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**A STUDY OF *PHYLLAPLYSIA TAYLORI*
IN THE EELGRASS ECOSYSTEM
OF PADILLA BAY**

Amy DeLorenzo

May 1999

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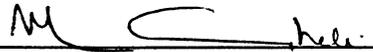
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ECOSYSTEM OF PADILLA BAY

by

Amy DeLorenzo

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science



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**A STUDY OF *PHYLLAPLYSIA TAYLORI* IN THE EELGRASS
ECOSYSTEM OF PADILLA BAY**

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
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May 1999

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ABSTRACT. In eelgrass systems, the interactions and relationships among *Zostera marina*, the epiphytes, and the macrofaunal grazers are important to the dynamics of the system. *Zostera* benefits from the presence of grazers which consume the epiphytic growth fouling the blades of *Zostera*. *Phyllaplysia taylori*, an opisthobranch mollusc, is one species of grazer in the eelgrass ecosystem of Padilla Bay. This research, consisting of a lab study and a field study, focused on the distribution patterns of *P. taylori* and its abundance within the eelgrass system of Padilla Bay.

The lab study assessed how the behavior of *P. taylori* affects its distribution. The response of *P. taylori* to two characteristics of substrate, orientation and color, was tested. *P. taylori* demonstrated a significant orientation preference and a significant color preference. The field study evaluated how specific factors affect the densities of *P. taylori* in its natural environment. Three sites (March Point, Kirby Beach, and Bayview) were sampled and compared with respect to epiphytic and macrofaunal communities on eelgrass. *P. taylori* was found abundantly at March Point, moderately at Bayview, and negligibly at Kirby Beach. Kirby Beach and Bayview were most similar with respect to epiphytic communities. March Point and Kirby Beach had more similar macrofaunal communities. The distribution and abundance of *P. taylori* is sensitive to a number of environmental factors, epiphytic and macrofaunal communities representing only two possibilities. No striking trends were apparent when considering the relationship between *P. taylori* and these two factors. Therefore, more extensive research is necessary to determine what factors affect the population size of *P. taylori* within Padilla Bay.

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INTRODUCTION

Eelgrass Ecosystem

The structure and function of eelgrass systems are of great ecological significance to coastal areas of the Pacific Northwest. Biologically, eelgrass systems are valuable due primarily to their high nutrient content. These ecosystems are one of the most productive systems, having a primary productivity level comparable to that of highly cultivated agricultural crops (McRoy and McMillan, 1977). Structurally, this system slows water movement created by currents and thereby creates a calm environment particularly important to larvae and juveniles (Kikuchi, 1980). Because of the reduced current velocity, sediments are trapped and erosion is prevented. Smaller organisms also rely on eelgrass beds for shelter and protection from larger predators (Wood *et al.*, 1969). Economically these systems are also extremely valuable because many major fisheries depend on organisms that have spent all or some of their life cycle within eelgrass systems (Phillips, 1984).

Zostera marina

The eelgrass itself is the foundation of the community since these marine angiosperms are the major source of food within this ecosystem. Consumption of the plant itself is minimal; it is grazed primarily by vertebrates (some species of fish, birds, and turtles) and a few invertebrates (urchins and some opisthobranchs). The majority of the plant material is either deposited on the bottom or transported out of the ecosystem, providing large quantities of detrital material and a basis for detrital food chains (Kikuchi, 1980; Orth and Van Montfrans, 1984).

Epiphytic Communities

Eelgrass provides substrate for a diverse assemblage of epiphytes. The term epiphyte is defined as any species of plant which colonizes the surfaces of other plants (Isaacs *et al.*, 1996). The relationship between epiphytes and their host macrophyte is hard to classify. Evidence of both the beneficial and detrimental influences epiphytes have on their host can be found in the literature (Borum *et al.*, 1984; Bronmark, 1985; Orth and Van Montfrans, 1984; Sand-Jensen, 1977).

Generally, if the fouling becomes too heavy, the host plant suffers. A dense community of epiphytic organisms dramatically increases photosynthesis levels in eelgrass habitats. Wood (1972) found that this increase of photosynthesis by the epiphytes caused the pH of the system to become too basic. Bicarbonate ions are therefore limiting and the process of photosynthesis cannot continue. Conversely, at night, increased respiration by the epiphytes causes an extremely low pH which in turn causes a drop in redox potential. Ultimately, these fluctuations can cause mortality in some animals and dramatically limit the growth of many plants.

Another result of heavy epiphytism is shading of the host plant. This shading often causes temperature stratification that is most noticeable in areas where there is limited water circulation as is the case in many eelgrass meadows. This pronounced layering of water temperatures will disrupt the life cycle of the eelgrass plant by postponing flowering, fruiting, and seed production. Shading of the host plant by the epiphytes can also restrict photosynthesis to the upper layer of the water column, thereby preventing oxygenated water from reaching the lower portions of the water column. Another outcome of epiphytic shading is a reduction of the incoming nutrient and light

levels available to the host plant. In addition to nutrient interception, Borum *et al.* (1984) found that less than 10% of incoming light was transmitted through the epiphytic layer to the host. Since both *Zostera* and the epiphytes which foul it use similar wavelengths of light, they are therefore in direct competition for light (Caine, 1980).

Not only are the processes carried out by the host disrupted, but the plants themselves can also be physically harmed. The blade will often decay underneath the epiphytes causing the blade to break off. In her study on *Odonthalia floccosa*, Ruesink (1998) found the presence of diatom epiphytes to have a detrimental effect. In addition to the negative physiological effects, epiphytes greatly increased the drag on the host plant. This typically caused pieces to break off and in some cases dislodged the entire plant.

Seagrasses have a variety of methods of preventing heavy epiphytism. Their high growth rate enables them to continually produce new blades and slough off the older, highly-fouled blades (Sand-Jensen, 1977). From May through August, when epiphytic biomass is greatest, the maximum age of leaves decreases dramatically from 200 days in winter and spring to 50-70 days. Borum *et al.* (1984) found an exponential relationship between leaf age and epiphytic biomass showing that even minor changes in the maximum ages of leaves substantially reduces the average biomass of epiphytes on the leaves. Epiphytic colonization could also be controlled chemically. The phenolic acid content of seagrasses is similar to land plants. *Zostera marina* has eight phenolic acids, some of which commonly function as growth inhibitors (Zapata and McMillan, 1979). A third highly effective method of regulating epiphytic biomass is accomplished by grazers which consume the epiphytes, physically removing them from the eelgrass blades (Thom *et al.*, 1991). This removal of epiphytic biomass improves the health of *Zostera*. In a

study done by Orth and Van Montfrans (1984), because of the removal of the epiphytes by grazers, *Zostera marina* produced more new shoots and had, on average, larger leaves when grazers of epiphytes were present.

Phyllaplysia taylori

Phyllaplysia taylori is one of many invertebrate grazers in eelgrass ecosystems. *Phyllaplysia taylori* (hereafter referred to as *Phyllaplysia*) was first discovered near Vancouver Island by George W. Taylor, after who this opisthobranch was subsequently named (MacFarland, 1966). *Phyllaplysia* and other members of the family Aplysiidae are commonly referred to as sea hares because of the resemblance of their body outline to that of a sitting hare and the resemblance of their rhinophores to the ears of a hare (Beeman, 1968). The normal habitat of *Phyllaplysia* is on *Zostera marina* in bays and estuaries (Beeman, 1963). They are fairly hardy animals in both habitat and diet. Beeman (1970) collected animals from Elkhorn Slough in water ranging in temperature from 13.5 – 21°C and with a salinity as low as 23.95 ppt. *Phyllaplysia* graze non-selectively on the film of small organisms colonizing the surface of *Zostera*. In their natural habitat, the diet of *Phyllaplysia* is composed primarily of sessile diatoms, and their jaws, radula and stomach teeth are well adapted for breaking the siliceous frustules of these organisms (Beeman, 1969). However, when forced to feed on a species of red algae not commonly found on *Zostera*, *Phyllaplysia* remained healthy. Also, when maintained in tanks without *Zostera*, *Phyllaplysia* grew much faster (Beeman, 1970).

Phyllaplysia taylori has a unique life cycle. It is one of the few known opisthobranchs and the only recorded anaspidean to have direct development (Bridges,

1975). Immediately upon hatching, the veliconchs settle directly onto the *Zostera* blades on which the egg cases were also laid. Because direct development is unusual, the life cycle of *Phyllaplysia* has been the focus of a number of studies. The nidosomes (egg masses) of *Phyllaplysia* are rectangular packets, laid on eelgrass blades, covering the entire width on the blade and usually extending down the blade for about 5 cm. The veliger develops within the egg capsule into a veliconch, at which time it hatches. Directly after hatching, the veliconchs settle and development continues on the eelgrass blades through the post larval and adult stages (Bridges, 1975).

Bridges (1975) made a few observations on settlement, although none were quantitative. She observed that once settled, even if not on blades of eelgrass, the larvae would not crawl onto eelgrass which indicates that preference for eelgrass as a substrate is not great in *Phyllaplysia* larvae. Because these opisthobranchs do not undergo a planktonic stage characteristic of the majority of members of the aplysiidae family, the ability to search for and recognize a favorable substrate for settlement is not important.

Direct development is common to species such as *Phyllaplysia taylori* whose food source is fairly abundant. In a study on nudibranch larvae, Hadfield (1963) determined that a long planktonic stage may be necessary for species which generally feed on food that is less abundant or widely spaced. Because the food sources of direct developers are generally more abundant, it is not necessary for the larvae to maintain a position in the plankton to search for a proper substrate. Direct development supplies the young with a suitable substrate immediately upon hatching (Thompson, 1962).

However, at some stage in the life cycle, *Phyllaplysia taylori* must show a preference for *Zostera* blades. These invertebrates are found almost exclusively in this

habitat. Their external appearance matches that of the eelgrass blades, not only in color but also with respect to the dorsal markings, composed of dark brown-black longitudinal stripes, which mimic the veins on the eelgrass blades (Beeman, 1968). When I observed these animals under epi-fluorescence, no trace of chlorophyll was evident in their cells. Therefore, since the green coloration of *Phyllaplysia* is not due to diet, the choice of eelgrass blades as a substrate could be for protection through camouflage.

Padilla Bay

Padilla Bay, a National Estuarine Research Reserve located in western Skagit County, Washington, contains one of the largest meadows of eelgrass in the Pacific Northwest, providing an ideal location for field work concerning eelgrass systems. Seagrasses cover approximately 3200 hectares (58%) of the area designated as Padilla Bay with a recorded density ranging from 61 to 441 shoots per square meter. *Zostera japonica*, *Ulva* sp., and *Enteromorpha* sp. are other common types of vegetative cover within this system (Bulthuis, 1991). Marine waters feed into Padilla Bay primarily through the Guemes Channel with some influence from the Swinomish Channel. Four sloughs: Joe Leary, Big Indian, Little Indian, and No Name, provide fresh water flow to this estuarine system (Bulthuis, 1993). Tides are mixed semi-diurnal, and ranged from -2.5 to 9.4 feet during the spring and summer of 1998.

Purpose of Study

Previous studies conducted in Padilla Bay have indicated that the invertebrate grazer community characteristic of eelgrass meadows is dynamic. T. Shaw (1994) found

significant temporal and diel variation of many species of grazers within one season. A study done the following year by M. Shaw (1995) found different trends in the abundance and behavior of caprellid grazers than those reported by T. Shaw (1994). The latter study was conducted at a site 600 meters south of the original study. These two studies lead to the speculation that the abundance of grazer taxa could vary either annually or with location, or as a result of a combination of these two factors.

With this background knowledge of the dynamics of grazer fauna, my study focused on *Phyllaplysia taylori*, one of the two dominant grazers in eelgrass meadows in Padilla Bay (T. Shaw, 1994). Preliminary sampling of eelgrass beds at three sites in Padilla Bay was done between July and September 1997. Sampling was done approximately every two to three weeks. The sites chosen were March Point (western region of the bay), Bayview State Park (southern region), and Kirby Beach (northern region). These sites were chosen because of their dispersed locations in the bay as well as their relative ease of access.

A homogenous distribution of *Phyllaplysia taylori* was not observed in Padilla Bay during the 1997 sampling period. *Phyllaplysia* were extremely rare at March Point, found in very low numbers and only in one of four samples taken from this site. However, the two other sites, Bayview and Kirby Beach, paralleled each other in the densities of *Phyllaplysia*. One of the major differences between March Point and the other two sites was the type of epiphytic fouling on the eelgrass blades. This preliminary sampling of Padilla Bay indicated that adult *Phyllaplysia* could show a selection preference for specific substrates.

More thorough sampling was done in 1998. Lab studies in which the behavior of *Phyllaplysia* was tested in reference to their substrate preferences were also designed to supplement the field work. The field work was conducted to trace the distribution of *Phyllaplysia* within Padilla Bay and the temporal pattern of *Phyllaplysia* densities in the areas in which they were found. The study by T. Shaw (1994) showed niche separation among the grazers, indicating that grazers could affect the distribution of *Phyllaplysia*. Therefore, additional focus was on the macrofauna comprising the communities of which *Phyllaplysia* is a member.

MATERIALS AND METHODS

Lab Study

The laboratory portion of this study was done to clarify the distribution and behavior of *Phyllaplysia taylori* in relation to its choice of substrate. The behavior of *Phyllaplysia* with respect to two characteristics of substrate, orientation and color, was examined under controlled conditions and used to supplement the information gained from the field study on the distribution of *Phyllaplysia* in its natural environment. All organisms used in these experiments were collected from Padilla Bay approximately four months before the experiments were run. They were maintained in flow-through seawater tables at Shannon Point Marine Center. Fresh eelgrass was also kept in the seawater tables. All experiments were run between 11:00 a.m. and 3:00 p.m.

Orientation Preference

This experiment was done to determine if *Phyllaplysia taylori* actively chooses a substrate based on the orientation of the substrate. In other words, is *Phyllaplysia* found on eelgrass blades for some reason other than the vertical, upright positioning of the leaves or is it attracted to any vertical surface?

Cubical Tupperware containers (16 x 16 cm) were used in this experiment. The left and right sides of the container allowed for vertical positioning; the top and bottom allowed for horizontal positioning. The front and back of the container were replaced with a wire mesh screening to allow for water flow. One organism was placed in each container, and the container was submerged in a seawater table. The water table was

covered with an opaque, black plastic sheet to remove any effects of changes in light intensity on the positioning of *Phyllaplysia*.

During preliminary testing over a four-hour time span, the position of the majority of the organisms did not change after the first hour. Therefore, experiments were run for one hour. At the end of the hour, the position of the organism within the container and the size of the organism were recorded. Sixty individuals were tested.

Color Preference

In this experiment, *Phyllaplysia taylori* was allowed to choose between five colors: green, brown, red, black, and white. Green, brown, and red are related to the principal colors of substrate found in the habitat of *Phyllaplysia*, green is the color of eelgrass with negligible fouling, brown when heavily fouled by diatoms, and red is representative of various species of red algae found in eelgrass beds. Black and white were used to determine if the organism detects color or reacts to contrast or light intensity.

One organism was placed in the center of a colored Plexiglas disc and submerged in a sea table under 6 cm of water. This disc was divided into five equal pie sections representing each of the five colors to be tested. Fluorescent lights (two 40 watt cool white bulbs, 2500 lumens) were set up over the sea table and black plastic was draped over the lights and table to keep the effect of light constant. The organism was watched for 60 minutes and the time spent on each color was recorded. Forty-five organisms were tested.

Field Study

Field work was conducted to examine the distribution of *Phyllaplysia* in its natural environment and to determine how certain habitat characteristics affect this distribution. Both the epiphytic community and the macrofaunal community present at the chosen sampling sites were analyzed.

Study Site

Three areas within Padilla Bay were used for field sampling (Fig. 1). The three study sites were chosen after preliminary sampling of eelgrass beds within Padilla Bay between July and September of the previous year (1997). These three sites, designated as March Point, Bayview, and Kirby Beach are spaced relatively evenly throughout the shallow, subtidal regions of Padilla Bay. All contain large beds of *Zostera marina*.

March Point. This area was the most heavily trafficked of the three sites. It is open to people with shellfish licenses and is easily accessible to the public. This area is also commonly used as a parking area for RV's.

Over the sampling period, the eelgrass blades ranged in width from 1.0 – 1.5 cm and reached a length of up to 150 cm on average. The temperature of the water varied from 12-18.7°C, the salinity varied from 26.7-30 ppt, and the D.O. varied from 9.15-17.98 mg/L.

Bayview. This area is also open to the public for recreation. However, people were very seldom seen close to the water's edge. At this site, during low tide, the mudflats leading out to the water are more extensive than at the other sites making access to the water more difficult.

