears to be somewhat greater (for most samples) during the spring growing season, suggesting that mimics are somewhat less efficient per sample unit at recovering epiphyte abundance patterns than are the direct measures. This lower efficiency is probably at least fully offset by the considerably lower effort of data collection per sample unit for mimics.

Longer period deployment (seasonal) for mimics (figures 54-61) may be advantageous in assessing epiphyte loading trends over an entire growing season with less effort, given the apparent short-term variability seen in short interval (~ 15 day) monitoring. As epiphyte abundance increases over a number of weeks or months, abundance is likely to become density limited; this effect is likely the cause of the leveling off of abundance curves at the Rum Point, Coards Marsh, Spence Cove, and Route 90 stations (Figures 55, 58-61, Appendix). Monitoring beyond this point would not likely yield much useful information because progressively worsening conditions (i.e., those as or more conducive to more epiphyte loading) would likely not be adequately manifested in increased light attenuation. Where the leveling of K_e occurred, it did not do so before the end of the spring growing season at any station; thus deploying mimics for the duration of the spring growing season, may be an effective strategy. However, shorter interval measurements of K_e should be considered. In more eutrophic situations than the Coastal Bays (or possibly under future conditions in the Coastal Bays), density limitation of epiphytes may occur much more rapidly (e.g., at 7-10 days in the Patuxent River (R. Stankelis, pers. comm.)).

Regression analysis of the 223 seasonal mimic sample units for which light attenuation and chlorophyll a concentration were measured is summarized in Figure 62 and shows a reasonably strong predictive relationship between epiphyte chlorophyll a concentration and light attenuation. Interestingly, the light attenuation properties of comparable concentrations of chlorophyll a appear to be similar for the mesohaline Patuxent River (R. Stankelis, unpublished data), even though fouling rates on the Patuxent River are much higher than in the Coastal Bays and the possibility that mesohaline epiphyte communities may be compositionally different. This relationship should be compared for both areas and for other areas

for which similar data has been collected. Similar, and perhaps stronger, relationships may exist if epiphyte biomass (dry weight) is substituted for chlorophyll a concentration (e.g., Stankelis et al. 2003).

Testing of artificial substrate methods under "worse case" scenarios than presently occur in the Maryland Coastal Bays (perhaps in the considerably more eutrophic Delaware Inland Bays) would be insightful in showing how well the monitoring might perform in the future.

National Park Service monitoring protocols stress the critical importance of understanding statistical power of a monitoring method both during the pilot phase (e.g., this study) and in the early years of an actual monitoring program. This study has generated several methods of measuring epiphyte abundance.

It was found to be reasonable for one person to make 80-100 sample measurements of mimics per day. This is an adequate number to detect a 20% (of sample mean) change in light attenuation (at 1999 and 2000 levels) (with both Type I (α) and Type II (β) error rates set at 0.2) for most samples encountered during the spring growing season (Figure 53, Appendix). A far smaller number of measurements (~ 35) could detect a similar percentage of change for most samples collected in summer. It would take three persons 2 days to make field collections of this many sample units, if distributed at 6 different stations.

In contrast, 40-50 sample units of either epiphyte chlorophyll a or epiphyte biomass (dry weight), as measured in 1998 and 2000, must be processed to consistently detect a comparable change level in those parameters. It takes about nine to ten person-days to process these in the laboratory. Chlorophyll a measurements add external laboratory costs at a rate of about $10/sample unit (2000). It would take three persons 3 days to make field collections of this many sample units, if distributed at 6 different stations.

An effective epiphyte monitoring program using mimics could likely be implemented at the park with little impacts to existing operations when current base-funded natural resource management staffing is full (it has not been since early 2001). A monitoring program using epiphyte biomass or chlorophyll could be implemented by restructuring some operations and adding 0.5 staff full time equivalent (FTE). If chlorophyll a is investigated, $2,000 - $3,000 for laboratory costs (this could probably be done for less, if it is feasible to make an initial investment in fluorometric laboratory equipment). Continuing both biomass and chlorophyll a measurement would require about 1.0 additional FTE annually.

Biological and logistical considerations for determining the best method of monitoring SAV epiphytes are summarized in Table 2.

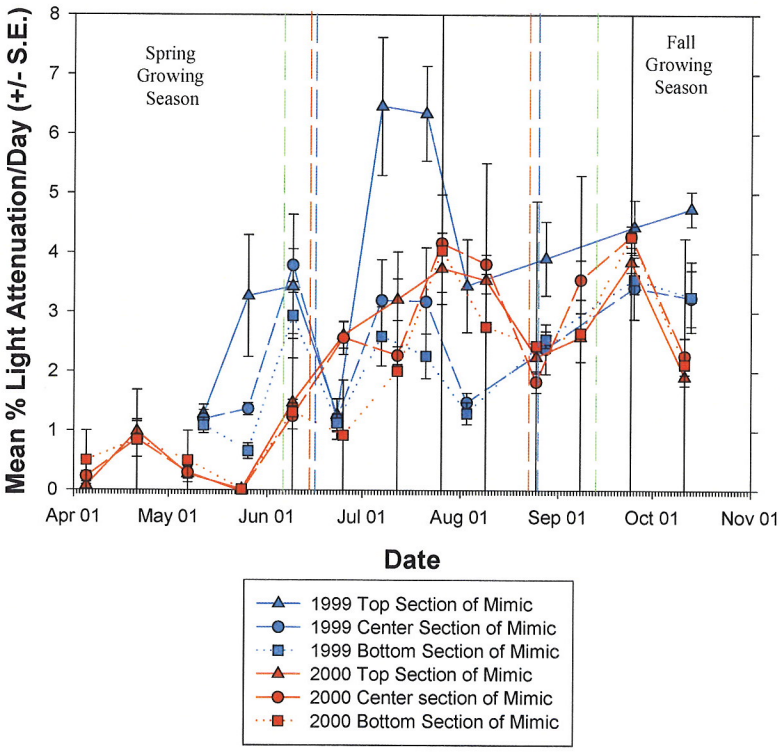


Figure 40: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics - Marker 25 (A)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