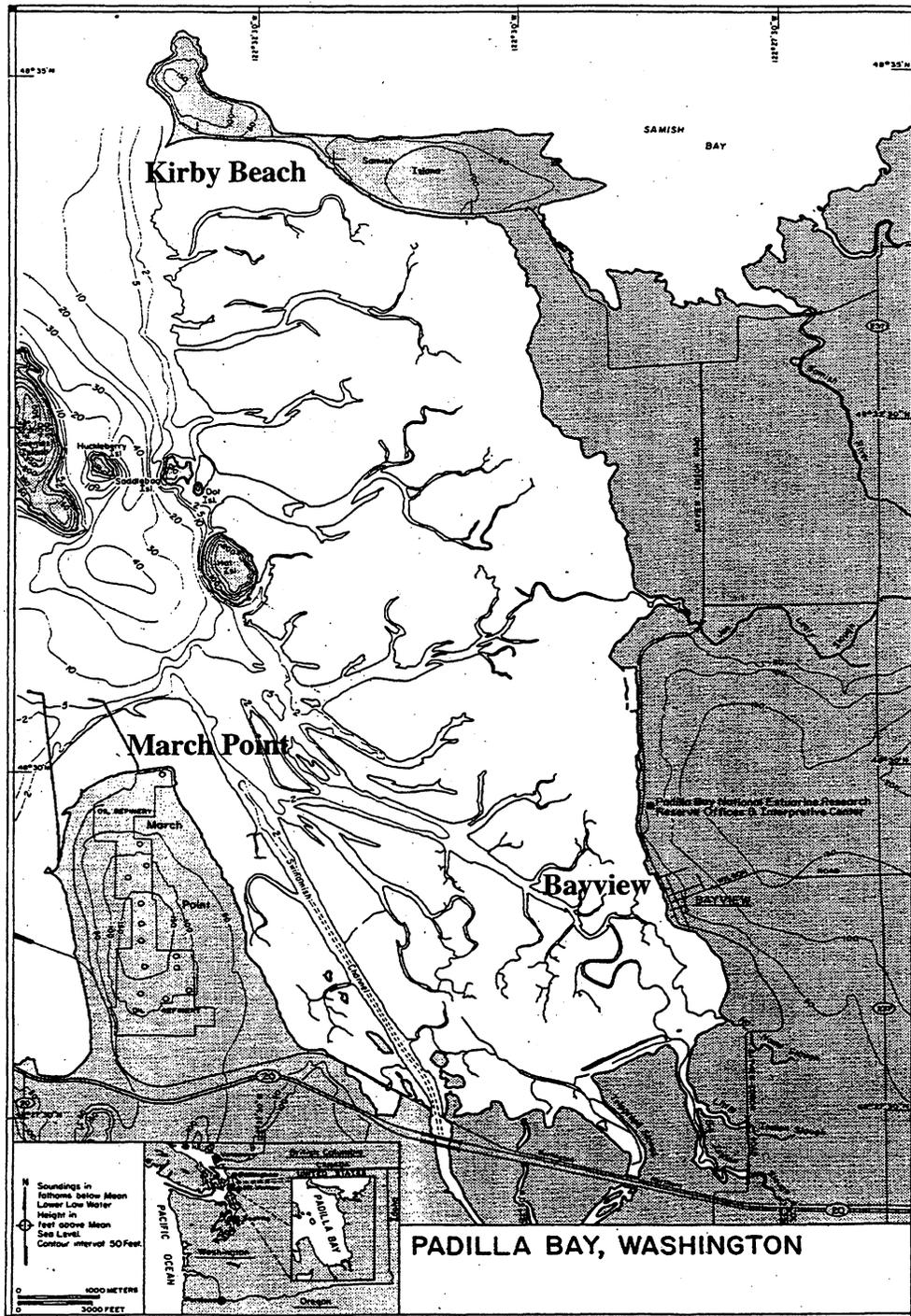


Figure 1. A map of Padilla Bay showing the location of the three sampling sites.

The eelgrass blades from this site were smaller. They ranged in width from 0.8-1.2 cm and up to 115 cm long. The temperature ranged from 11.8-21°C, the salinity varied from 25.5-29.4 ppt, and the D.O. varied from 6.52-11.96 mg/L.

Kirby Beach. There is limited access to this beach, the majority of which is private. Only during one of the sampling times were other people present.

The eelgrass blades from this site varied in width from 0.7-1.0 cm and on average grew no longer than 100 cm. The temperature of the water was generally warmer at this site compared to the others, and ranged from 16-22.4°C. The salinity varied from 26-30.4 ppt, and the D.O. ranged from 8.4-15.74 mg/L.

Field Sampling

Eelgrass beds at the three sites were sampled biweekly, beginning at the end of March and continuing through the first week of September. This six-month period is the most productive for the eelgrass (Thom *et al.*, 1991). Sampling was conducted during low tide so, although the sites were always inundated, the water was never more than 1.5 m deep. Twelve replicates were taken from each site during each of the twelve sampling periods. The twelve replicates were chosen randomly from within the site using the method employed by Shaw (1994). In this method, sample addresses, consisting of a direction (0-360°, in increments of 10°) and a distance (1-10 m, in increments of 1 m) from a set reference point located in the center of the site, were generated from a random numbers table.

Samples were collected by hand using 0.5-meter-long funnel-nets with a 16 cm diameter opening at one end. Typically, one shoot consisting of approximately 4-8

leaves was collected within each net. The shoot was detached from the rhizome to discourage the collection of any organisms other than those associated with the eelgrass leaves. After collection, the shoots were held in the net in a bucket of sea water, each replicate kept separate, and transferred to the water tables at Shannon Point Marine Center for identification and counting of the macrofauna and analysis of the epiphytic community.

All work was done with live specimens. The samples were processed within a maximum of 48 hours after collection. All motile macrofauna in each sample were identified (Kozloff, 1987) and counted. A few specimens of the fauna more difficult to key were preserved in 70% isopropyl alcohol for later study. The entire length of each blade and the width of each blade at its midpoint were recorded for all eelgrass blades in the samples. One blade from each sample was kept separate for epiphyte analysis. This blade was selected randomly by using a randomly generated list of numbers ranging from 1-7.

Measurements of Epiphytes

Dry Weight. The blade selected for epiphyte analysis was rinsed in filtered seawater to dislodge sediment trapped in the epiphytes. Macrofauna were also removed from the blade. Using a rubber spatula, both sides of the blade of eelgrass were scraped and rinsed with filtered seawater. The method described by Parsons *et al.* (1984) was used to determine the dry weight of the epiphytes. This epiphyte / filtered seawater solution was centrifuged for 5 minutes, and the supernatant was discarded. The pellet was then re-suspended in a known amount of filtered seawater. A known volume of this

solution was filtered onto pre-weighed Whatman GF/C (pore size = 1.0 μm) glass fiber filters. The filters were rinsed three times with deionized (DI) water to dissolve the salt retained by the filters. To function as a control, clean pre-weighed filters were also saturated with filtered seawater and rinsed three times with DI water. All filters were dried in an oven at 60°C for a minimum of 24 hours. The filters were then re-weighed and the dry weight of the epiphytes calculated from the difference in weights.

Organic Content. To determine the organic content, the oven-dried filters were then placed in a muffle furnace at 450-500° C for 24 hours. This temperature completely oxidizes any organic matter without altering the weight of the glass fiber filter. The filters were weighed a final time and the organic content was calculated according to standard formulas. The control filters of seawater / DI water blanks were also ashed and weighed.

Chlorophyll Analysis. The procedure followed was modified from the method described by Parsons *et al.* (1984). A known volume of the epiphyte/filtered seawater solution was filtered onto a second Whatman GF/C glass fiber filter and set aside for chlorophyll analysis. This filter was stored in an aluminum foil packet and kept frozen until analysis.

Using a tissue homogenizer, the filter was ground in a 90% acetone solution. The homogenate was kept cold and dark for an extraction period of 24 hours. After this period, the acetone extracts were centrifuged for approximately 5 minutes. Using a diode-array spectrophotometer, readings were taken of the supernatant at wavelengths of 630, 647, 664, and 750 nm. The absorbance reading at 750 nm was used to correct for turbidity. All other corrected absorbance readings were used in the formulas in Parsons

et al. (1984). The amount of chlorophyll a, b, and c in each sample was calculated per cm^2 of blade. Chlorophyll ratios a/c and a/b were calculated and graphed for each site.

Data Analysis of Lab Study

Orientation Preference

Because any analysis which requires the subject to make a choice violates the assumption of independence, a non parametric test was used on this data set. A log likelihood test (G test) is more robust than a Chi-Square test with data sets which contain small values (Winer, 1971). Therefore, a G-test for goodness of fit was used. If a significant preference was indicated, residual analysis was used to test the significance of the individual choices (Whittam and Siegel-Causey, 1981). For each choice, the standardized residuals (e) were adjusted to the variance (v) giving a normal standard deviate (d). These results were then compared to a normal distribution. If the value of the normal standard deviate is greater than 1.96, this indicates significance at $p=0.05$. A value greater than 2.58 is significant at $p=0.01$.

To determine if size was a factor in the horizontal or vertical distribution of *Phyllaplysia*, the results were graphed by size class (small: 1.0-1.3 cm, medium: 1.4-2.2 cm, and large: 2.3-3.5 cm). A G-test of independence was done to determine if there was a significant difference in orientation based on size.

Color Preference

The analysis was done the same as in the analysis on orientation preference. A G-test for goodness of fit was calculated by comparing the length of time in minutes that the subject spent on each color. Each subject was evaluated separately. When the subject showed a significant preference, a residual analysis was done to determine which colors elicited a significant response. The value of the normal standard deviate (d) used to determine significance was 1.96, indicating a significant preference at $p=0.05$. Size was not tested in this set of experiments because there was little difference in size among the subjects. The majority of the subjects belonged to the medium size class, ranging in size from 1.5-2.0 cm.

Data Analysis of Field Study

Epiphytic Community

A two-way analysis of variance (ANOVA) was performed on the data collected from the epiphytes to determine if epiphytic biomass on the eelgrass was significantly different among sites and if there was significant temporal variation of the epiphyte standing stock during the sampling period. Each characteristic (weight, organic content, and pigments) was analyzed by site and by date. Because the assumption of homogeneous variance was violated in the analysis of chlorophyll a, b, and c, there was a greater possibility of making a Type I error in these analyses. Although ANOVA is robust and operates well even with considerable heterogeneity of variances in the data set (Zar, 1999), alpha was adjusted to 0.01 to decrease the possibility of incorrectly rejecting the null hypothesis. All other assumptions of ANOVA were met.

In a two-way ANOVA, the significance of the interaction between the two factors must be analyzed before the main effects (site and date) can be interpreted. If the interaction is statistically significant, then the difference among sites is not constant at all levels of the second factor, the date at which the sample was taken. When the interaction term was significant, a main effects analysis was done by site and by date separately to determine what factors were significant without the confounding effect of the other term (Winer, 1971). The data were first analyzed within each site using a one-way ANOVA by date and then within each date using a one-way ANOVA by site. For all one-way ANOVAs, the value for the mean square error term was taken from the two-way ANOVA. Any results showing significance were further analyzed using simple contrasts to specify where the differences lay. The p value from the T-statistic was used to determine the significance of the contrasts because the contrast coefficients were chosen *a priori*. The sequential Bonferroni technique was used to control the group-wide error rate by adjusting α for the number of tests included each analysis (Rice, 1988).

Species Diversity, Evenness, and Richness of the Macrofaunal Communities

Shannon's index (H') was used as a measure of species diversity of the macrofauna collected in the samples. Although this measure is sensitive to both species richness and evenness, it does not discriminate between low richness/high evenness and high richness/low evenness. For this reason, richness and evenness indices were measured separately to supplement the information gained from the calculation of Shannon's index (Ludwig and Reynolds, 1988). Species richness was measured with the Menhinick index ($R2$). This measurement is based on the assumption that a functional

relationship exists between the number of species in a sample and the number of individuals observed. From my experience sampling these sites over the past two years, I believe this assumption is applicable to my data. Species evenness was measured with the J' of Pielou index ($E1$), the evenness index most widely used by ecologists. All indices (diversity, evenness, and richness) were analyzed using a two-way analysis of variance (ANOVA) by date and by site. Significance was determined at $\alpha < 0.05$. Replicates were not taken. Therefore, Tukey's Test for Additivity was used to determine if the assumption of additivity was violated. All other assumptions of ANOVA were met.

Comparisons of Species Compositions of Study Sites

Analyses done on the epiphytic parameters as well as on the indices of species diversity, evenness, and richness reveal much about the temporal changes in the community organization of the three sites and help distinguish differences in faunal communities among these sites. However, these measures do not give information on which species are characteristic of a given sample during different times of the year, nor do they give information on what species are characteristic of a given site. Therefore, a hierarchical cluster analysis was used as a classification technique for placing similar samples into clusters. To account for variation in the amount of eelgrass collected in each sample, the species density measure, equal to the number of individuals of a species collected divided by the total surface area of eelgrass (m^2) sampled, was calculated for each replicate. For this analysis, the species density measures of the 12 replicates were summed for each sample, leaving a total of 12 samples for March Point and Kirby Beach and 11 samples for Bayview. (The first collection was not completed at Bayview due to

the inaccessability of that site at that time.) This resulted in a total of 35 samples to be clustered.

The first step in this type of analysis is to compute a similarity matrix between samples. The Bray-Curtis dissimilarity measure was used. Because my data included a few extremely abundant species, the data were log-transformed. Clustering was done using the group average method.

Distribution of *Phyllaplysia taylori*

One of the goals of my research was to determine what habitat characteristics affect the distribution and abundance of *Phyllaplysia taylori*. First, however, it is necessary to determine if there is a difference in the occurrence of *Phyllaplysia* on eelgrass shoots among the three sites. A two-way ANOVA by site and date was done on the number of *Phyllaplysia* collected during the sampling period. Because the assumption of homogeneous variances was violated, alpha was adjusted to 0.01. The assumption of normality was also violated, but ANOVA is robust so it is unlikely that this affected the results. All other assumptions were met.

When the interaction between site and date was significant, simple contrasts were used to test the main effect of time on the abundance of *Phyllaplysia* within each site and the main effect of site on the distribution of *Phyllaplysia* during July, August, and September, the peak times for *Phyllaplysia*. As in the analysis of epiphyte characteristics, the data were first analyzed within each site using a one-way ANOVA by date and then within the five specific dates using a one-way ANOVA by site. The p value calculated from the T-statistic was used to determine the significance of the simple

contrasts because the contrast coefficients were chosen *a priori*. Alpha was adjusted using the sequential Bonferroni test.

Correlations

To determine if change in the density of *Phyllaplysia taylori* was correlated with any of the other blade factors measured during the field sampling, the data were analyzed using Spearman Rank Correlations. The density of *Phyllaplysia* (individuals / m²) was measured against the biomass, organic content, and chlorophyll content of the epiphytes, and against the density of the twelve most common species collected at the three sites. Of these twelve species, there were four species of gastropods (*Alia carinata*, *Haminoea vesicula*, *Lacuna variegata*, and *Lottia alveus*), four species of amphipods (*Isochyrocerus anguipes*, *Isochyrocerus* sp., *Caprella californica*, and green amphipod), two types of polychaetes (nereid sp., and hesionid sp.) one species of isopod (*Idotea ressecata*), and one species of flatworm (*Phylloplana viridis*). Correlations were calculated by site. Kirby Beach was not analyzed separately because of the extremely low numbers of *Phyllaplysia* collected there.

Trends in Epiphytic Biomass and Macrofaunal Density

In addition to the correlations run between *Phyllaplysia taylori* and the various site characteristics, Spearman Rank Correlations were run on eight of the more common species of macrofauna with respect to epiphytic biomass. The data were also graphed to show any trends in densities of these organisms in relation to the temporal variation of epiphytic biomass.

RESULTS

Lab Study

Orientation Preference

Initial analysis of the results in which horizontal was compared to vertical surfaces indicated that *Phyllaplysia* had no significant preference for orientation (Table 1). When the results were further divided so that a distinction was made between the top and the bottom a significant preference was evident. *Phyllaplysia* demonstrated a significant preference for the top, a significant avoidance of the bottom, and no significant preference or avoidance for the vertical surfaces (Table 2). No significance was found for orientation in relation to size of the subject (Table 3, Fig. 2).

Color Preference

Of the forty-five subjects tested, one did not show a response to color. Table 4 gives the number of individuals, based on the value of the normal standard deviate, that demonstrated a significant color preference. Of the remaining forty-four subjects, over half demonstrated a significant preference for green. Red, brown, and white were significantly avoided by the majority of the subjects. Relatively equal numbers of subjects avoided or demonstrated no response to black (Fig. 3).

Table 1. G-test for goodness of fit on the orientation of *Phyllaplysia*.
Significance was determined with a Chi Square value ($\alpha=0.05$, 1df) of 3.84.

	Horizontal	Vertical	Totals
Observed	32	22	54
Expected	27	27	54

$$G = 1.86$$

Table 2. G-Test for goodness of fit on the orientation of *Phyllaplysia*. Horizontal positioning was subdivided into top and bottom. Significance was determined with a Chi Square ($\alpha=0.05$, 2df) of 5.99. In the lower table, e=standardized residuals, v=variance, and d=normal standard deviate.

	Top	Bottom	Vertical	Totals
Observed	27	5	22	54
Expected	18	18	18	54

$$G = 17.92$$

	e	v	d
top	3.67	0.31	6.59 *
bottom	-2.31	0.41	-3.61 *
vertical	-0.96	0.27000	-1.85

*significant at $p=0.01$ ($d>2.58$)

Table 3. G-test of independence on the effect of size on the orientation preference of *Phyllaplysia*. Significance was determined at a Chi Square ($\alpha=0.05$, 2df) of 5.99.

	Small	Medium	Large	Totals
Horizontal	2	21	9	32
Vertical	4	16	2	22
Totals	6	37	11	54

G = 4.31

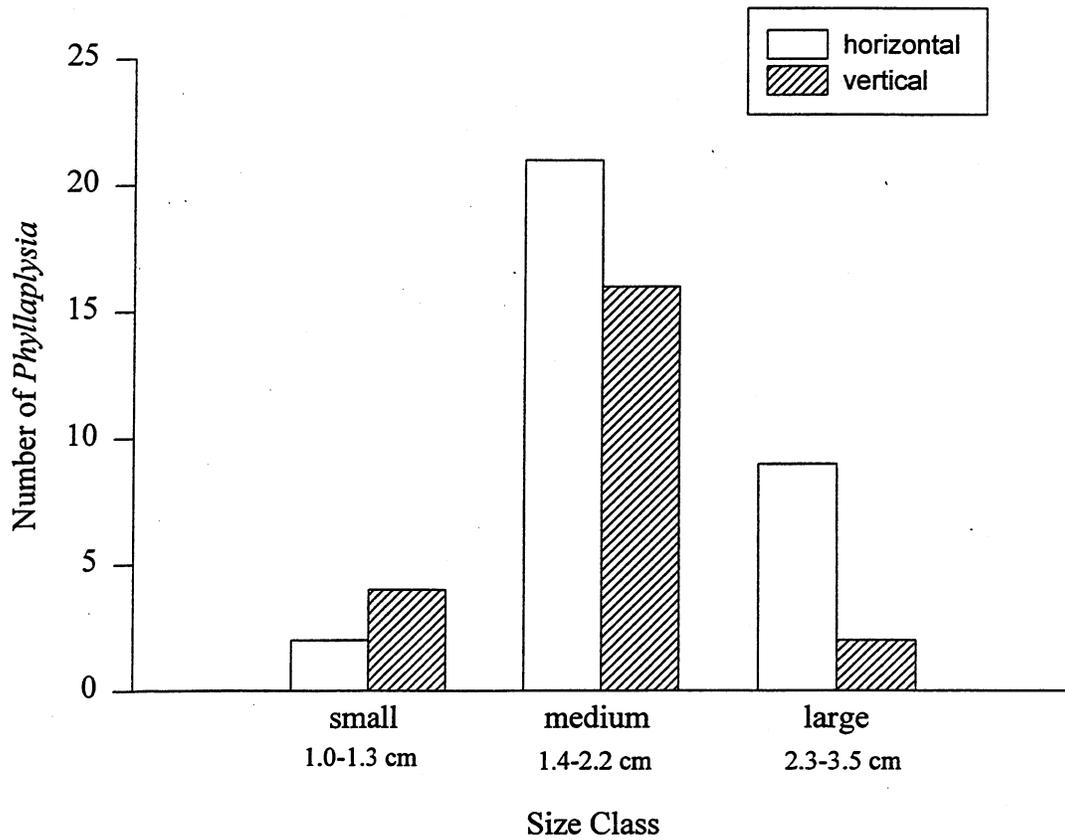


Figure 2. The distribution of three size classes of *Phyllaplysia* in reference to their choice of orientation.

Table 4. The number of *Phyllaplysia* that showed a significant preference, significant avoidance, or no significant preference in the test on color preference. Significance, determined at $\alpha=0.05$, was based on the value of the normal standard deviate calculated from residual analysis.

	Preferred	Avoided	No Preference
Green	24	13	7
Brown	11	22	11
Red	9	18	7
White	1	26	17
Black	7	18	19

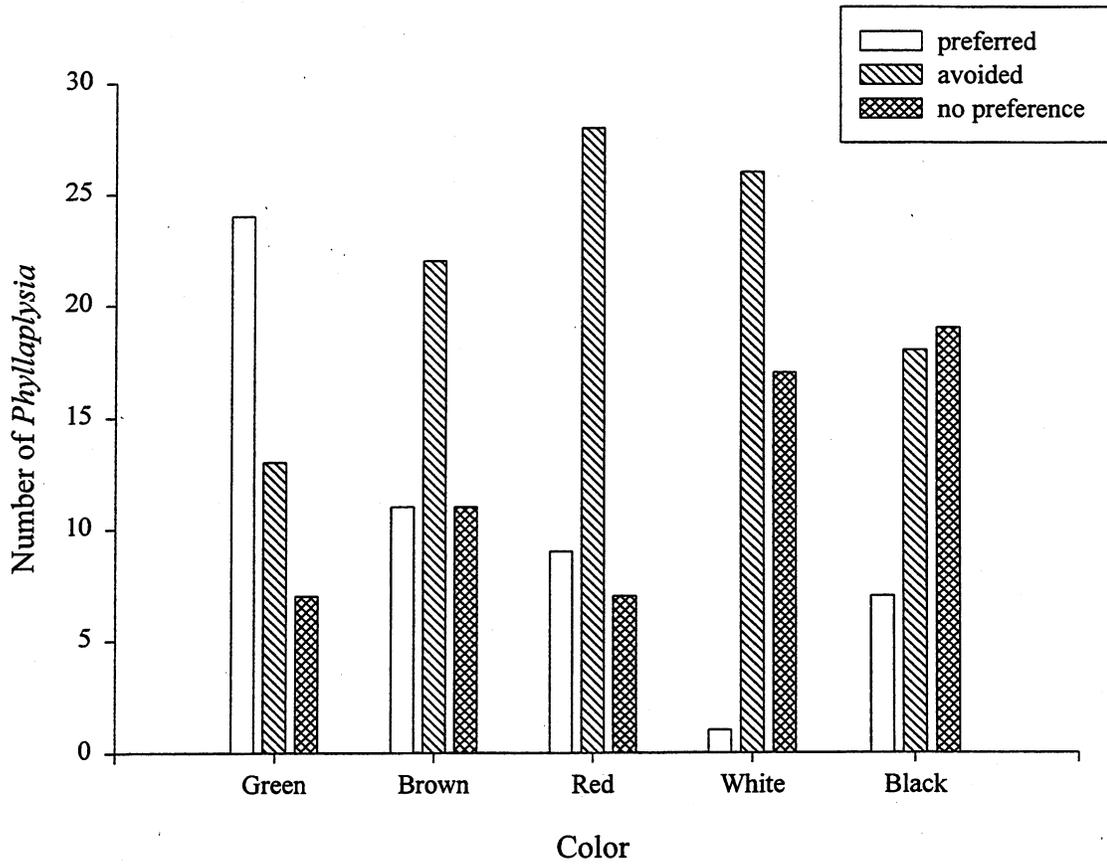


Figure 3. The number of *Phyllaplysia* that showed a response to each color.

Field Study

Epiphytic Community

Generally the epiphytic communities differed among the three sites and also showed variation over time during the sampling period. These trends were determined using a main effects analysis. Because the interaction term in the two-way ANOVA on epiphyte measurements by date and by site was significant in all tests ($p < 0.0001$), it was necessary to analyze the data separately by site and by date using a main effects analysis.

Within Sites. At each site, different measures of epiphytic biomass varied significantly over time (Table 5). At March Point, all measures of epiphytic growth (dry weight, organic content, and chlorophyll a, b, and c) showed significant variation over time. At Kirby Beach and Bayview the organic content of the epiphytes varied significantly over the sampling period. No other measurement of epiphytic growth showed significant temporal variation at these two sites.

Within Sampling Dates. Main effects were tested on ten sampling dates to determine if there were significant differences in the epiphytic communities among the three sites. (Table 6). No data on epiphytes were collected for either the first or the sixth sampling period (late March and early June). The dry weight of the epiphytes showed significant differences among sites on two sampling dates (Fig. 4). Organic content differed the most among sites throughout the sampling period (Fig. 5). Chlorophyll a, b, and c differed among sites primarily in the latter portion of the sampling period (Fig. 6).

Simple Contrasts. Because the one-way ANOVAs within sampling dates showed that significant differences existed among the three sites, simple contrasts among

Table 5. The p values calculated from main effects analysis by date on the epiphytic measurements taken from each site during the sampling period. The sequential Bonferroni test adjusted α from 0.01 to 0.0014. Significant values are shown in boldfaced type.

	March Point	Kirby Beach	Bayview
Dry Weight	<0.0001	0.0022	0.7604
Organic Content	<0.0001	<0.0001	<0.0001
Chlorophyll a	<0.0001	0.0122	0.7551
Chlorophyll b	<0.0001	1.00	1.00
Chlorophyll c	<0.0001	0.8556	0.9971

Table 6. The p values calculated from main effects analysis by site on the epiphytic measurements taken during each sample date. The sequential Bonferroni test adjusted α from 0.01 to 0.0007. Significant contrasts are shown in boldfaced type.

sample date	dry weight	organic content	chlorophyll a	chlorophyll b	chlorophyll c
early April	0.402	<0.0001	0.013	0.469	0.346
late April	0.431	0.017	0.001	0.789	0.304
early May	0.499	0.056	0.035	0.783	0.416
late May	0.0199	<0.0001	0.015	0.896	0.314
late June	0.799	<0.0001	0.143	0.721	0.524
early July	0.944	<0.0001	0.283	0.947	0.765
late July	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
early August	0.002	0.008	0.04	0.858	0.452
late August	<0.0001	<0.0001	<0.0001	0.028	0.002
early September	0.042	<0.0001	0.001	0.395	0.047

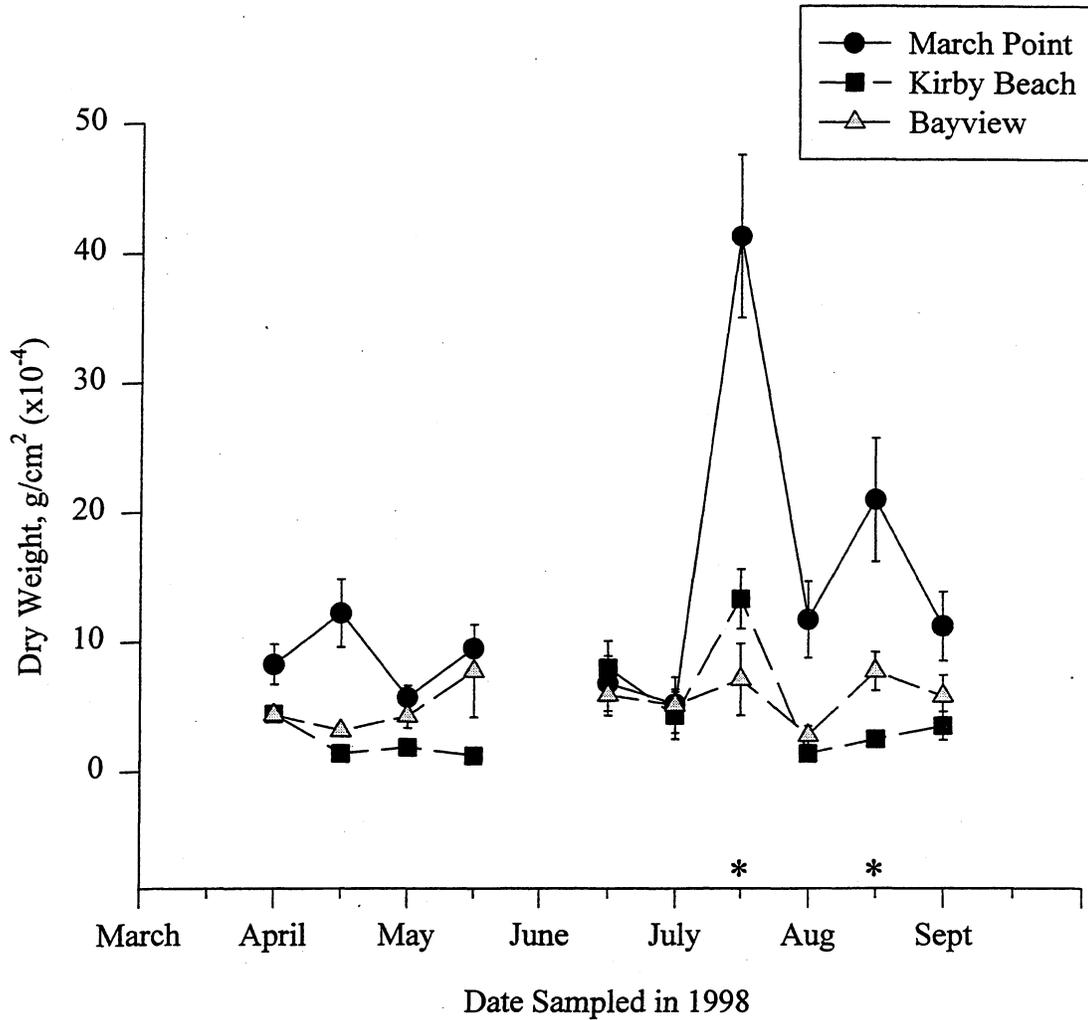


Figure 4. The dry weight (mean \pm SE) of epiphytes on the eelgrass collected from three sites in Padilla Bay during the 1998 sampling period. An asterisk above the x-axis indicates a significant difference among sites on that sampling date.

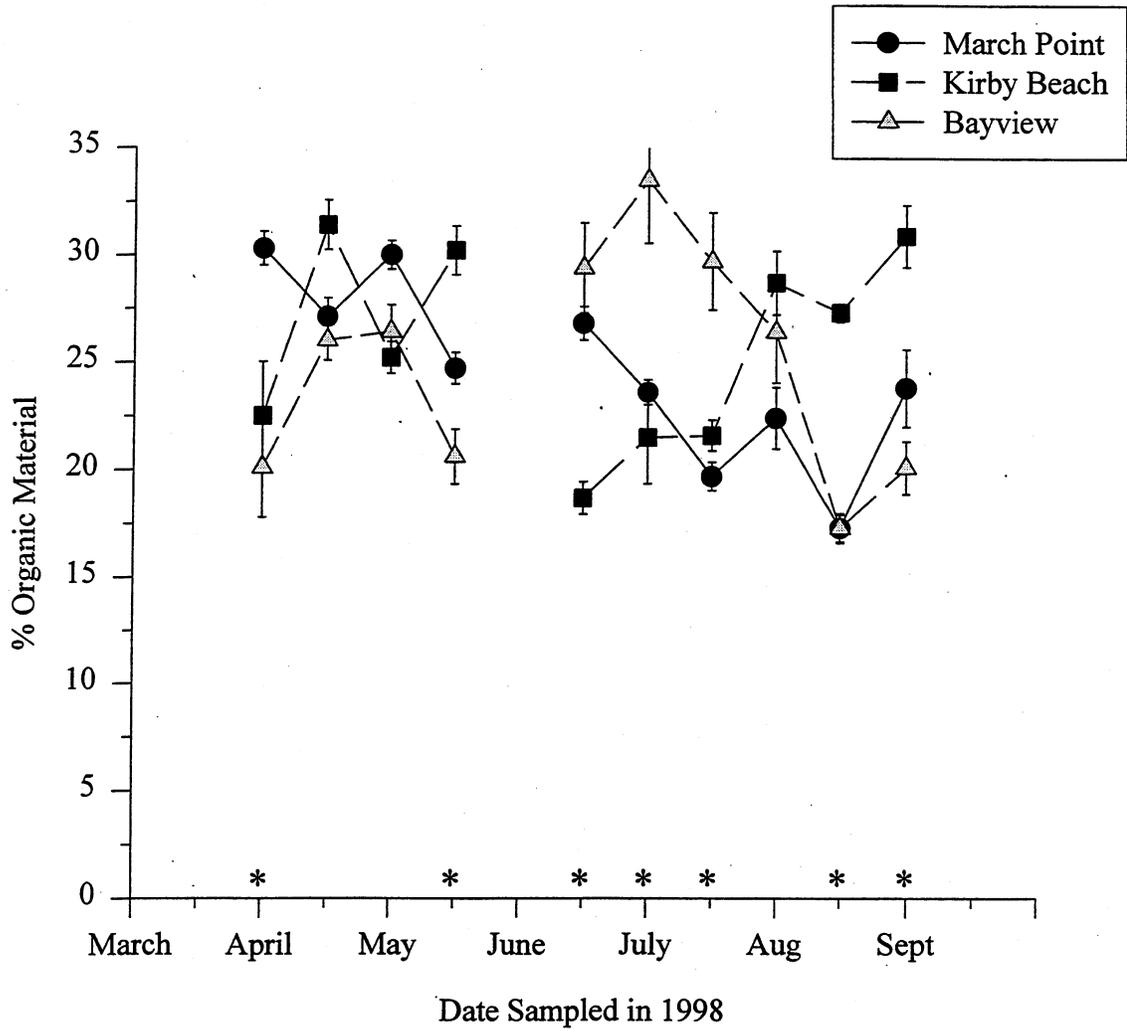


Figure 5. The percent organic material (mean \pm SE) of epiphytes on the eelgrass collected from three sites in Padilla Bay during the 1998 sampling period. An asterisk above the x-axis indicates a significant difference among sites on that sampling date.

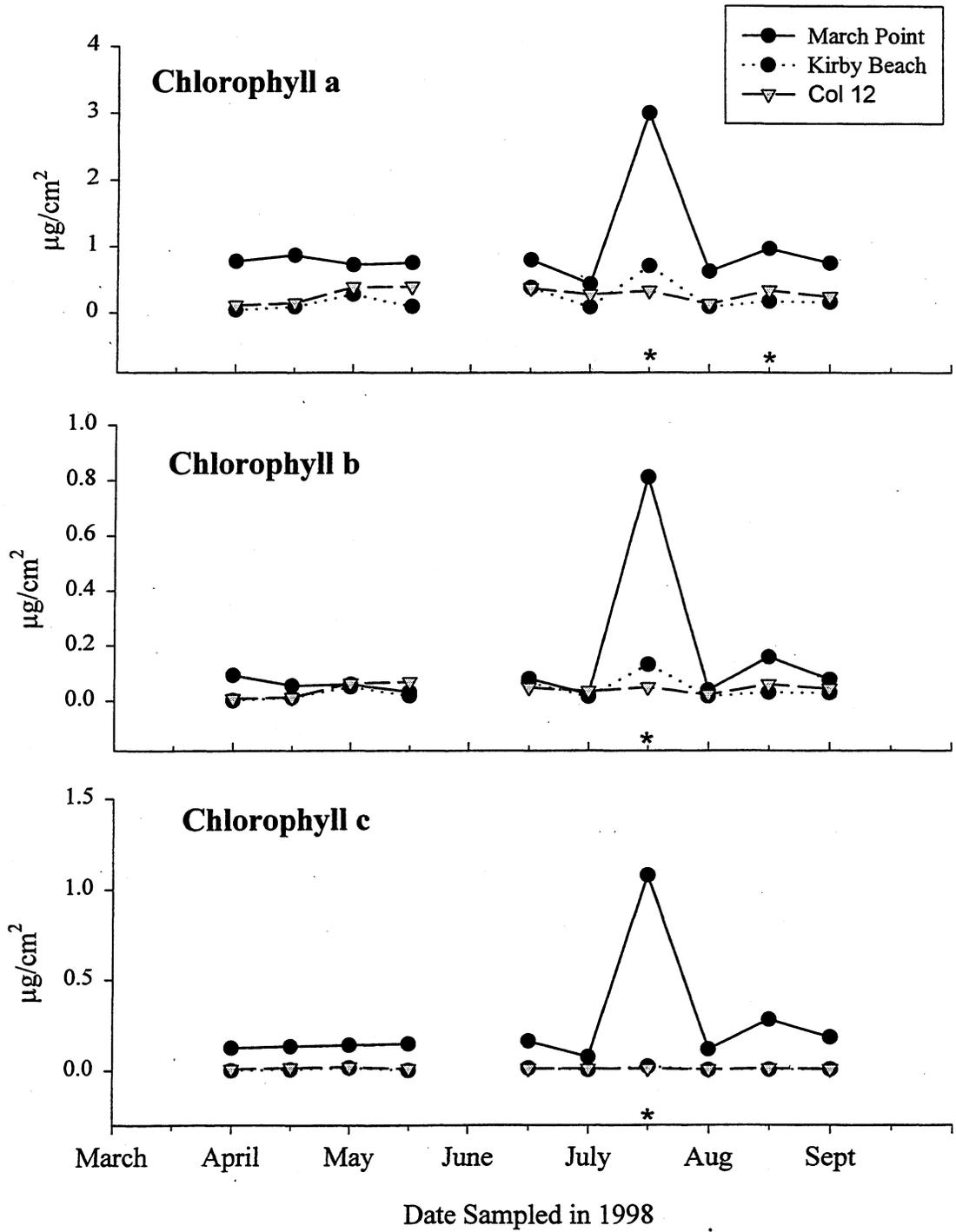


Figure 6. The content of chlorophyll a, b, and c in the epiphytes collected from three sites in Padilla Bay during the 1998 sampling period. An asterisk above the x-axis indicates a significant difference among sites at that sampling date.

the sites were done on each of the epiphytic measures to determine where these differences were (Table 7). The first contrast compared Kirby Beach to Bayview. The second and third contrasts compared March Point to Bayview and March Point to Kirby Beach respectively. The comparisons of the epiphytic measurements taken from March Point to those taken from Kirby Beach showed more significant differences than any other comparisons of sites. Kirby Beach and Bayview seemed to be the most similar with respect to characteristics of their epiphytic communities.