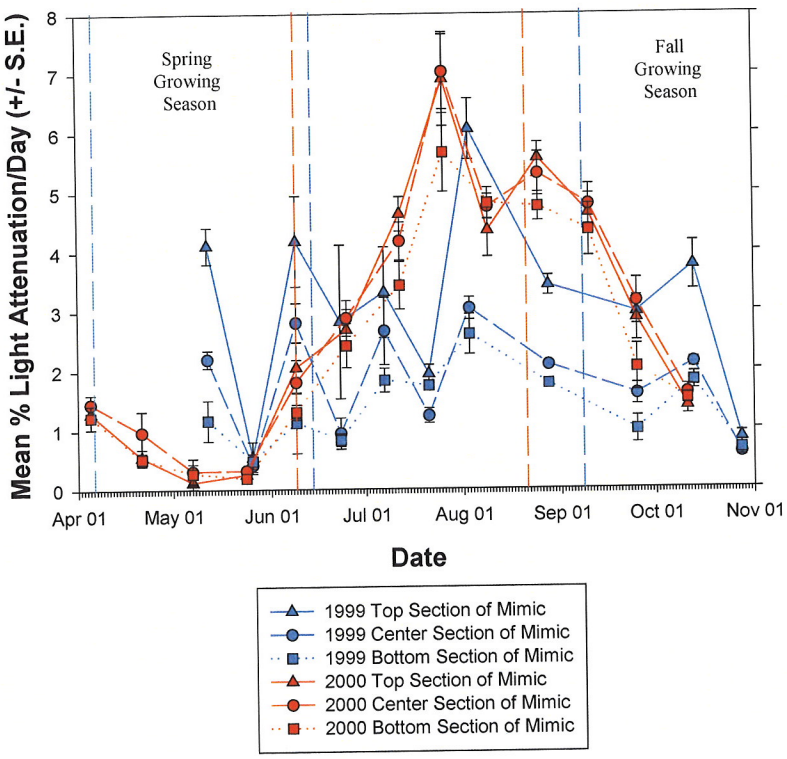


Figure 41: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics - Rum Point (B)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

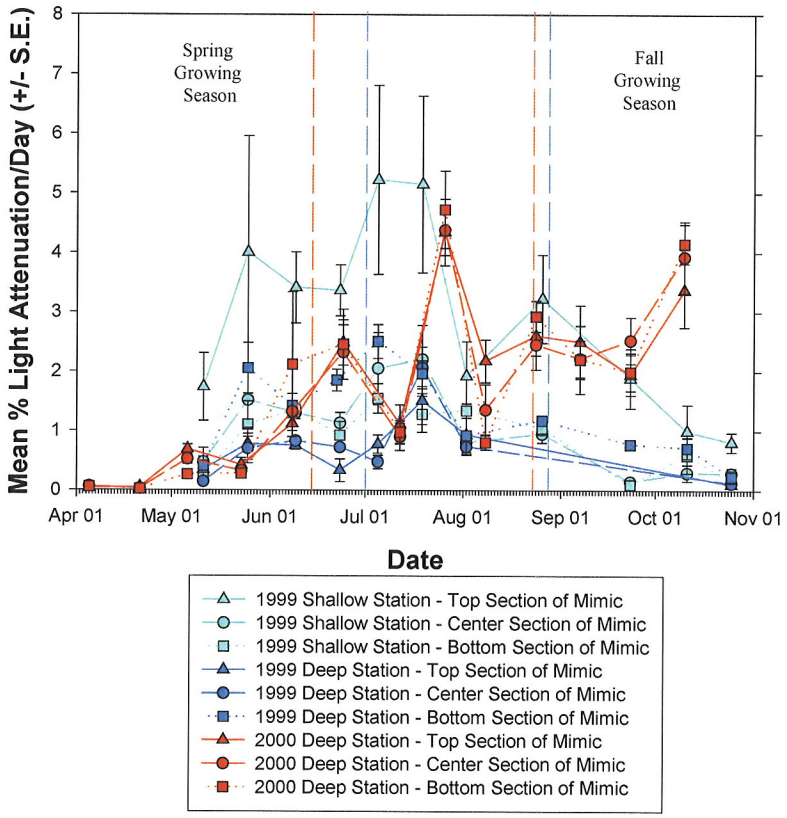


Figure 42: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics - Tingles Island (Ds and Dd)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

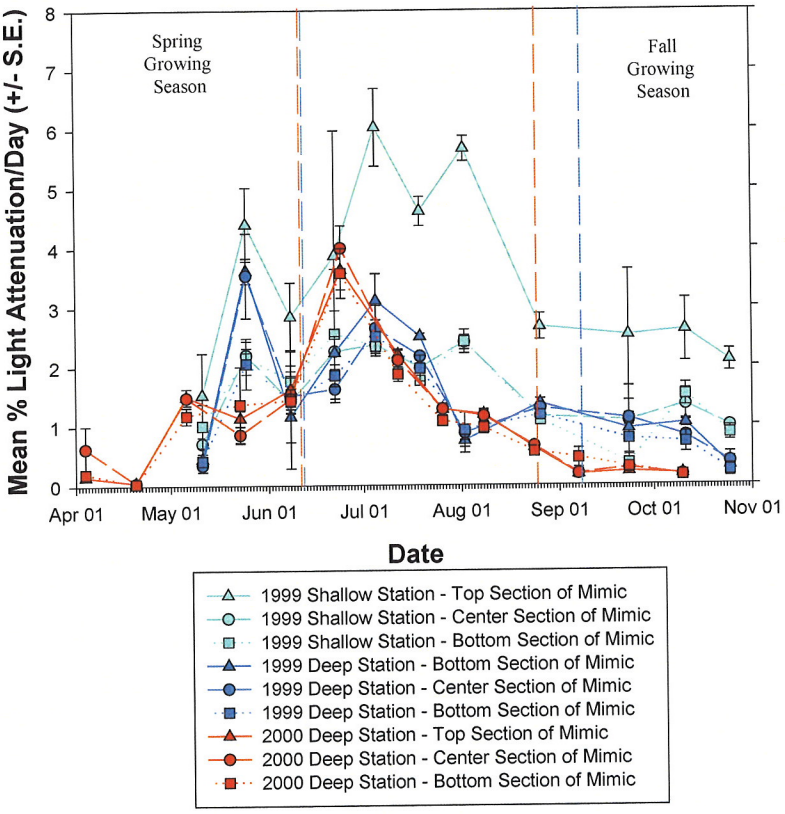


Figure 43: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics - Coards Marsh (Es and Ed)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

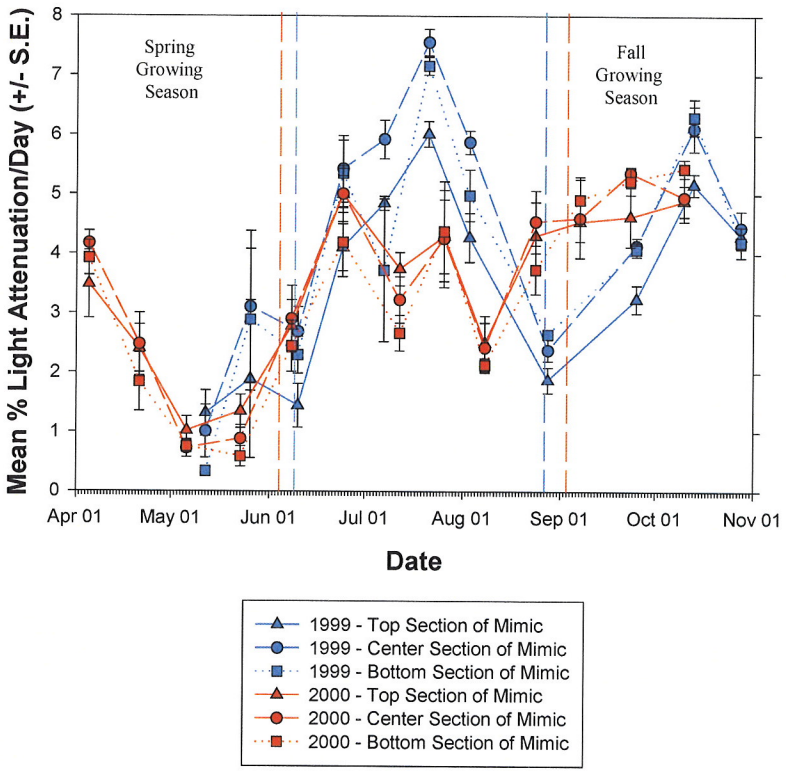


Figure 44: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics - Spence Cove (G)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