Chlorophyll Ratios.

Excluding the early samples taken during March, April, and May, the ratio of chlorophyll a to chlorophyll c stayed fairly low and remained fairly constant at all three sites. The ratio of chlorophyll a to chlorophyll b was generally higher and fluctuated throughout the sampling period (Fig. 7).

Species Diversity, Evenness, and Richness of the Macrofaunal Communities

The two-way ANOVAs on the species indices calculated from the distribution of the macrofauna showed that the only significant difference among sites was in species richness. Neither evenness nor diversity of species varied among the three sites (Fig. 8). The date sampled was not statistically significant for any of the measurements, showing that time had little effect on these indices during the sampling period (Table 8).

Table 7. Simple contrasts by date of epiphytic measurements. Contrasts were decided *a priori* so significance ($\alpha=0.05$) was determined with the p value calculated from the T-statistic. The adjusted values of α are shown with each of the epiphytic measurements. Significant contrasts are shown in boldfaced type

Contrast	March Point	Kirby Beach	Bayview
1	0	-1	1
2	1	0	-1
3	1	-1	0

Dry Weight / cm² ($\alpha=0.0063$)

sample date	Kirby Beach : Bayview	March Point: Bayview	March Point: Kirby Beach
early April	0.9852	0.2173	0.2242
late April	0.5718	0.0042	0.0006
early May	0.4392	0.6514	0.2208
late May	0.0381	0.5771	0.0087
late June	0.5040	0.7809	0.6964
early July	0.7970	0.9791	0.7768
late July	0.0482	<0.0001	<0.0001
early August	0.6570	0.0046	0.0011
late August	0.0944	<0.001	<0.0001
early September	0.4655	0.0860	0.0147

% Organic Content ($\alpha=0.0036$)

sample date	Kirby Beach : Bayview	March Point: Bayview	March Point: Kirby Beach
early April	0.2372	<0.0001	0.0001
late April	0.0073	0.5928	0.0310
early May	0.5440	0.0726	0.0166
late May	<0.0001	0.0438	0.0059
late June	<0.0001	0.1811	0.0001
early July	<0.0001	<0.0001	0.2868
late July	0.0001	<0.0001	0.3598
early August	0.2598	0.0491	0.0021
late August	<0.0001	0.9768	<0.0001
early September	<0.0001	0.0694	0.0005

Table 7 (cont.)

Chlorophyll a ($\alpha=0.005$)

sample date	Kirby Beach : Bayview	March Point: Bayview	March Point: Kirby Beach
early April	0.7256	0.0010	0.0003
late April	0.7794	0.0004	0.0001
early May	0.5945	0.1652	0.0553
late May	0.1355	0.0754	0.0012
late June	0.9555	0.0331	0.0379
early July	0.3393	0.4353	0.0832
late July	0.0626	<0.0001	<0.0001
early August	0.8179	0.0154	0.0080
late August	0.4137	0.0017	0.0001
early September	0.6773	0.0119	0.0034

Chlorophyll b ($\alpha=0.0167$)

sample date	Kirby Beach : Bayview	March Point: Bayview	March Point: Kirby Beach
early April	0.9220	0.1564	0.1299
late April	0.8639	0.5385	0.4318
early May	0.9570	0.5621	0.5263
late May	0.8918	0.7312	0.6316
late June	0.9711	0.2527	0.2680
early July	0.9254	0.8235	0.7515
late July	0.8431	<0.0001	<0.0001
early August	0.9733	0.5947	0.5717
late August	0.9258	0.0146	0.0113
early September	0.9828	0.2516	0.2429

Chlorophyll c ($\alpha=0.0125$)

sample date	Kirby Beach : Bayview	March Point: Bayview	March Point: Kirby Beach
early April	0.9348	0.1124	0.0951
late April	0.9900	0.0972	0.0947
early May	0.8543	0.3793	0.2880
late May	0.4987	0.2761	0.0779
late June	0.8036	0.1265	0.2003
early July	0.7880	0.5915	0.4207
late July	0.2687	<0.0001	<0.0001
early August	0.8416	0.2003	0.1758
late August	0.6952	0.0028	0.0007
early September	0.8316	0.0600	0.0370

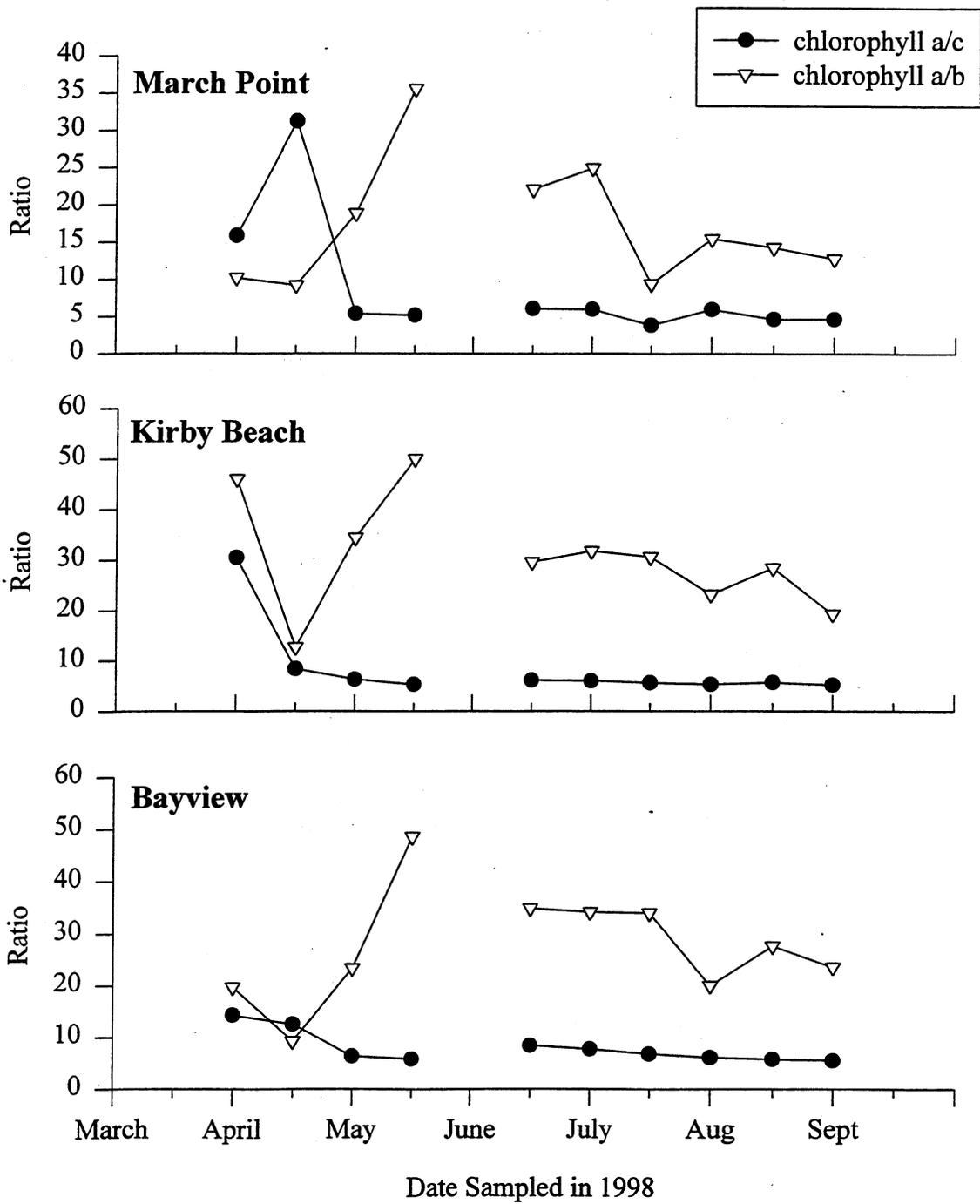


Figure 7. The ratio of chlorophyll a to c and chlorophyll a to b during the 1998 sampling period at March Point, Kirby Beach, and Bayview.

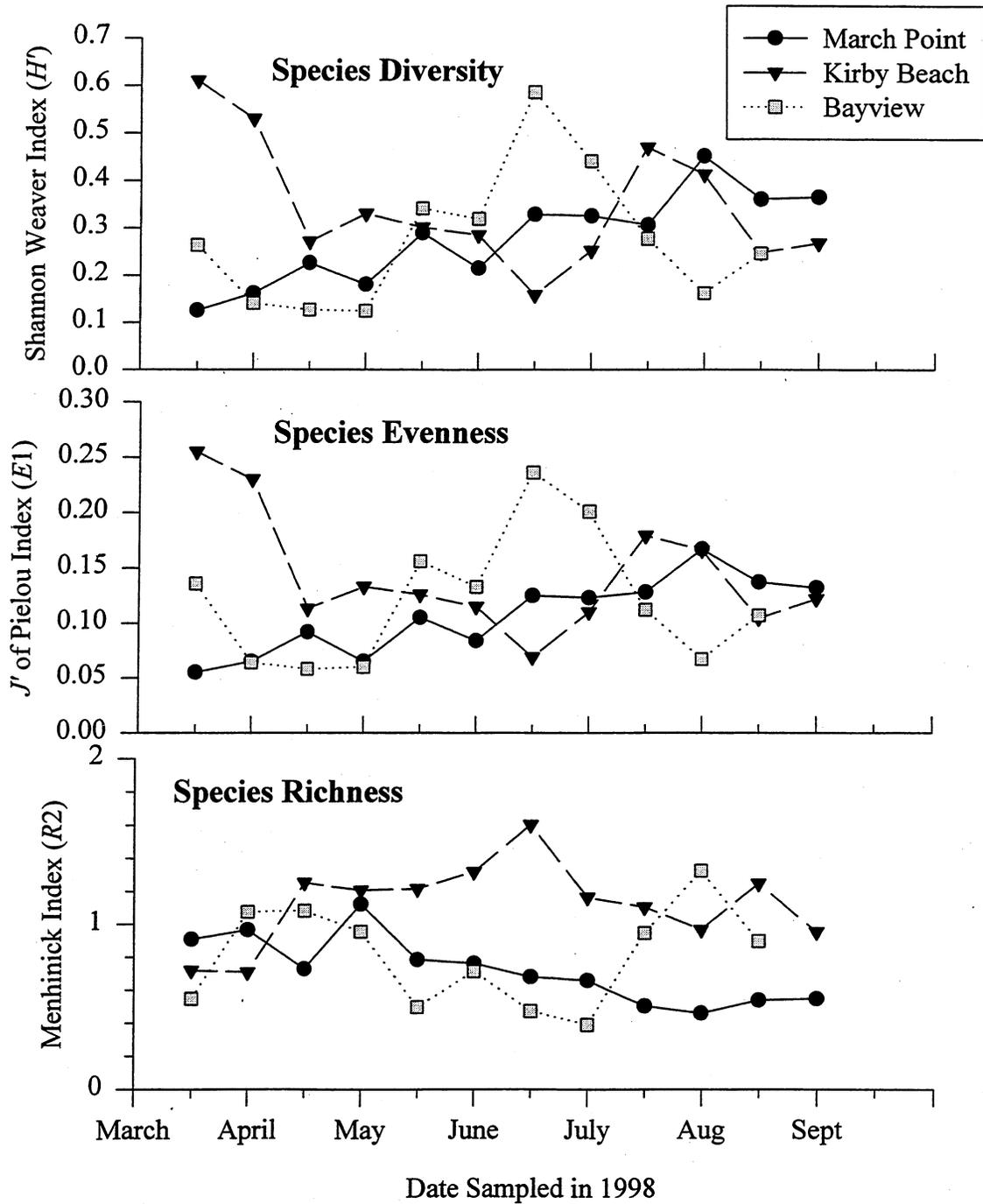


Figure 8. The changes over time in the macrofaunal species indices measured at the three sites during the 1998 sampling period. Only species richness differed significantly among the three sites.

Table 8. The p values calculated from the two-way ANOVA by site and by date on species indices. Significance was determined at $\alpha = 0.05$. Significant values are shown in boldfaced type. Because no replicates were taken, the interaction term could not be evaluated.

	Species Diversity	Species Evenness	Species Richness
Site	0.3782	0.2289	0.0023
Date	0.6131	0.5093	0.474

Comparisons of the Composition of Macrofaunal Communities on Eelgrass from the Study Sites

A cluster analysis on the samples resulted in the dendrogram shown in Figure 9. The Bray-Curtis dissimilarity index does not have a statistical basis and so cannot be tested for significance (Nichols, 1970). Therefore, the identification of specific groups after clustering is a subjective decision (Ludwig and Reynolds, 1988; Hughes and Thomas, 1971). While dividing too finely can lead to uninterpretable results, too broad a division makes it hard to find characteristics unique to the groups. For these reasons, a distance of 0.4 was used as a reference point for dividing the dendrogram into nine groups.

Tables 9, 10, and 11 give the densities of the 28 species identified in the samples. The presence or absence as well as the relative densities of the species were the determining factors in the formation of these nine groups (Table 12). For example, in the samples clustered together in group 4 (samples taken from March Point from late July through early September), the nereid polychaete and *Phyllaplysia taylori* occur in higher densities than in any of the other samples. Also, the low numbers of *Caprella californica* and *Idotea resicata* separate the samples in this group from the other samples. Conversely, group 7 (samples taken from Bayview from late May through late July), is characterized by samples containing low densities of the nereid polychaete and high densities of *Caprella californica*.

In most cases, dividing this dendrogram at a distance of 0.4 gives a clear distinction among the groups. The separation between groups 5 and 6 is not as definite, being joined at a distance only slightly greater than 0.4. Therefore, sample 10, taken

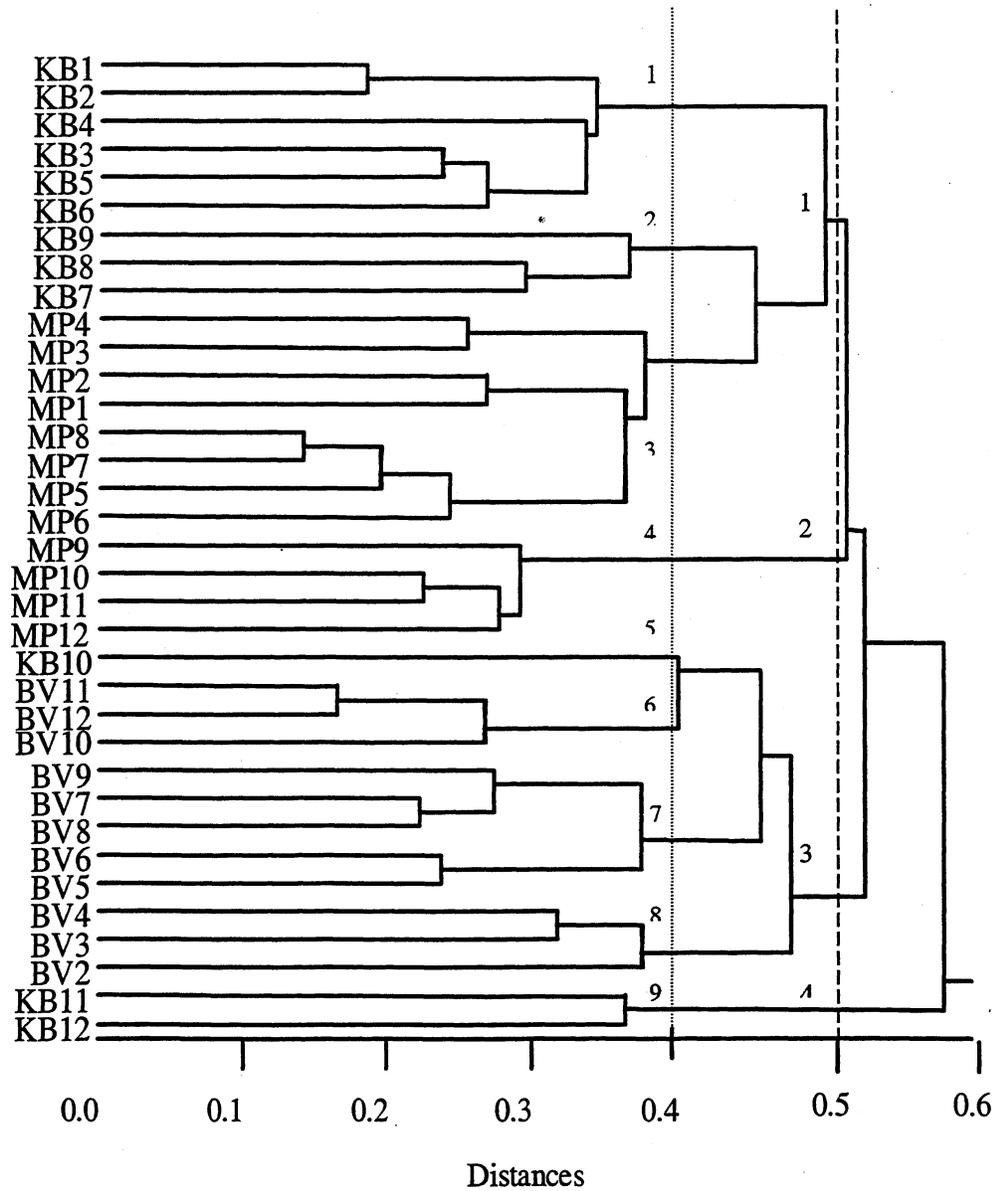


Figure 9. The dendrogram resulting from the clustering of samples taken from the three sites during the sampling period with respect to species densities. The labels on the y axis represent the twelve sampling periods (1-12) at each of the three sites (MP=March Point, KB=Kirby Beach, BV=Bayview). The first division is at a distance of 0.4 and results in 9 groups. The second, broader division is at a distance of 0.5 and results in 4 groups.

Table 9. The number of organisms collected per square meter of *Zostera* at March Point.

sample date sampled	1 late March	2 early April	3 late April	4 early May	5 late May	6 early June	7 late June	8 early July	9 late July	10 early Aug	11 late Aug	12 early Sept
Phylum Platyhelminthes												
<i>Phylloplana viridis</i>	6.4	5.1		1.3	2.7		2.8	1.4	5.2	9.0	14.5	6.2
Phylum Cnidaria												
<i>Epiactis prolifera</i>												
Phylum Mollusca												
<i>Alia carinata</i>	1.3	3.4		1.3	1.4	1.3	2.8	2.8				
<i>Lacuna variegata</i>	38.4	56.2	8.6	15.0	363.4	199.2	214.0	329.2	130.4	43.3	7.3	19.7
<i>Lottia alveus</i>	1.3		2.9	1.3		1.3				7.5	7.3	1.2
<i>Phyllaplysia taylori</i>	2.6			2.5					20.9	73.2	97.0	13.5
<i>Haminoea vesicula</i>					8.2	10.3	21.1	16.6	5.2	3.0		
<i>Hermisenda crassicornis</i>										7.5		1.2
<i>Hermea vancouverensis</i>		5.1	2.9		1.4		4.2	1.4	2.6			
<i>Eubranchus olivaceus</i>												
<i>Melibe leonina</i>												
Juvenile Bivalve										1.5	1.2	1.2
Phylum Nemertea												
Green Nemertean										1.5	1.2	2.5
Phylum Annelida												
Nereid	51.2	46.0	11.5	6.3	13.7	10.3	108.4	123.1	384.8	1351.8	655.9	961.3
Polychaete 2	1.3	8.5						1.4			3.6	6.2
Hesionid		3.4	1.4	2.5		1.3		1.4				1.2
Polychaete 4		1.7										
<i>Lepidonotus squamatus</i>			8.6	7.5	2.7	3.9	4.2	2.8				
Terebellidae				1.3	2.7					3.0	1.2	1.2
Phylum Arthropoda												
<i>Idotea resicata</i>	10.2	13.6	5.7	11.3	12.3	9.0	5.6	11.1	3.9	3.0	2.4	2.5
<i>Caprella californica</i>	16.6	63.1	202.3	149.1	34.2	3.9	136.6	49.8	2.6	6.0		2.5
<i>Metacaprella anomala</i>				8.8	5.5	1.3	4.2					
<i>Isochrocerus anguipes</i>	25.6	27.3	70.3	17.5	87.4	105.4	71.8	55.3	32.6	34.4	13.3	13.5
<i>Corophium</i> sp.												4.9
<i>Isochrocerus</i> sp.		29.0	70.3	10.0	2.7							
Green Amphipod			1.4		1.4	1.3	1.4			4.5	2.4	3.7
Barnacle			4.3	5.0	4.1		2.8	9.7	5.2	16.4	6.1	
Phylum Echinodermata												
<i>Leptasterias hexactis</i>				3.8	4.1	2.6	1.4	1.4	1.3		1.2	
Total number of species	10	12	12	16	16	13	14	14	11	15	14	16
Total number of individuals	154.7	262.5	390.2	244.4	547.8	350.9	581.5	607.2	594.8	1565.4	814.7	1042.5

Table 10. The number of organisms collected per square meter of *Zostera* at Kirby Beach

sample date sampled	1	2	3	4	5	6	7	8	9	10	11	12
	late March	early April	late April	early May	late May	early June	late June	early July	late July	early Aug	late Aug	early Sept
Phylum Platyhelminthes												
<i>Phylloplana viridis</i>	96.5	36.4	24.8	24.5	10.4	5.7		2.2	4.0	100.6	112.0	155.4
Phylum Cnidaria												
<i>Epiactis prolifera</i>		2.3		3.5								
Phylum Mollusca												
<i>Alia carinata</i>	280.2	209.6	7.6	66.5	17.9	22.9		11.2	8.1	2.2		
<i>Lacuna variegata</i>	80.9	41.0	28.6	14.0	10.4	7.2	5.9	4.5		4.5		
<i>Lottia alveus</i>	115.2	36.4	45.8	26.3	17.9	5.7	3.9	2.2	4.0		7.8	17.3
<i>Phyllaplysia taylori</i>	6.2		1.9							2.2	2.6	
<i>Haminoea vesicula</i>					3.0	5.7					2.6	14.4
<i>Hermisenda crassicornis</i>	9.3								12.1			
<i>Hermea vancouverensis</i>												
<i>Eubranchus olivaceus</i>												
<i>Melibe leonina</i>											5.2	
Juvenile Bivalve												
Phylum Nemertea												
Green Nemertean									6.1			5.8
Phylum Annelida												
Nereid	40.5	22.8	3.8	8.8	6.0	2.9	21.7	69.7	74.7	154.3	39.1	23.0
Polychaete 2				1.8							2.6	
Hesionid		2.3	7.6	12.3	14.9	7.2	3.9	18.0	4.0	4.5		11.5
Polychaete 4												
<i>Lepidonotus squamatus</i>				3.5		2.9	2.0	2.2	2.0			
Terebellidae	3.1								2.0	2.2		
Phylum Arthropoda												
<i>Idotea resecata</i>	80.9	72.9	17.2	7.0	22.3	12.9	2.0		16.2	8.9	7.8	
<i>Caprella californica</i>	3.1	15.9	1.9	1.8	6.0	27.2	15.8	22.5	127.2		2.6	8.6
<i>Metacaprella anomala</i>							5.9		38.4			
<i>Isochyrocerus anguipes</i>	12.5	13.7	5.7		6.0	8.6	13.8	29.2	24.2	4.5		2.9
<i>Corophium</i> sp.										51.4	18.2	14.4
<i>Isochyrocerus</i> sp.				3.5								
Green Amphipod			1.9		6.0	1.4	2.0	4.5	2.0	6.7	2.6	
Barnacle										2.2		
Phylum Echinodermata												
<i>Leptasterias hexactis</i>												
Total number of species	11	10	11	12	11	12	10	10	14	12	11	9
Total number of individuals	728.5	453.3	146.8	173.3	120.5	110.3	77.0	166.3	325.1	344.3	203.2	253.2

Table 11. The number of organisms collected per square meter of *Zostera* at Bayview.

sample date sampled	1	2	3	4	5	6	7	8	9	10	11	12
	late March	early April	late April	early May	late May	early June	late June	early July	late July	early Aug	late Aug	early Sept
Phylum Platyhelminthes												
<i>Phylloplana viridis</i>		140.8	70.8	35.7	16.3	13.2	3.4	3.8		1.7	11.3	12.4
Phylum Cnidaria												
<i>Epiactis prolifera</i>			1.9									
Phylum Mollusca												
<i>Alia carinata</i>						1.9						
<i>Lacuna variegata</i>							1.7	19.2	1.9			
<i>Lottia alveus</i>		4.0								3.4	1.9	1.8
<i>Phyllaplysia taylori</i>		8.0	1.9	4.7	5.4		1.7	32.7	9.7	5.1	3.8	8.9
<i>Haminoea vesicula</i>				1.6	5.4	5.7	8.4	5.8				
<i>Hermisenda crassicornis</i>										1.7		
<i>Hermea vancouverensis</i>												
<i>Eubranchus olivaceus</i>							1.7	7.7				
<i>Melibe leonina</i>												
Juvenile Bivalve												
Phylum Nemertea												
Green Nemertean										1.7		
Phylum Annelida												
Nereid		5.3	3.8			1.9	13.5	32.7	7.7	25.7	13.2	78.0
Polychaete 2												
Hesionid		2.7		1.6	1.8	1.9	1.7	9.6				
Polychaete 4												
<i>Lepidonotus squamatus</i>						1.9		3.8			1.9	
Terebellidae										5.1	1.9	1.8
Phylum Arthropoda												
<i>Idotea resecata</i>		19.9	17.2	24.9	10.8	62.2	28.6	55.7	40.6	15.4	7.6	5.3
<i>Caprella californica</i>		37.2	3.8	4.7	43.3	427.5	291.1	982.3	926.8	169.9	34.0	56.7
<i>Metacaprella anomala</i>				7.8	1.8							
<i>Isochyrocerus anguipes</i>			26.8	4.7	14.4	15.1	20.2	50.0	27.1	1.7	22.7	17.7
<i>Corophium</i> sp.										30.9	5.7	30.1
<i>Isochyrocerus</i> sp.			1.9	1.6					7.7			
Green Amphipod			1.9				10.1	11.5	3.9	13.7	26.5	7.1
Barnacle									1.9			
Phylum Echinodermata												
<i>Leptasterias hexactis</i>												
Total number of species	0	7	9	9	8	9	11	12	9	12	11	10
Total number of individuals	0	218	130.1	86.98	99.3	531.1	382	1215	1027	276.29	130	219.79

Table 12. The mean number of individuals of each species per square meter of *Zostera* in each of the nine groups as designated by cluster analysis. KB=Kirby Beach, MP=March Point, BV=Bayview.

group	1	2	3	4	5	6	7	8	9
samples	KB 1-6	KB 7-9	MP 1-8	MP 9-12	KB 10	BV 10-12	BV 5-9	BV 2-4	KB 11-12
species									
Phylum Platyhelminthes									
<i>Phylloplana viridis</i>	33.1	2.1	2.5	8.7	100.6	8.5	7.3	82.4	133.7
Phylum Cnidaria									
<i>Epiactis prolifera</i>	1.0							0.6	
Phylum Mollusca									
<i>Alia carinata</i>	100.8	6.4	1.8		2.2		0.4		
<i>Lacuna variegata</i>	30.4	3.5	153.0	50.2	4.5		4.6		
<i>Lottia alveus</i>	41.2	3.4	0.8	4.0		2.4		1.3	12.5
<i>Phyllaplysia taylori</i>	1.4		0.6	51.1	2.2	5.9	9.9	4.8	1.3
<i>Haminoea vesicula</i>	1.5		7.0	2.1			5.0	0.5	8.5
<i>Hermissenda crassicornis</i>	1.6	4.0		2.2		0.6			
<i>Hermea vancouverensis</i>			1.9	0.7					
<i>Eubranchus olivaceus</i>							1.9		
<i>Melibe leonina</i>									2.6
Juvenile Bivalve				1.0					
Phylum Nemertea									
Green Nemertean		2.0		1.3		0.6			2.9
Phylum Annelida									
Nereid	14.1	55.4	46.3	838.5	154.3	39.0	11.2	3.0	31.0
Polychaete 2	0.3		1.4	2.4					1.3
Hesionid	7.4	8.7	1.3	0.3	4.5		3.0	1.4	5.8
Polychaete 4			0.2						
<i>Lepidonotus squamatus</i>	1.1	2.1	3.7			0.6	1.1		
Terebellidae	0.5	0.7	0.5	1.4	2.2	2.9			
Phylum Arthropoda									
<i>Idotea resecata</i>	35.5	6.0	9.9	2.9	8.9	9.4	39.6	20.7	3.9
<i>Caprella californica</i>	9.3	55.2	81.9	2.8		86.9	534.2	15.2	5.6
<i>Metacaprella anomala</i>		14.8	2.5				0.4	2.6	
<i>Isochyrocerus anguipes</i>	7.7	22.4	57.6	23.5	4.5	14.0	25.4	10.5	1.4
Corophium sp.				1.2	51.4	22.2			16.3
<i>Isochyrocerus</i> sp.	0.6		14.0				1.5	1.2	
Green Amphipod	1.5	2.8	0.7	2.6	6.7	15.8	5.1	0.6	1.3
Barnacle			3.2	6.9	2.2		0.4		
Phylum Echinodermata									
<i>Leptasterias hexactis</i>			1.7	0.6					

from Kirby Beach in early August is closely connected with the last three samples taken from Bayview in August and September, and probably belongs in that group instead of its own separate group.

A broader division of this dendrogram at a distance of 0.5 forms four major clusters (Fig. 9). The first cluster consists of the first nine samples taken from Kirby Beach (March – July) and the first eight samples taken from March Point (March – early July). The second cluster contains the remaining samples taken from March Point (late July – early September). The third cluster is composed of all samples taken from Bayview and the sample taken in early August (sample 10) from Kirby Beach. The third cluster contains the last two samples (samples 10 and 11) taken from Kirby Beach in late August and early September.

Distribution of *Phyllaplysia taylori*

The two-way ANOVA on the distribution of *Phyllaplysia* was significant in both factors (site and date) and the interaction term ($p=0.0001$). Therefore, a main effects analysis was used to determine the significance of the individual factors. Only the densities of *Phyllaplysia* collected at March Point showed significant temporal variation ($p<0.0001$). Samples taken from July through September were tested for significant differences among sites. The samples taken in early July, early August, and late August had significant differences in the densities of *Phyllaplysia* (Table 13, Fig. 10). The simple contrasts (Table 14) indicate that March Point was significantly different from Kirby Beach and Bayview for both samples taken in August, Kirby Beach was significantly different from Bayview for the sample taken in early July, and March Point

Table 13. The p values calculated from main effects analysis by site of the distribution of *Phyllaplysia taylori* during July through September. The sequential Bonferroni adjusted α from 0.05 to 0.017. Significant values are in boldfaced type.

date	p
early July	0.00738
late July	0.261
early August	<0.0001
late August	<0.0001
early September	0.564

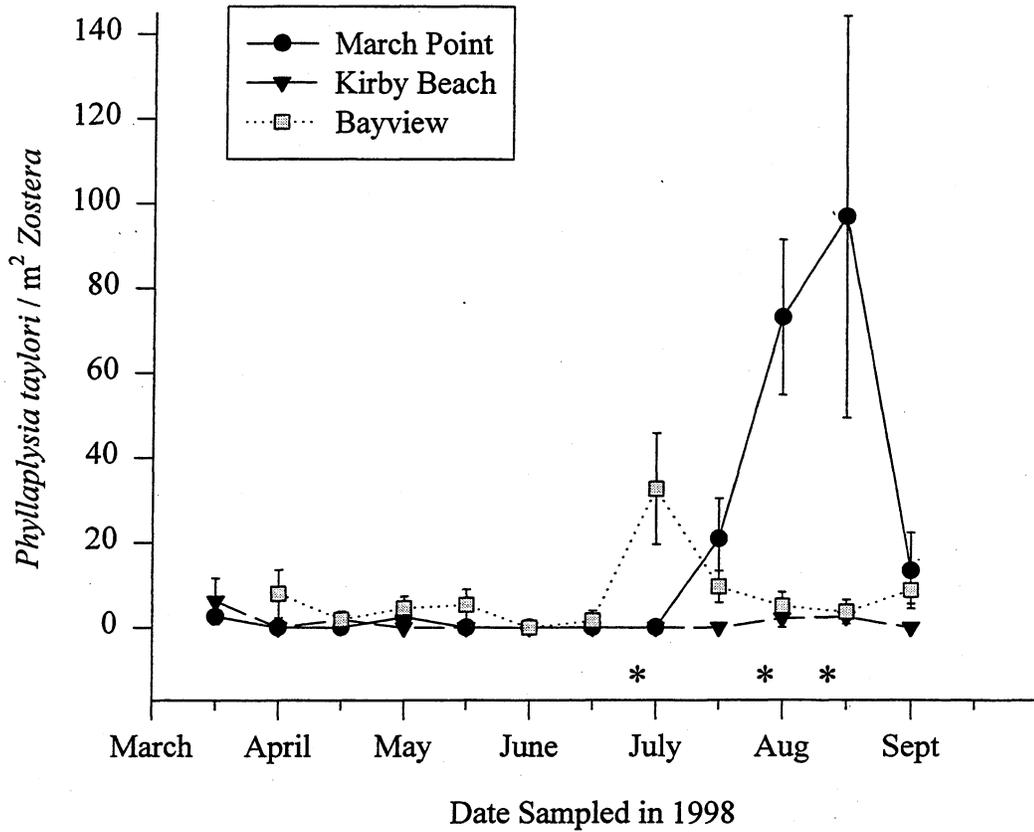


Figure 10. The density of *Phyllaplysia* (mean \pm SE) on eelgrass collected during the sampling period of 1998. An asterisk above the x-axis indicates a significant difference among sites on that sampling date.

Table 14. Simple contrasts by date of the distribution of *Phyllaplysia* among the sites. Because contrasts were determined *a priori*, the p value is calculated from the T-statistic. The sequential Bonferroni test adjusted α from 0.05 to 0.0083. Significant values are in boldfaced type.

Contrast	March Point	Kirby Beach	Bayview
1	2	-1	-1
2	0	-1	1
3	1	0	-1

	March Point: Kirby Beach & Bayview	Kirby Beach: Bayview	March Point: Bayview
early July	0.115	0.0065	0.0065
early August	<0.0001	0.8522	<0.0001
late August	<0.0001	0.8501	<0.0001

was significantly different from Bayview for samples taken in early July and early and late August.