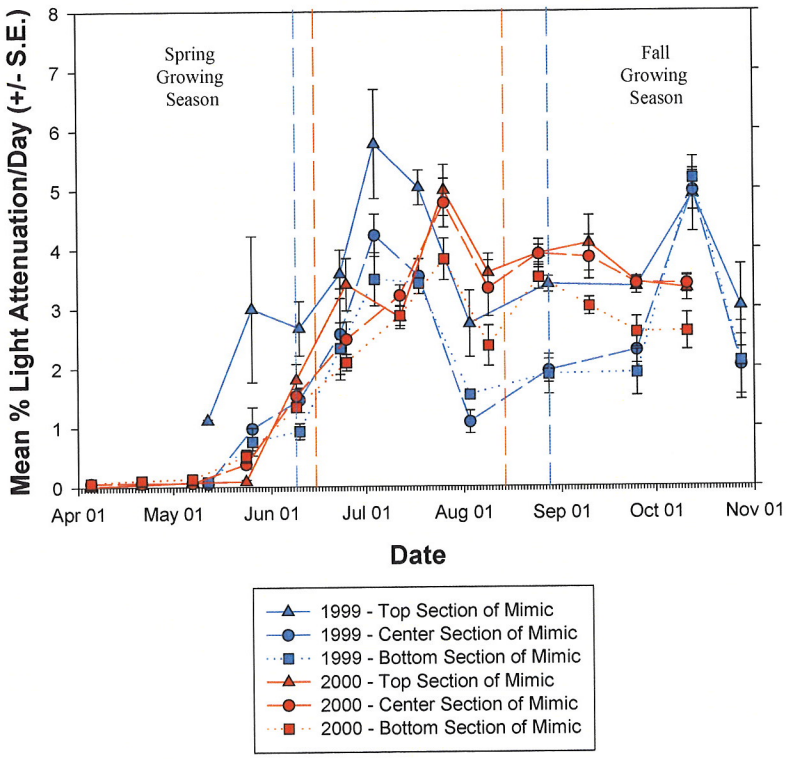


Figure 45: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics - Route 90 (Z)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

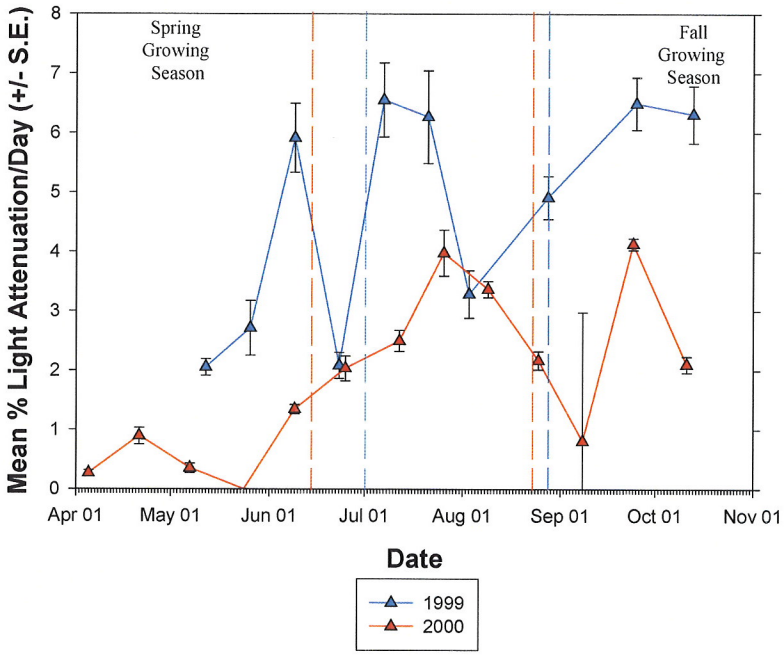


Figure 46: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics (pooled) - Marker 25 (A)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

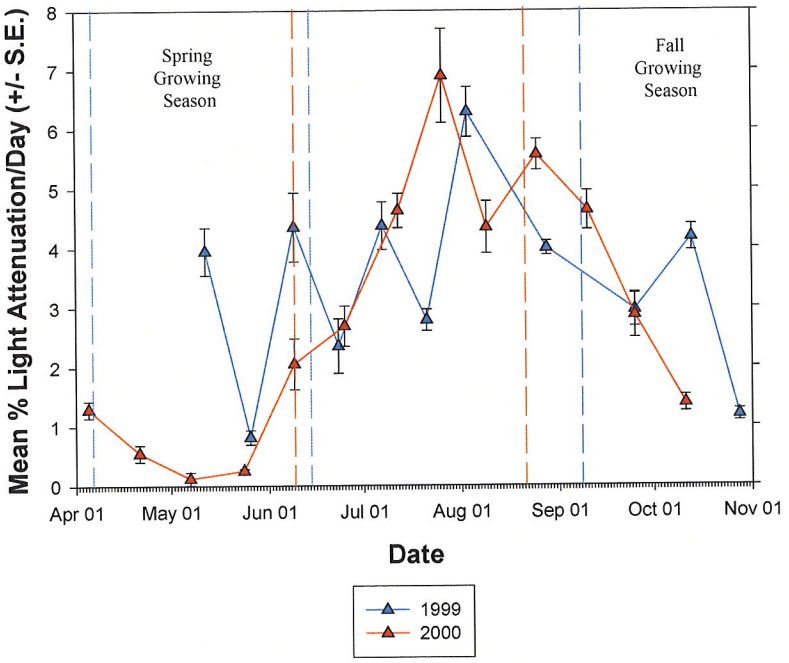


Figure 47: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics (pooled) - Rum Point (B)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