Yearly Trends in Populations of *Phyllaplysia*

The sampling procedure followed in 1997 was not as rigid and did not involve the determination of the surface area of the eelgrass collected. Therefore, only the numbers of organisms, and not the densities, were recorded during the preliminary sampling and precise comparisons between the two years cannot be made. However, general trends are apparent. Preliminary sampling from 1997 indicated that while almost no *Phyllaplysia* were collected from March Point, Kirby Beach and Bayview had very high numbers in mid-July (Fig. 11). During the sampling period of 1998, the density of *Phyllaplysia* at Bayview was relatively steady with a small peak during early July. Although this site did not show significant variation over time, simple contrasts of this site indicated that this peak was significant. At March Point, the peak in *Phyllaplysia* (August) was much more pronounced, and, during the 1998 sampling period, much higher numbers of *Phyllaplysia* were collected. Very few *Phyllaplysia* were collected from Kirby Beach during 1998.

Correlations

Correlations were run to determine any general associations between a number of factors and the density of *Phyllaplysia*. The data set contained a number of zeroes. Not only can this cause spurious results in correlations, but it also causes the assumption of homogeneous variances to be violated. Therefore, a non-parametric test for correlations, Spearman's Rank Correlation, was used to determine the magnitude of the correlations

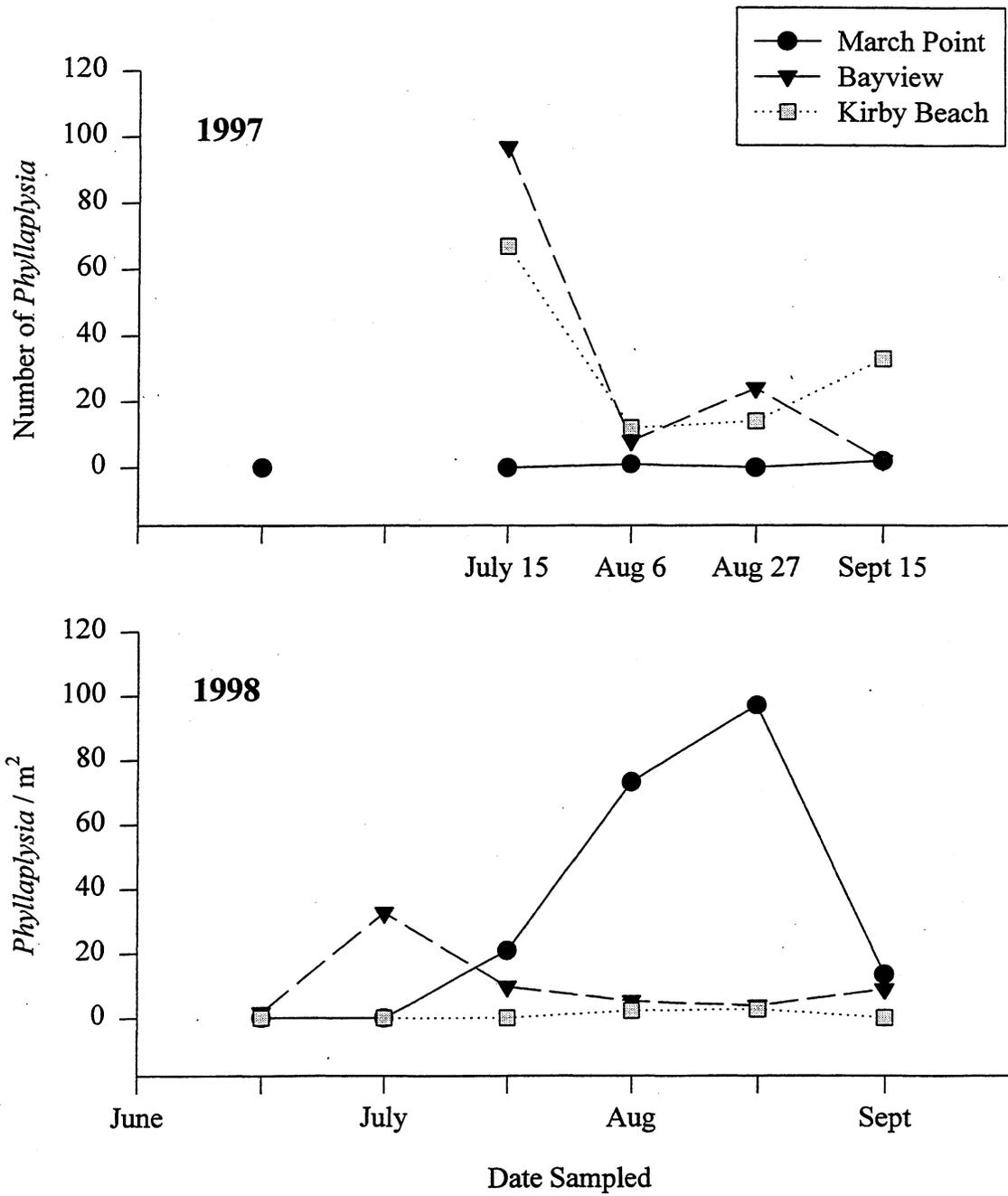


Figure 11. The abundance of *Phyllaplysia* collected from three sites in Padilla Bay during the 1997 and 1998 sampling periods.

that existed within the data set. Using this method, the degree of correlation was calculated from the ranked scores of the data instead of the actual data.

Because inherent differences exist among the three sites, the sites were analyzed separately for correlations. Kirby Beach was not analyzed because numbers of *Phyllaplysia* collected from this site were negligible. In general, correlation coefficients were low.

March Point. Some of the strongest correlations, both positive and negative, occurred at March Point (Tables 15 and 16). At March Point *Phyllaplysia* was negatively correlated only with the organic content of the epiphytes (Fig. 12).

With respect to other species of macrofauna, *Phyllaplysia* had a significant positive correlation with one other gastropod, *Lottia alveus*, and was also positively correlated with *Phylloplana viridis*, and the nereid polychaete (Fig. 13). *Phyllaplysia* showed a significant negative correlation with one species of gastropod, *Lacuna variegata*, and with two species of amphipods, *Isochyrocerus anguipes* and *Caprella californica* (Fig. 14).

Bayview. The correlations with the density of *Phyllaplysia* that were found at Bayview were not as strong (Tables 15 and 16). *Phyllaplysia* density did not fluctuate as dramatically at Bayview as it did at March Point. The peaks in population were not as pronounced. *Phyllaplysia* was also found more regularly at this site. Therefore, it was harder to find strong correlations with other factors that did have a greater degree of fluctuation. The only significant positive correlation found between *Phyllaplysia* and the different epiphytic parameters was with the organic content of the epiphytes.

Table 15. Correlation coefficients calculated from Spearman's Rank Correlations for the relationship between *Phyllaplysia* and the epiphytic parameters measured at March Point and Bayview. Significance was determined at $\alpha=0.05$ ($r=0.178$). Significant values are shown in boldfaced type.

	March Point	Bayview
Dry Weight	0.097	-0.095
Organic Content	-0.439	0.212
Chlorophyll a	-0.008	-0.127
Chlorophyll b	0.015	-0.293
Chlorophyll c	0.036	-0.127

Table 16. Correlation coefficients calculated from Spearman's Rank Correlations for the relationship between *Phyllaplysia* and the other species of macrofauna on eelgrass collected at March Point and Bayview. Significance was determined at $\alpha=0.05$ ($r=0.168$). Significant values are shown in boldfaced type.

	March Point	Bayview
<i>Alia carinata</i>	-0.14	-0.049
<i>Haminoea vesicula</i>	-0.103	-0.058
<i>Lacuna variegata</i>	-0.227	0.172
<i>Lottia alveus</i>	0.19	0.098
<i>Phylloplana viridis</i>	0.373	-0.11
<i>Isochyrocerus anguipes</i>	-0.205	0.034
<i>Isochyrocerus</i> sp.	0.113	-0.005
Green Amphipod	0.122	-0.015
<i>Caprella californica</i>	-0.357	0.114
<i>Idotea resicata</i>	-0.113	0.005
Nereid sp.	0.534	0.079
Hesionid sp.	-0.124	0.038

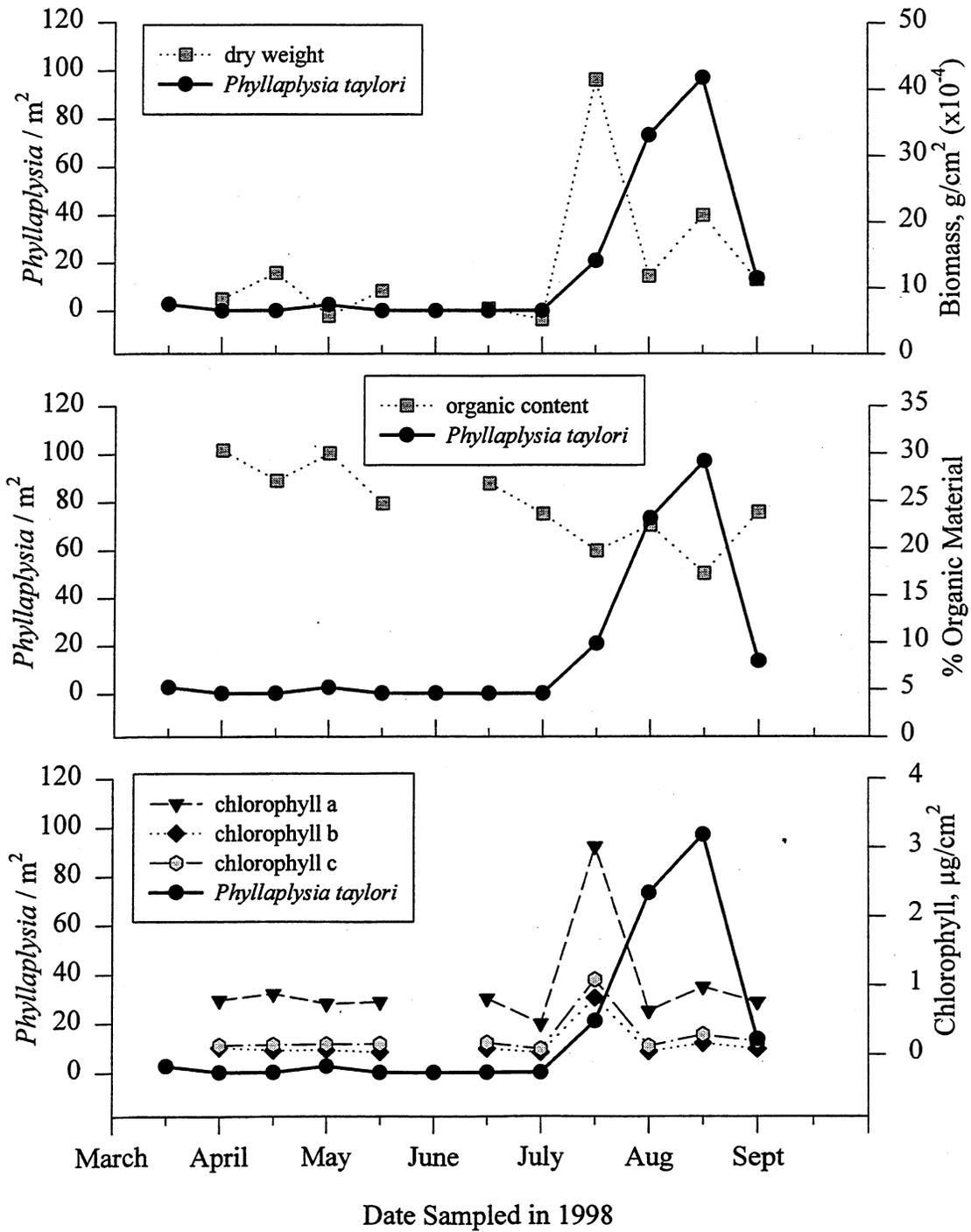


Figure 12. The relationship between *Phyllaplysia* and the measurements of epiphytic biomass at March Point during 1998. *Phyllaplysia* showed a significant negative correlation with the percent organic material of the epiphytes.

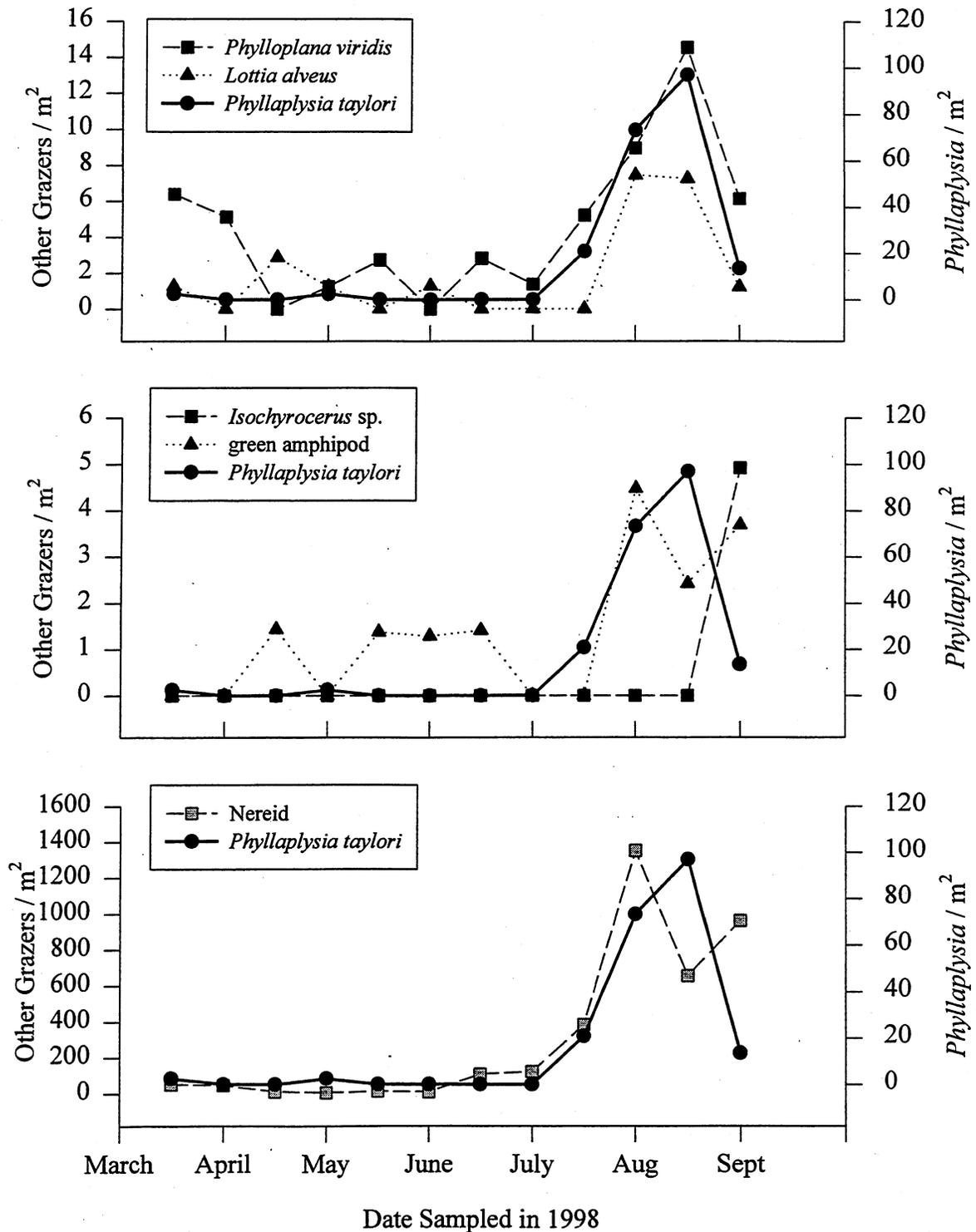


Figure 13. The relationships between *Phyllaplysia* and other species of macrofauna on eelgrass collected at March Point during 1998. Significant positive correlations occur between *Phyllaplysia* and three other species: *Lottia*, *Phylloplana*, and *Nereid*.

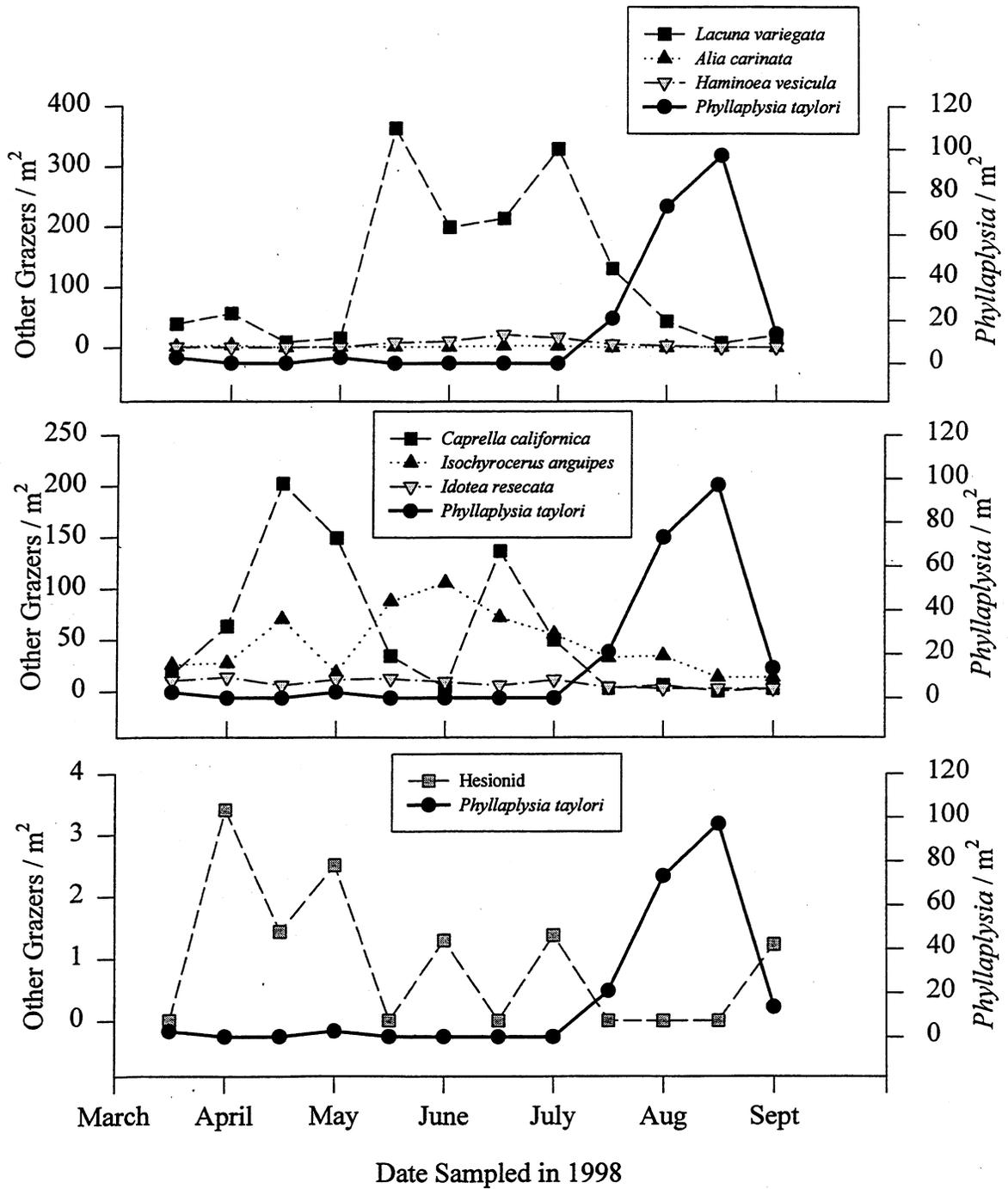


Figure 14. The relationships between *Phyllaplysia* and other species of macrofauna collected on eelgrass at March Point during 1998. Significant negative correlations occur between *Phyllaplysia* and three other species: *Lacuna*, *Isochyrocerus*, and *Caprella*.