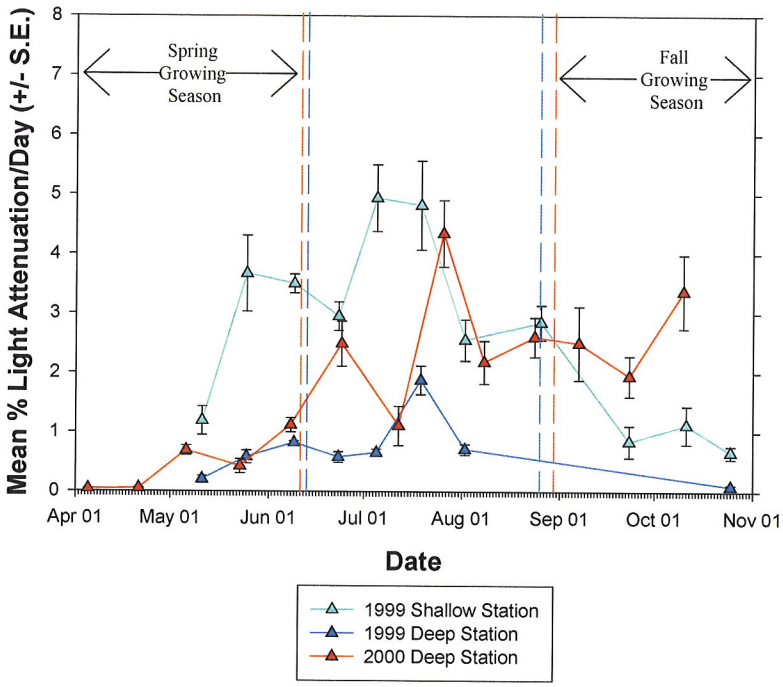


Figure 48: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics (pooled) - Tingles Island (Ds and Dd)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

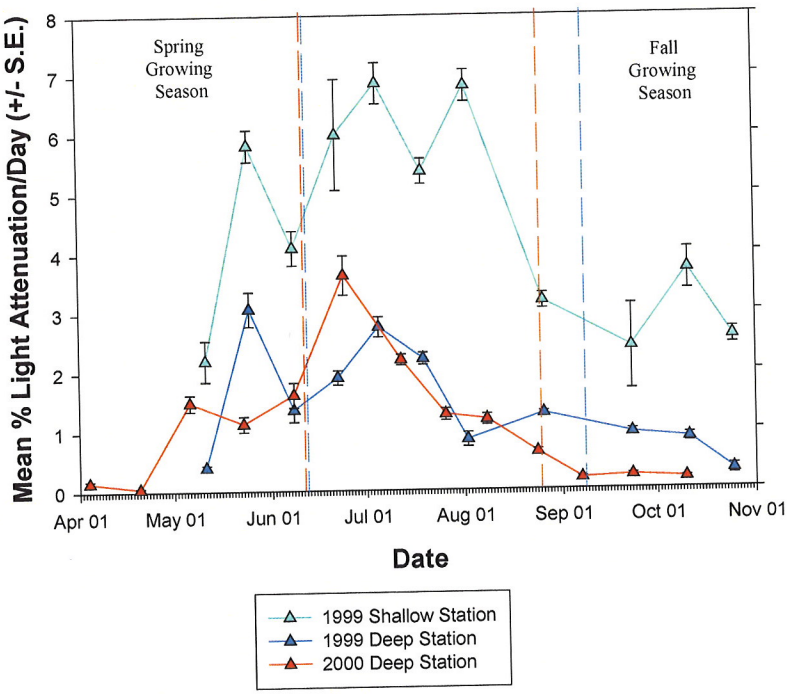


Figure 49: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics (pooled) - Coards Marsh (Es and Ed)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

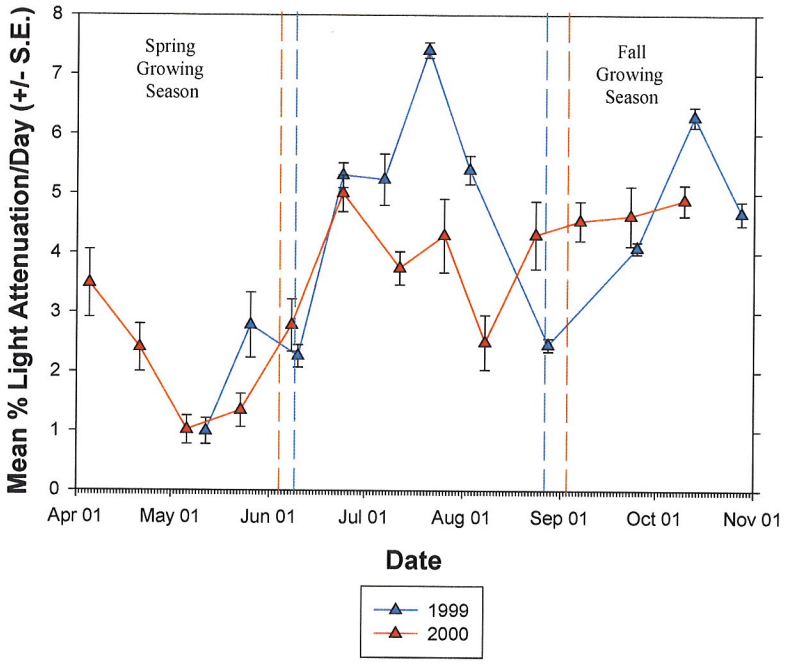


Figure 50: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics (pooled) - Spence Cove (G)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

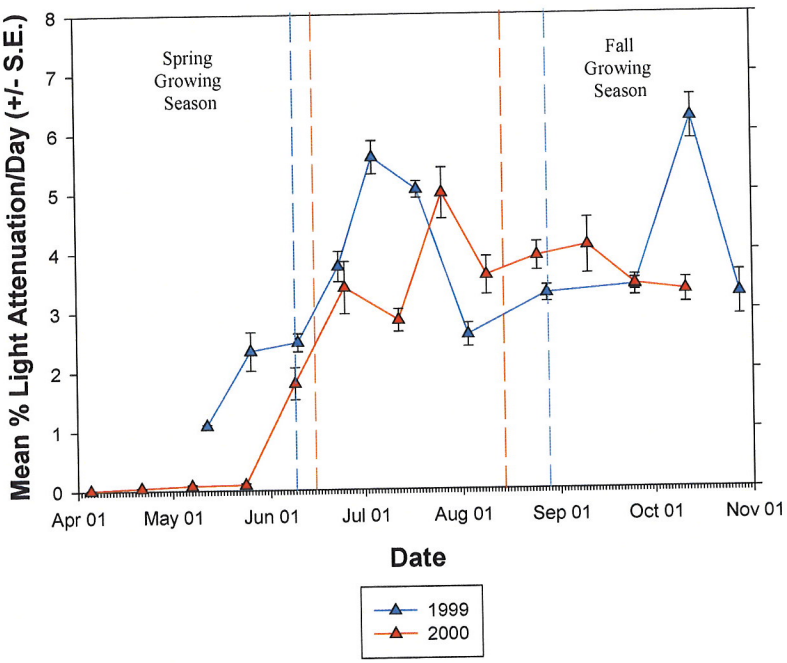


Figure 51: Light attenuation from epiphytes on on SAV mimics - interval monitoring, all sections of SAV Mimics (pooled) - Route 90 (Z)

Vertical dash lines represent end of spring growing season and start of fall growing season: 1999 (blue), 2000 (red).

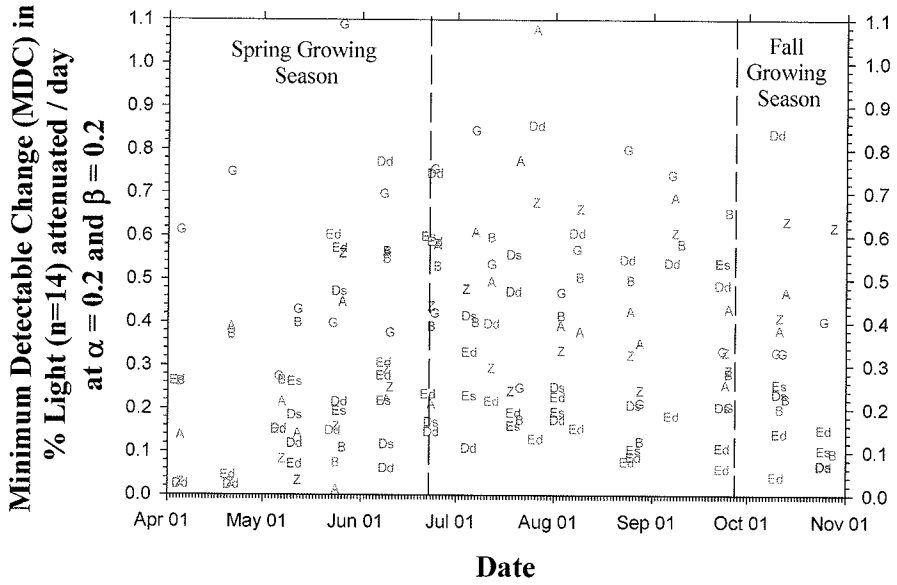


Figure 52: Light attenuation minimum detectable change for sample against date. Letters denote stations. Blue letters denote 1999 data; red letters denote 2000 data.

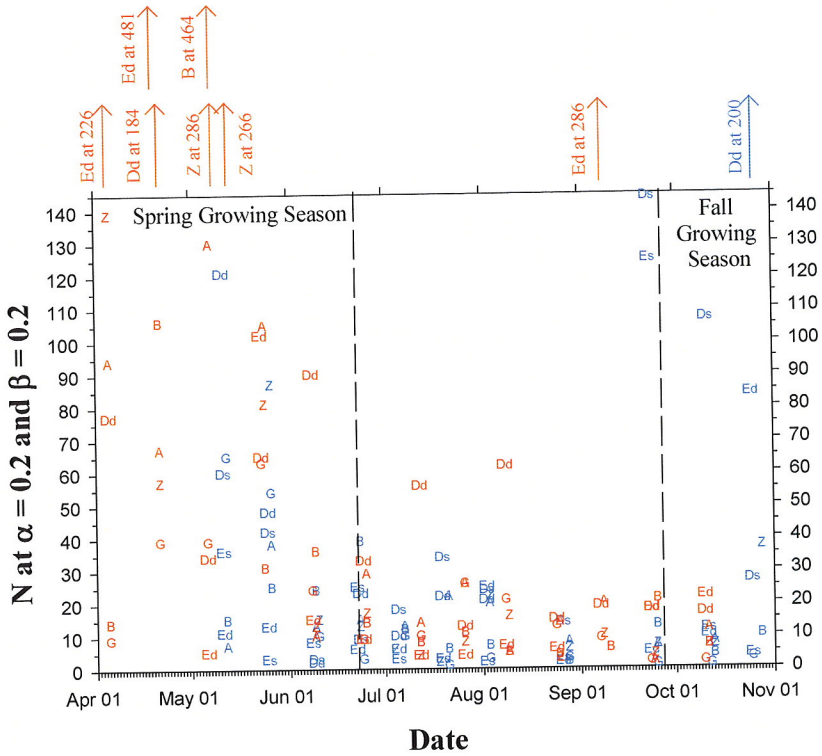


Figure 53: Minimum numbers of sample units required to detect change of 20% of sample mean against date. Letters denote stations. Blue letters denote 1999 data; red letters denote 2000 data.

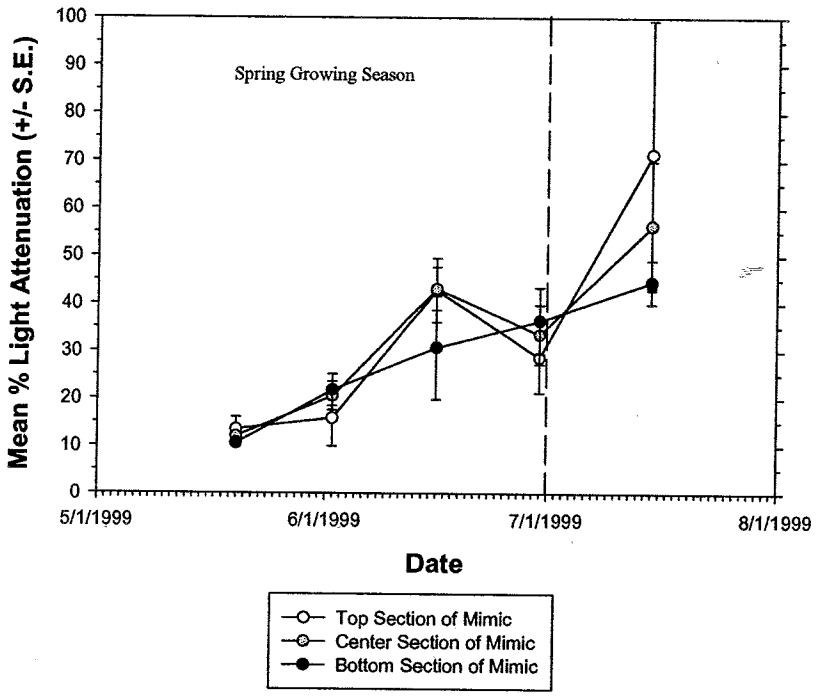


Figure 54: Light attenuation from epiphytes on on SAV mimics - seasonal monitoring, all sections of SAV Mimics - Marker 25 (A), 1999. Vertical dash line indicates calculated end of spring growing season.