Chlorophyll b was negatively correlated with the density of *Phyllaplysia* (Fig. 15). No species had a significant negative correlation with *Phyllaplysia* (Fig. 16). One species of macrofauna, *Lacuna variegata*, showed a significant positive correlation with *Phyllaplysia* at this site (Fig. 17).

Overall, the correlations that existed at March Point did not correspond with those at Bayview. While organic content was negatively correlated with *Phyllaplysia* density at March Point, these two factors were positively correlated at Bayview. Correlations between *Phyllaplysia* and *Lacuna* were also reversed at these two sites.

Trends in Epiphytic Biomass and Macrofaunal Density

Of the 28 species of macrofauna identified in the samples, eight species seemed to potentially have the most influence on the epiphytic biomass of the eelgrass collected from the three sites I sampled. Some of these species were prevalent at all sites while others appeared to have an impact on biomass at only one of the sites. Although there were no significant correlations between the dry weight of the epiphytes and the densities of these species of macrofauna (Table 17), some patterns do emerge.

March Point. In the most samples taken from late April through early July, the numbers of *Isochyrocerus anguipes* were high. The biomass of the epiphytes also remained low during this period and did not peak until late July (Fig. 18). Likewise, *Lacuna* density could also contribute to this trend, the highest numbers of *Lacuna* being collected from late May through July. In mid July, the epiphytic biomass shows a substantial peak. During this time none of these species were collected in high densities. The most dramatic decline in epiphytic biomass at this site occurred in early August. The

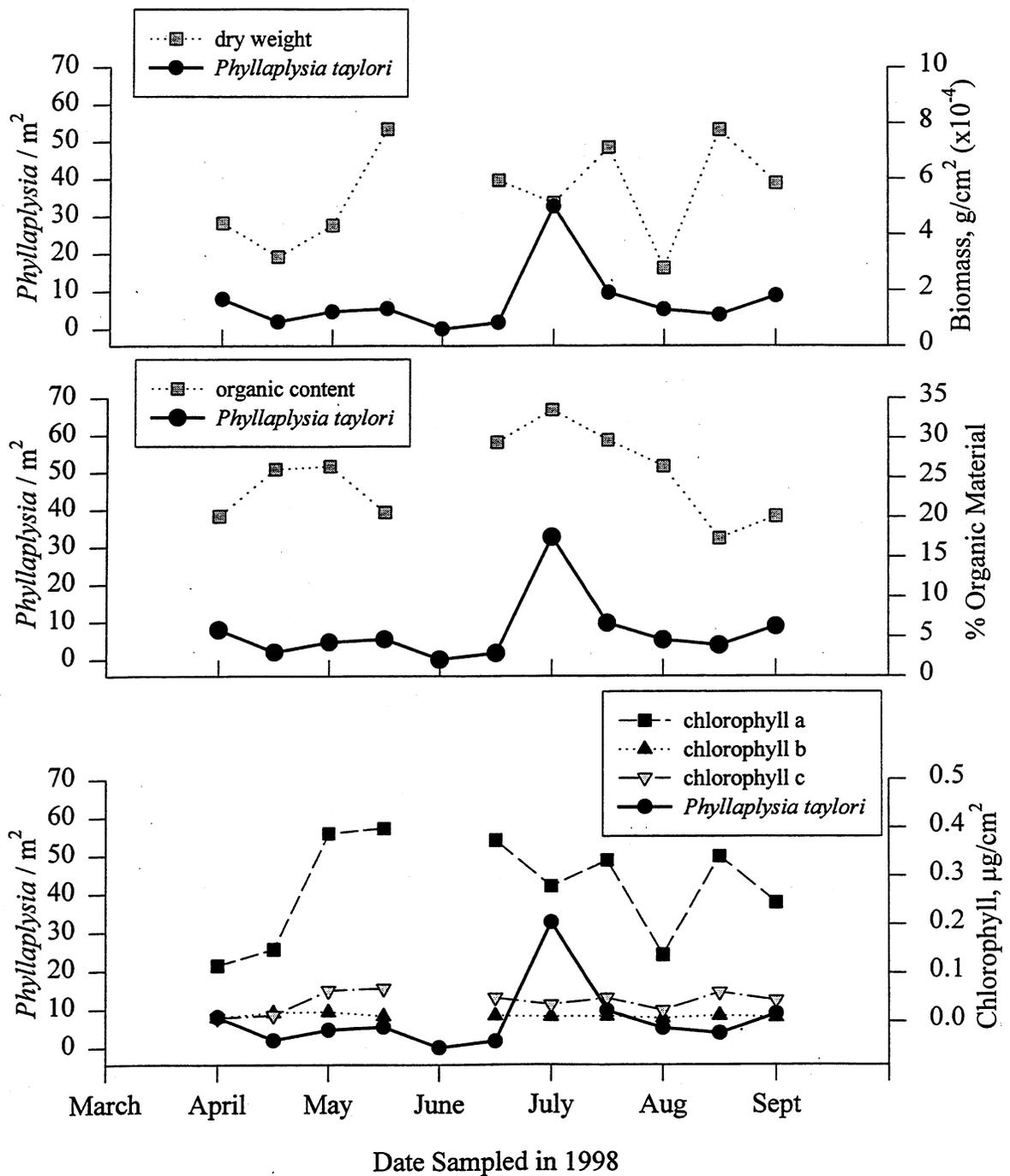


Figure 15. The relationships between *Phyllaplysia* and the measurements of epiphytic biomass at Bayview during 1998. *Phyllaplysia* showed a significant positive correlation with the percent organic material of the epiphytes and a significant negative correlation with the content of chlorophyll b in the epiphytes.

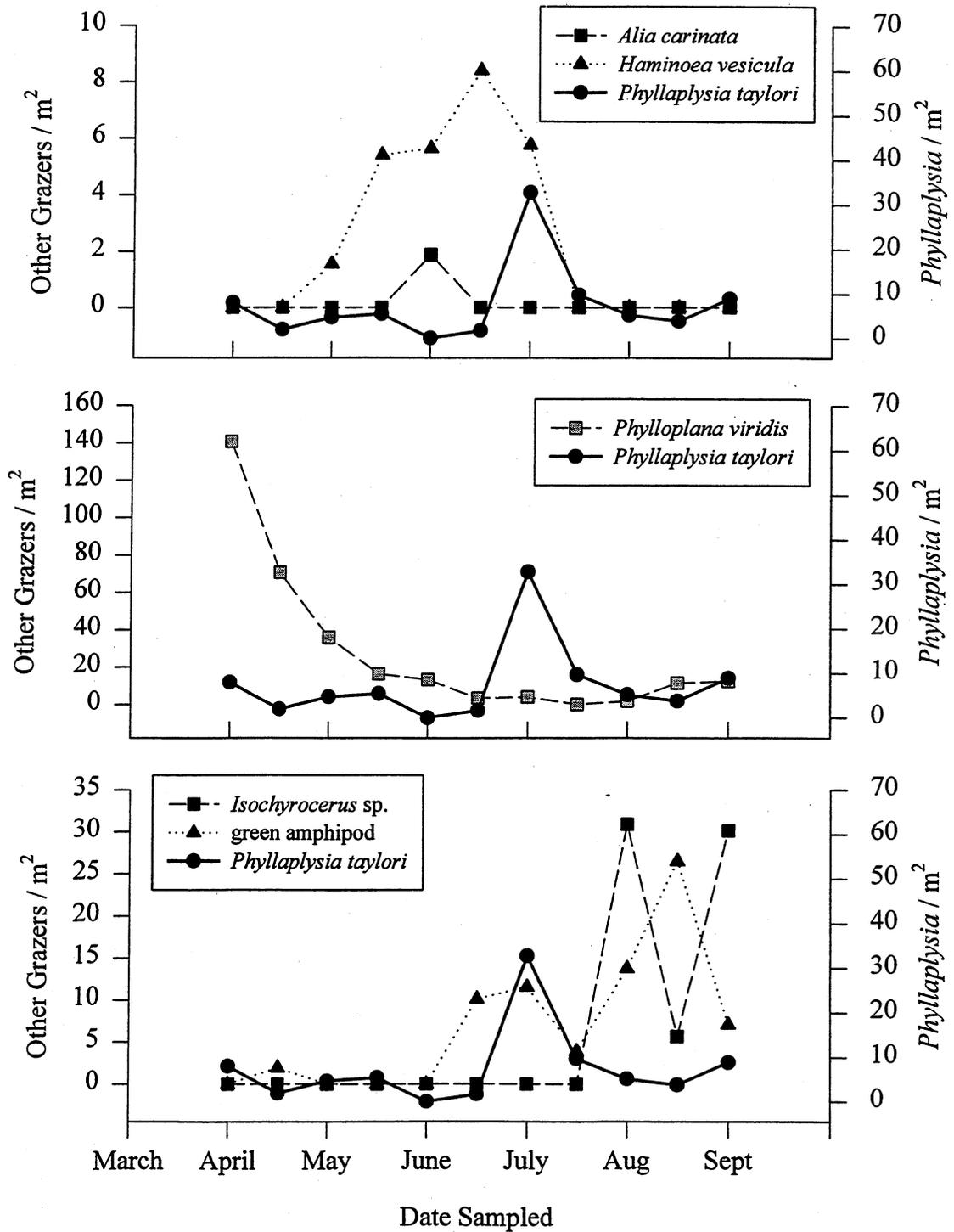


Figure 16. The relationships between *Phyllaplysia* and the macrofauna collected at Bayview during 1998. None of these negative correlations are statistically significant.

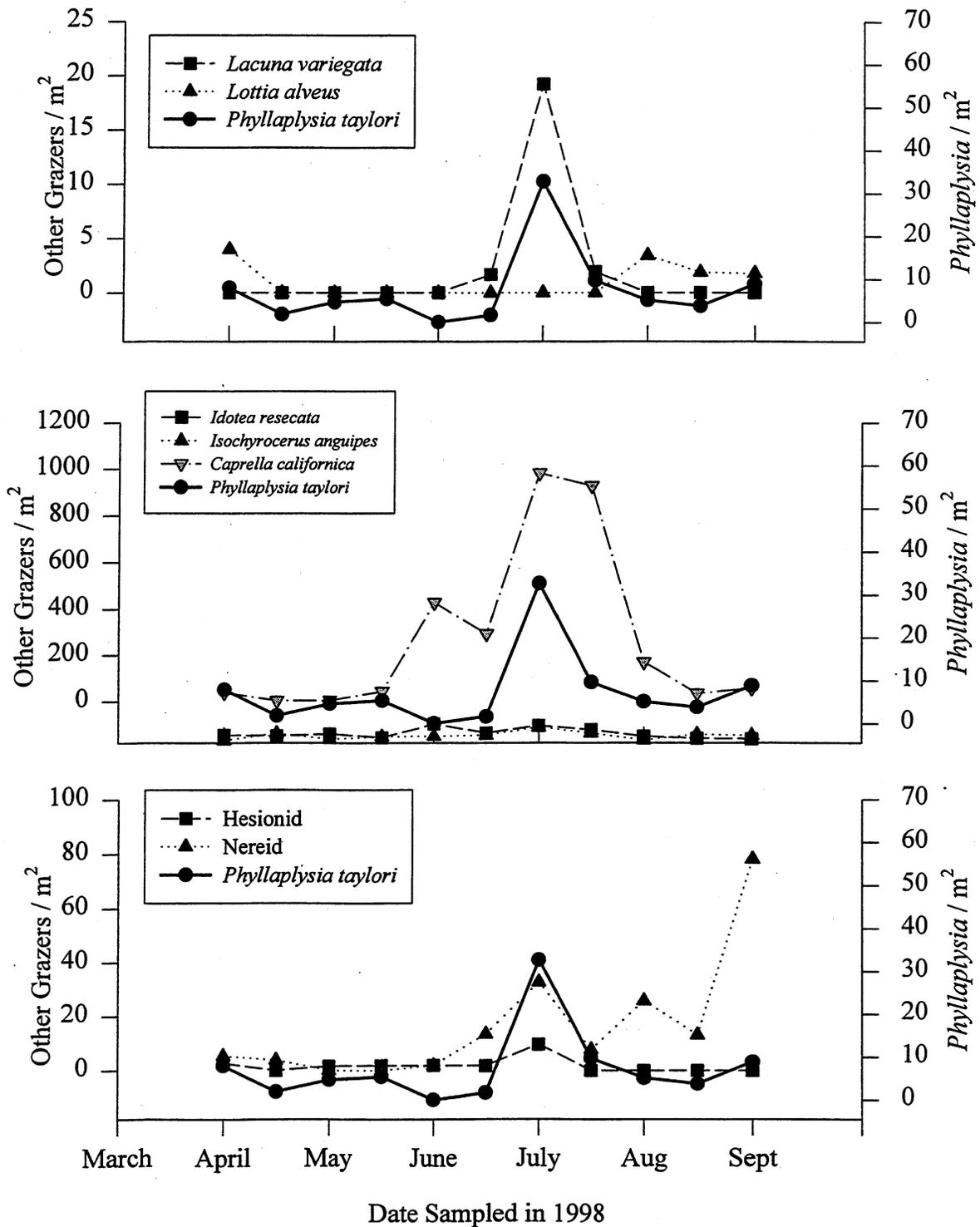


Figure 17. The relationships between *Phyllaplysia* and the other species of macrofauna collected on eelgrass at Bayview during 1998. Only *Lacuna* shows a significant positive correlation with *Phyllaplysia*.

Table 17. Correlations of epiphytic dry weight and the dominant macrofauna at the three sites. No correlations were significant. Significance was determined at $\alpha=0.05$ ($r=0.178$).

	March Point	Kirby Beach	Bayview
<i>Haminoea vesicula</i>		-0.074	
<i>Lacuna variegata</i>	-0.120		
<i>Lottia alveus</i>		-0.105	
<i>Phyllaplysia taylori</i>	0.097		0.095
<i>Idotea resecata</i>			-0.105
<i>Isochyrocerus anguipes</i>	-0.174	0.141	0.113
<i>Isochyrocerus</i> sp.		-0.067	-0.054
Nereid sp.	0.125		

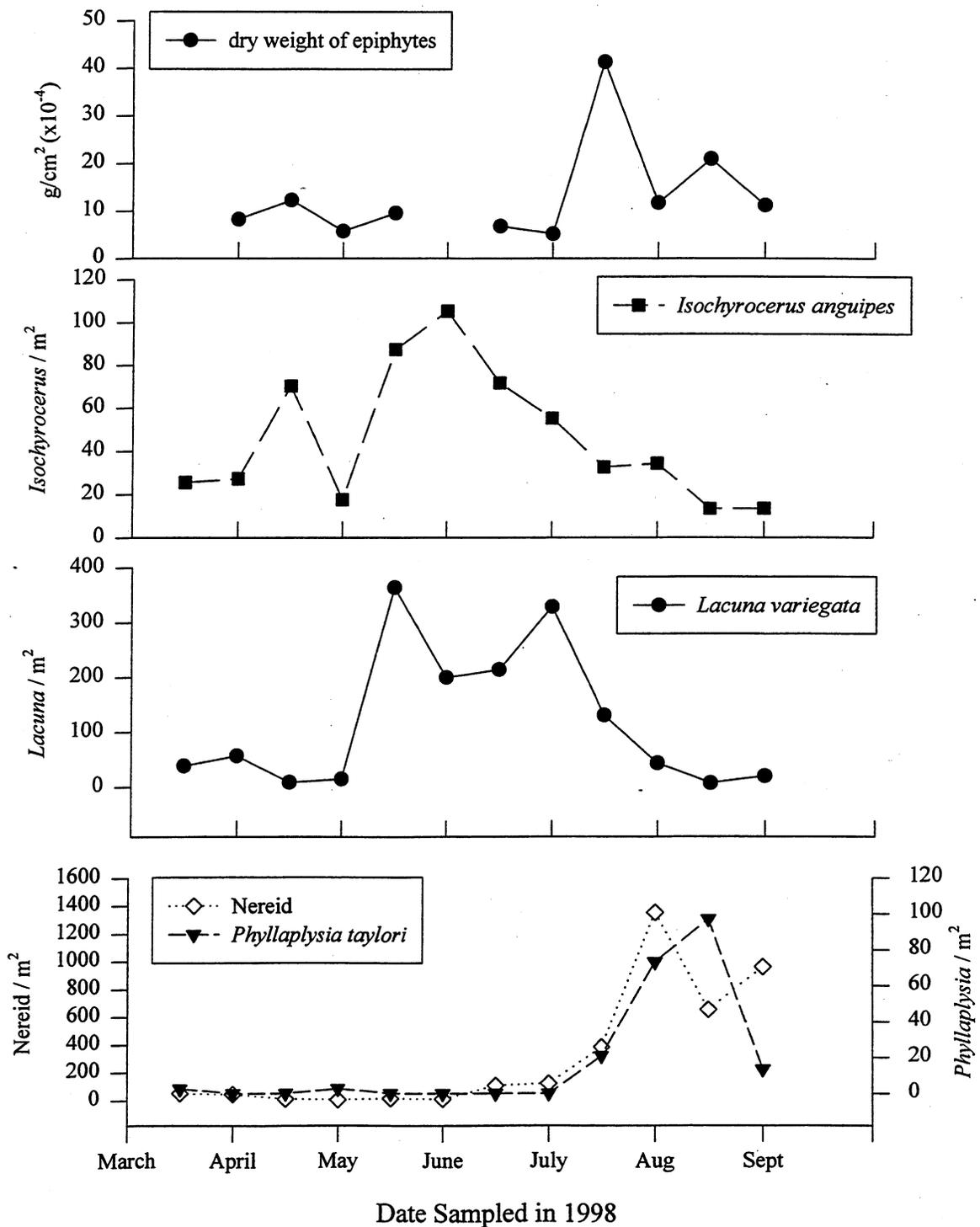


Figure 18. The trends in densities of the dominant macrofauna at March Point with respect to the biomass of the epiphytes at this site during the 1998 sampling period.

peak densities of two species, *Phyllaplysia taylori* and a nereid polychaete, occurred simultaneously with this decline.