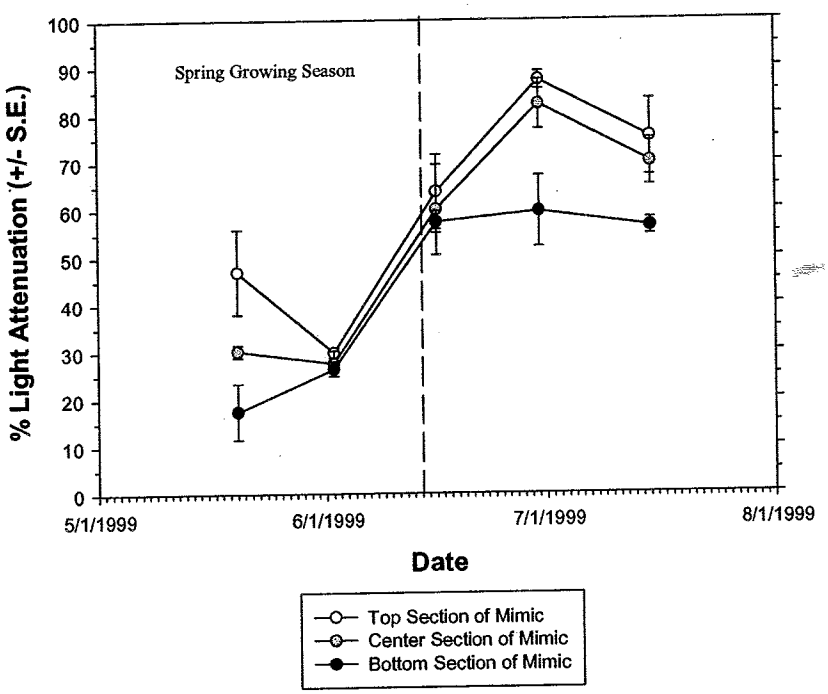


Figure 55: Light attenuation from epiphytes on on SAV mimics - seasonal monitoring, all sections of SAV Mimics - Rum Point (B), 1999. Vertical dash line indicates calculated end of spring growing season.

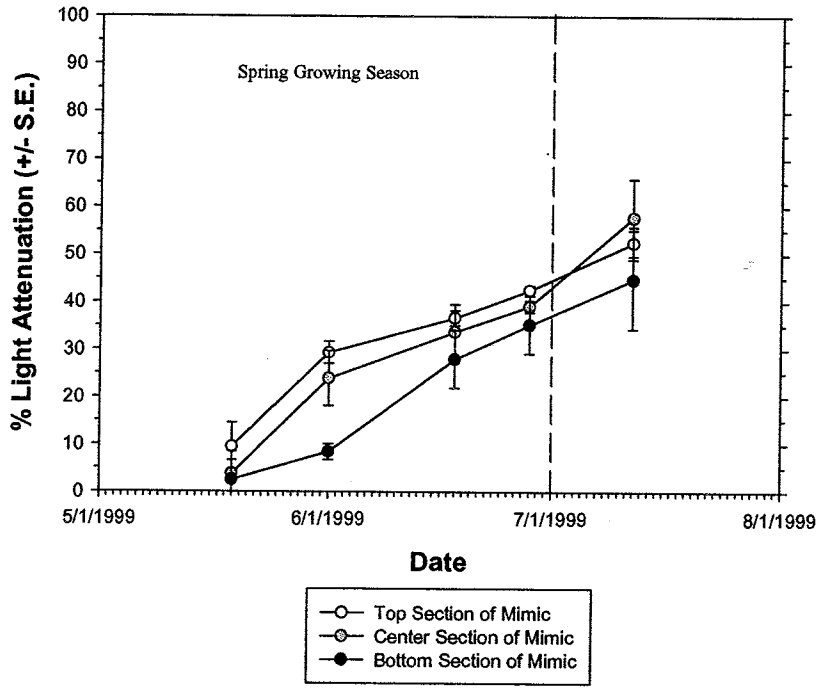


Figure 56: Light attenuation from epiphytes on on SAV mimics - seasonal monitoring, all sections of SAV Mimics - Tingles Island Deep Station (Dd), 1999. Vertical dash line indicates calculated end of spring growing season.

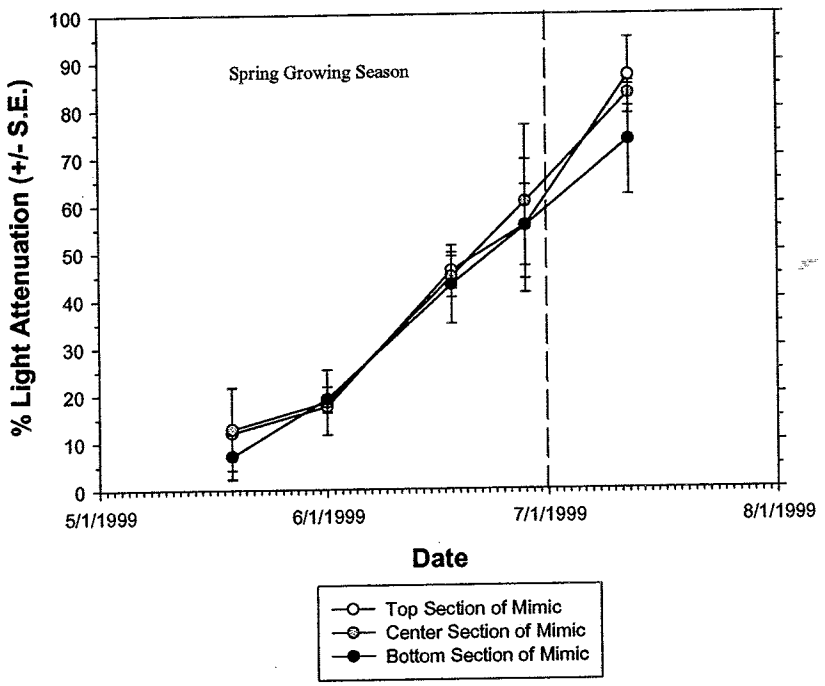


Figure 57: Light attenuation from epiphytes on on SAV mimics - seasonal monitoring, all sections of SAV Mimics - Tingles Island Shallow Station (Ds), 1999. Vertical dash line indicates calculated end of spring growing season.

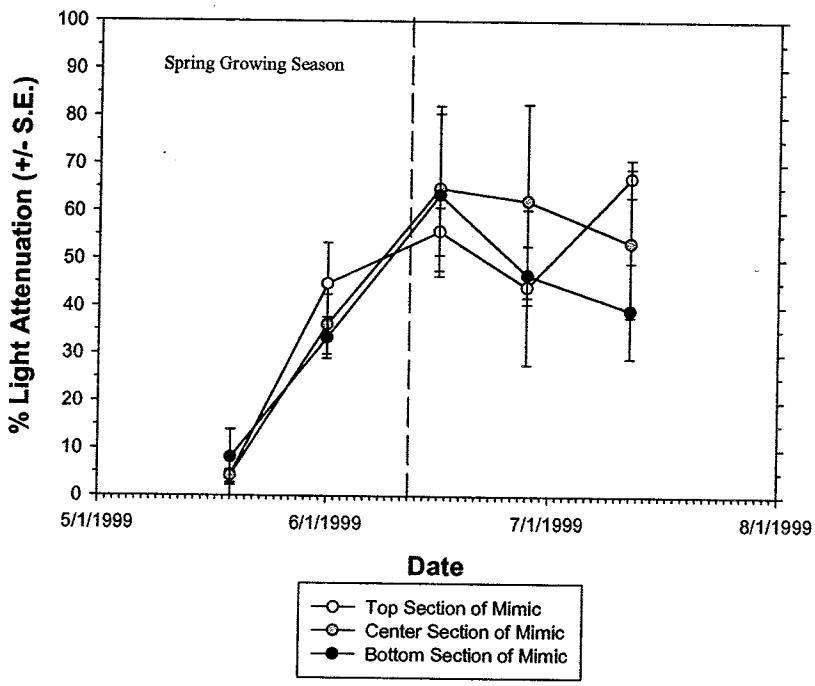


Figure 58: Light attenuation from epiphytes on on SAV mimics - seasonal monitoring, all sections of SAV Mimics - Coards Marsh Deep Station (Ed), 1999. Vertical dash line indicates calculated end of spring growing season.

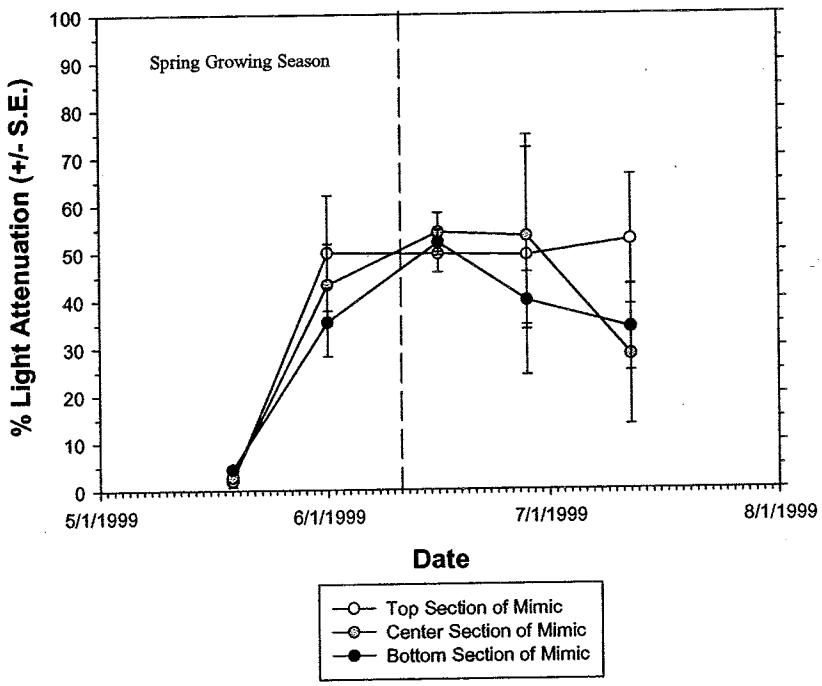


Figure 59: Light attenuation from epiphytes on on SAV mimics - seasonal monitoring, all sections of SAV Mimics - Coards Marsh Shallow Station (Es), 1999. Vertical dash line indicates calculated end of spring growing season.

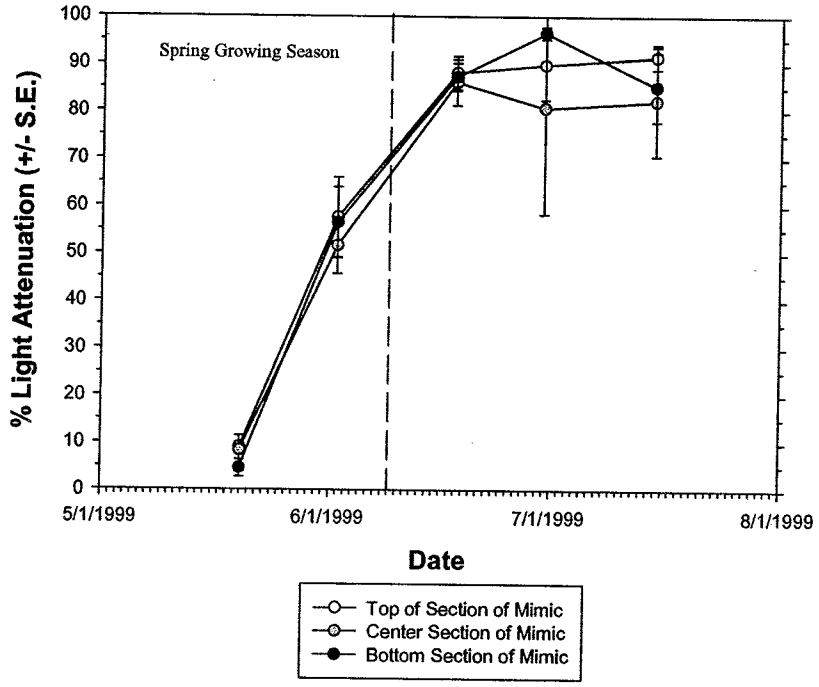


Figure 60: Light attenuation from epiphytes on on SAV mimics - seasonal monitoring, all sections of SAV Mimics - Spence Cove (G), 1999. Vertical dash line indicates calculated end of spring growing season.

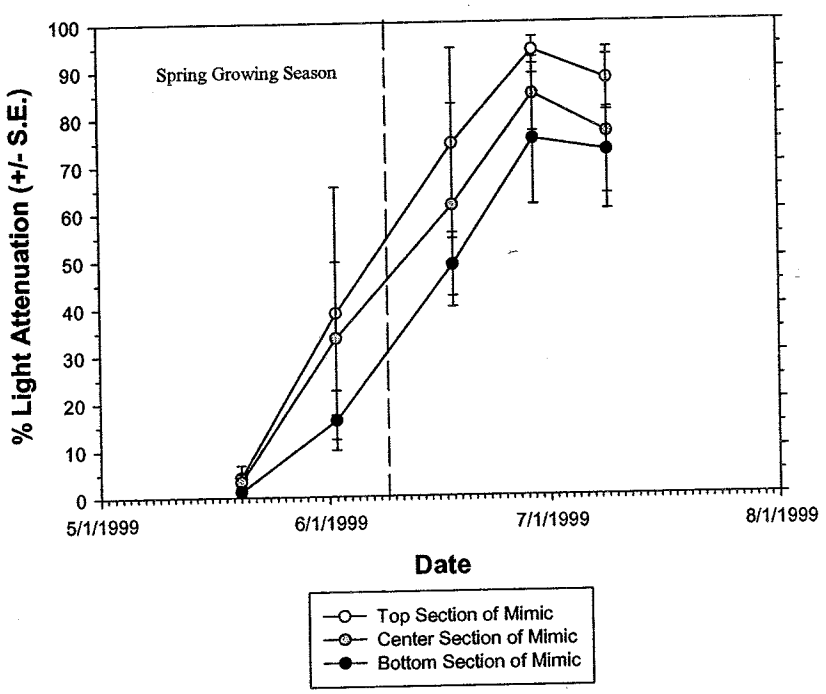


Figure 61: Light attenuation from epiphytes on on SAV mimics - seasonal monitoring, all sections of SAV Mimics - Route 90 (Z), 1999. Vertical dash line indicates calculated end of spring growing season.

Table 2. Summary of differences between direct (left column) and indirect (light attenuation through mimics) (right column) measures of SAV epiphyte monitoring that have practical implications for monitoring programs. Bolded column in each row reflects the more favorable situation for monitoring.

CONSIDERATION	Direct Measures of Epiphyte Abundance	Measures of Light Attenuation through Mimics
Ecological breadth of method	SAV must be present at monitoring station (e.g., conditions cannot be monitored after loss of SAV)	**SAV need not be present**
Data collecting investment	**(If SAV is present), data is always available**	Measuring devices are vulnerable to loss or disturbance; data can be lost.
Biological aspects of parameter	Is a measure that considers biological interaction between epiphytes and SAV (advantage if considering parameter a net ecological effect)	Does not consider biological interactions between epiphytes and SAV (advantageous if considering the parameter a stress). Epiphytes growing on mimics may differ from those on live SAV (although the relationship between the two may be reasonably well-correlated)
Characteristics of epiphyte communities	Removal of epiphytes destroys structure (and shading characteristics) of epiphyte community (Brush and Nixon 2002)	**Method largely retains structure of epiphyte community; results may be better correlated with stress on SAV.**
Repeatability	Observer (data collection) bias more of concern (e.g., completeness of scraping, filtering); more training and technique QA/QC needed.	**Achieving repeatability of technique across observers less difficult.**
Cost	More expensive and logistically more involved to conduct (more so for epiphyte chlorophyll a abundance than for epiphyte biomass (dry weight) abundance	**Once initial equipment is acquired, method is fairly inexpensive**

RESULTS AND DISCUSSION

Relationship between direct measures of epiphyte abundance and measurements of light attenuation through mimics.

The logarithmic regression model (Figure 62) provided a strong predictive relationship between chlorophyll a concentration on mimic surfaces and light attenuation, with more than 67% of the variation in light attenuation being explained by the model ($r^2 = 0.674$). Using similar methods, Stankelis (1999) found a similar relationship between epiphyte concentration on mimics and light attenuation in the Patuxent River, a tidal freshwater to mesohaline estuary. Interestingly, the light attenuation response is quantitatively very similar for given concentrations of epiphyte chlorophyll a in both estuary systems, even though salinities and fouling rates by epiphytes on mimics for the two systems differ (in the Patuxent River, mimics often acquire epiphyte concentrations after 7-10 days of exposure (Stankelis, 1999) that are comparable to those acquired in 180 days in the Coastal Bays. This suggests that artificial substrate (mimic) methods, as employed in this and the Patuxent River studies may yield models that are consistent predictors of epiphyte abundance from light attenuation through mimics across a number of estuaries in this region. A combined analysis of the data sets from these estuaries and others from which similar data has been or may be collected would give more insight on how strong and how universal this relationship may be.

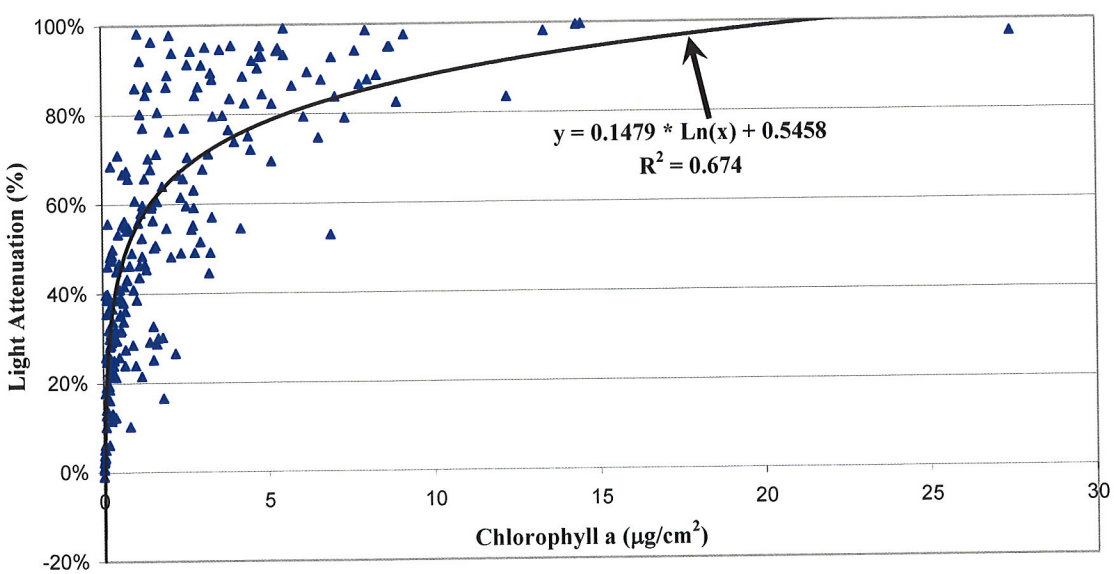


Figure 62. Relationship of epiphyte chlorophyll a concentration on mimic to light attenuation through mimic for each of 223 sample units, exposed from 15 to 181 days in Coastal Bays, 1999.

RESULTS AND DISCUSSION

Determination of growing season limits.

Spring and fall growing season limits for eelgrass, as predicted by regression are depicted by station by year (Figures 5-28 and 40-51, Appendix) and by year (stations pooled) (Table 3 and Figures 32-35 and 52-53, Appendix).

Table 3. Eelgrass (*Zostera marina*) growing season limits, Gaussian regression constants, and coefficient of determination (r^2) for Maryland Coastal Bays SAV monitoring station temperature data, by year, 1998-2000.

Year	Start, Spring growing season	End, Spring growing season	Start, Fall growing season	End, Fall growing season	a	b	x_0	r^2
1998	Mar 12	Jun 12	Aug 24	Nov 23	25.71	99.36	212.70	0.908
1999	Mar 29	Jun 13	Aug 30	Nov 10	26.47	86.15	212.76	0.828
2000	Mar 11	Jun 11	Aug 24	Nov 22	25.71	99.36	212.70	0.824

It is recommended that additional investigation to improve the fit of the predicted functional response of temperature to date be conducted. The predicted start of the fall growing seasons appeared to be somewhat earlier than temperature data for that period suggest. It should be noted that temperature data from SAV beds were not collected after October 21 in any year, which is likely before the end of the fall growing season. This truncation of data may degrade the reliability of the predicted response, including that part extrapolated beyond October 21. Functional models that allow for skewness in the response curve (e.g., Weibull distributions) may also improve the fit.

Appropriately conservative interim spring growing season limits for eelgrass in the Maryland Coastal Bays would be March 29 through June 11. For the fall growing season, September 8 to November 10 may be used.

"NEXT STEP" RECOMMENDATIONS

It is recommended that these findings be reviewed by subject-matter experts, primarily to assess the biological significance of the various possible monitoring methods and schedules. This should include review by the Estuarine Nutrient Enrichment Working group of the National Park Service Northeast Coastal and Barrier monitoring network.

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As the nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

NPS D-81 August 2003