Kirby Beach. *Lottia alveus* was most abundant at Kirby Beach in the early samples. A decrease in the biomass of epiphytes at Kirby Beach (Fig. 19) corresponded with an increase in the density of this gastropod. The density of *Haminoea vesicula* began to increase in late May, corresponding with a decrease in epiphytic biomass. No data on epiphytic biomass was collected in early June, one of the peak times for this gastropod. From late June to early July, a slight decrease in biomass was evident. This coincided with the increase in density of *Isochyrocerus anguipes*. The highest recorded density of *Isochyrocerus* sp. occurred in early August which was also the period of greatest decline of epiphytic biomass at this site.

Bayview. The first observed decline in epiphytic biomass at Bayview occurred in late April (Fig. 20). This was also when the density of *Isochyrocerus anguipes* began to rise. Biomass continued to decline through early July, corresponding with a relatively high density of *Isochyrocerus anguipes*, *Idotea resicata* and *Phyllaplysia taylori*. Similarly, in August, the peak density of *Isochyrocerus* sp. coincides with the lowest recorded epiphytic biomass at this site.

Three of the more abundant macrofauna did not contribute to these trends. At the three sites, the peak densities of *Phylloplana viridis*, *Caprella californica*, and a hesionid polychaete generally occur simultaneously with an increase in epiphytic biomass. One other common species of macrofauna, *Alia carinata*, was abundant in the first samples taken from Kirby Beach. Since epiphytic parameters were not measured during the first sampling period, it was impossible to ascertain the effects of this grazer at Kirby Beach.

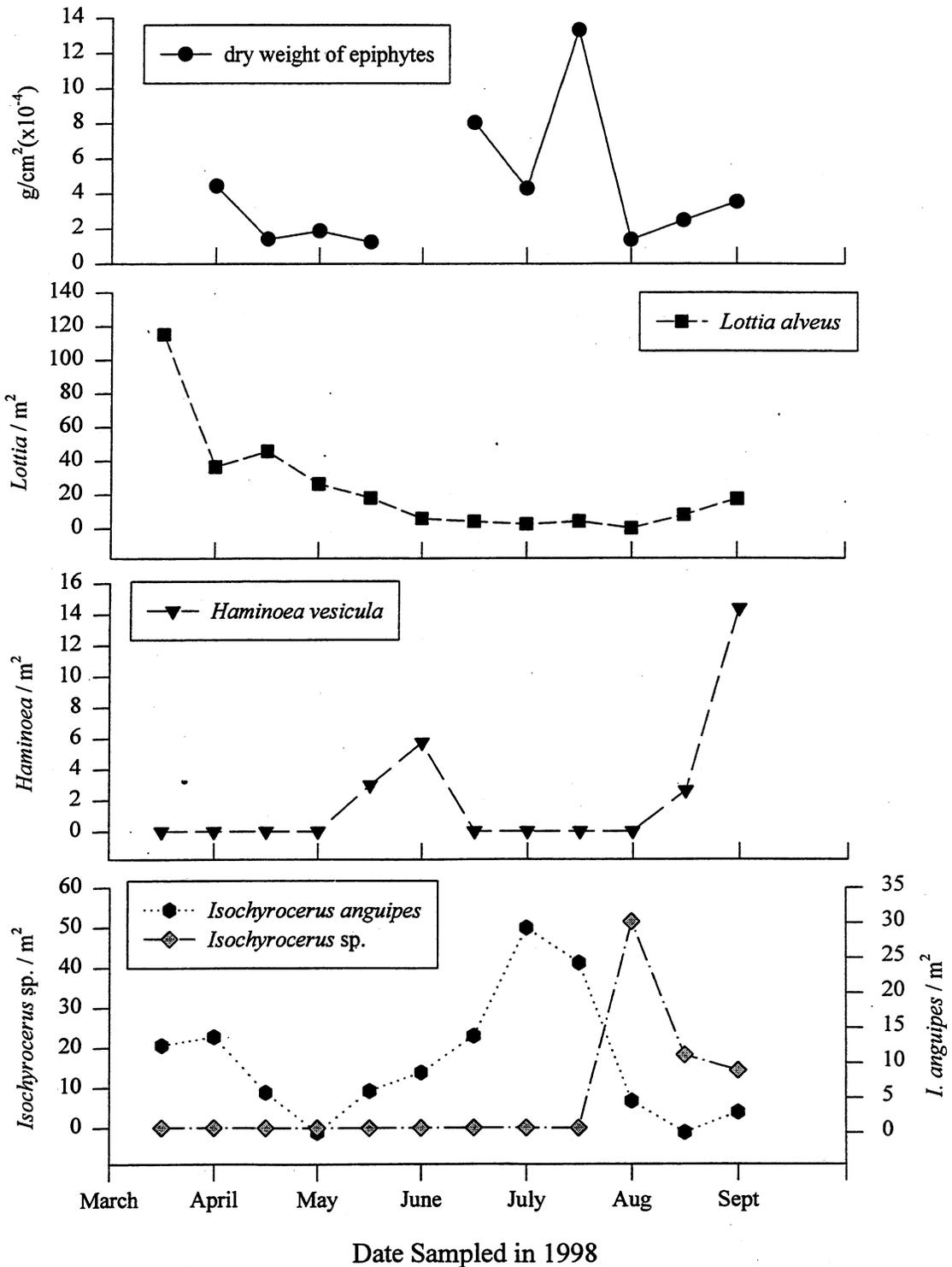


Figure 19. The trends in the densities of the dominant macrofauna with respect to the biomass of the epiphytes at Kirby Beach during the 1998 sampling period.

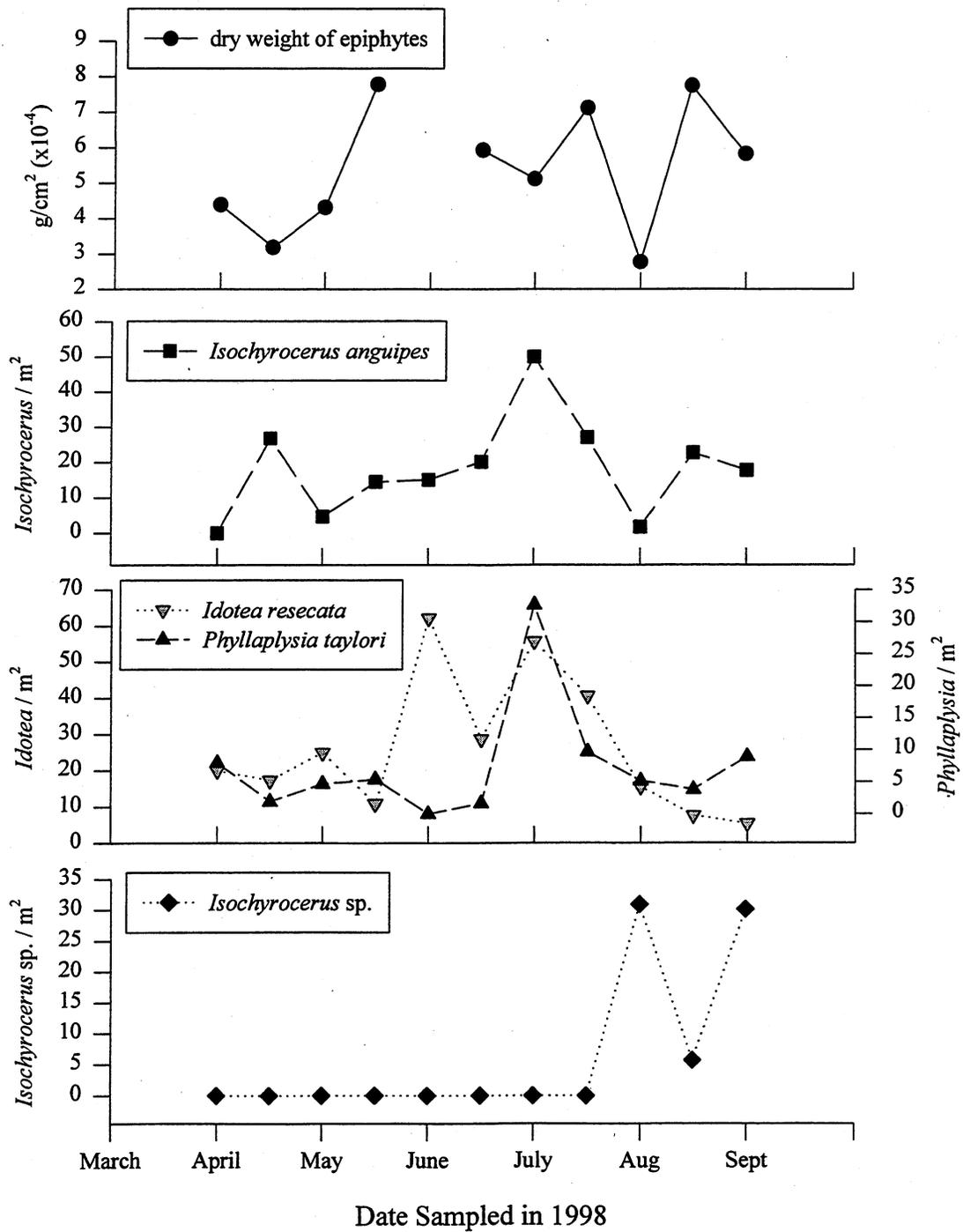


Figure 20. The trends in the densities of the dominant macrofauna collected at Bayview with respect to the biomass of the epiphytes at this site during the 1998 sampling period.

DISCUSSION

Phyllaplysia taylori

Phyllaplysia taylori is one of the dominant species of macrofauna on the eelgrass in Padilla Bay. The densities collected belie its importance. Its size relative to the other species of macrofauna is also relevant in determining its significance as a grazer in these systems. When compared to other species, for example the nereid polychaete, the densities of *Phyllaplysia* seem low. However, *Phyllaplysia* is a much larger organism. The average size of the nereid was approximately 1-2 cm long. *Phyllaplysia*, however, was substantially larger in both length and width. The typical length of adult organisms was 2-3 cm and the width can equal that of the entire eelgrass blade. The consumption rate of one *Phyllaplysia* is most likely comparable to that of a large number of the smaller species. Therefore, as a grazer in these eelgrass systems, *Phyllaplysia* is apt to have a strong influence on the epiphytic community.

Significant differences in both the distribution of *Phyllaplysia* among the three sites and the density of *Phyllaplysia* over time were found in this study (Figure 10). The distribution of *Phyllaplysia* could be affected by a number of factors such as the inherent behavior of this organism as well as its response to the presence and temporal variation of both food supply and other species of macrofauna.

Effect of the Behavior of *Phyllaplysia* on its Distribution

The behavior of *Phyllaplysia* was examined in the lab study. Thompson (1962) believed opisthobranchs have the ability of preferentially selecting a specific substrate. This ability would contribute to the successful establishment of populations in areas

which are favorable for colonization. *Phyllaplysia* are found almost exclusively on *Zostera*, and, because they are direct developers, *Phyllaplysia* uses *Zostera* blades as a substrate through its entire life cycle. Their specificity for eelgrass as a substrate was examined by testing the preference of *Phyllaplysia* with respect to two characteristics of eelgrass, orientation and color.

Orientation Preference. When no distinction was made between the top and the bottom of the test chamber, and only vertical versus horizontal positioning was analyzed, the results showed that *Phyllaplysia* did not indiscriminantly crawl up any vertical surface (Table 1). This would have supported my hypothesis that the choice of eelgrass as a substrate for *Phyllaplysia* is not a passive one. However, once a distinction was made between the top and the bottom, a different conclusion was reached. From these results, it is apparent that *Phyllaplysia* avoided being on the bottom (Table 2).

Phyllaplysia has a number of organs that would help in the determination of orientation. The cephalic tentacles and the labial lappets which project ventrally from the cephalic tentacles seem to function in sensing food and substrate (Beeman, 1968). Also, the rhinophores are utilized for current detection which could be helpful in determining position (Frings and Frings, 1965).

One of the most obvious reasons for anaspideans such as *Phyllaplysia* to stay off the bottom has to do with siltation. Many gastropods have organs in their mantle cavity called osphradia which are patches of sensory epithelium. One of the functions of this organ is to monitor the amount of suspended sediment in the water taken into the mantle cavity (Brusca and Brusca, 1990). Unlike nudibranchs, anaspideans have both a mantle

fold and ctenidia (MacFarland, 1966). Since water is continually circulated over the ctenidia, it is important for *Phyllaplysia* to monitor the amount of silt in the water.

When on the bottom of the test chamber, *Phyllaplysia* were oriented with their dorsal surface facing upward. When they crawled to the top of the test chamber, they were in essence, upside down with their dorsal surface facing downward. The opening to their mantle cavity is on their dorsal surface. Therefore, when these organisms are oriented with their dorsal side facing upwards, they are more exposed to sediment deposition. An upside down orientation gives *Phyllaplysia* more protection from sediment, preventing their ctenidium from becoming clogged.

The results of the tests run on orientation preference do not suggest that *Phyllaplysia* will actively choose eelgrass as a substrate. Instead, these results indicate that orientation is what determines their position on a substrate. *Phyllaplysia* seem to prefer being oriented so that their dorsal surface is not facing upward. Therefore, whatever substrate provides this favorable orientation appears to be the preferred substrate.

During my field sampling I noticed that the juvenile *Phyllaplysia* were almost always found along the basal portion of the eelgrass blades. The larger organisms were found higher up along the blade. The basal portions of eelgrass remain vertical during both high and low tides. The apical sections and sometimes the mid-sections of the blades are positioned horizontally during low tide. From these observations, I would expect that smaller organisms would prefer a vertical position and larger organisms would prefer a horizontal position. When size was considered as a determining factor in the positioning of *Phyllaplysia*, no significant preferences were found (Table 3).

Although insignificant, a trend is apparent (Figure 2). Four out of six small animals preferred a vertical orientation, but only two out of eleven larger animals preferred a vertical over a horizontal orientation. Perhaps if the size classes were more distinct and if more individuals of each class could be tested, a significant preference would be found as was noticed in the field.

The field observations on size as a determining factor of orientation do have ecological significance. T. Shaw (1994) observed diel migration in *Phyllaplysia*. He found significantly more *Phyllaplysia* along the mid-sections at night while during the day, the majority of *Phyllaplysia* collected were found on the basal portions of the eelgrass shoots. T. Shaw (1994) interpreted this diel migration pattern as a method of avoiding predation. This could also help explain the size dependent orientation observed in the field. The smaller organisms, being more susceptible to predation, may tend to stay in the safer, less exposed areas of the eelgrass blades, the basal portions, which remain vertical throughout the tidal cycle. The mid-sized organisms have less predation risk and, therefore, have the opportunity to explore further up along the blade for food. The primary concerns for the larger organisms are reproduction and feeding. During low tide, the apical sections of the leaves have a horizontal positioning. When these sections are horizontal, they overlap with other eelgrass blades. During this time, *Phyllaplysia* have a greater chance of coming into contact with possible mates. At this size, they are also less susceptible to predation and are able to crawl to these apical sections where the biomass of epiphytes, their food supply, is much denser.

Color Preference. Opisthobranchs are one of the more colorful groups of invertebrates. The variety of these colors has caused much speculation as to their

function. One of the primary functions of external colors in opisthobranchs is for interspecific signals (Edmunds, 1987). Cryptic coloration, for example, emits signals that are indistinguishable from the background noise thereby functioning to reduce the chance of predator detection. However, Edmunds (1987) believes that opisthobranchs lack color vision and therefore would be unable to visually select a background of the appropriate color. *Aplysia juliana*, for example, does not use visual cues to detect food (Frings and Frings, 1965). In most cases the ability of opisthobranchs to match their background color is attributed to their ability to sequester pigments from their food source (Edmunds 1987).

Phyllaplysia taylori do not have the ability to sequester pigments from their food. Not only do they not feed on the eelgrass, but when I observed them under epifluorescence, they showed no retention of chlorophyll. However, they are a good example of cryptic coloration. Their flattened form, their green coloration, and their longitudinal white lines which resemble the veins in the eelgrass blades help this animal blend in almost perfectly with eelgrass (Edmunds, 1987). Since they do not feed directly on the eelgrass, nor do they sequester pigments from their food source, *Phyllaplysia* could have the ability to visually select their background by color.

The eyes of gastropods vary in complexity from simple pigment-cup eyes found in primitive gastropods such as the abalone to the more complex eyes of littorines which have evolved both a cornea and a lens (Brusca and Brusca, 1990). Opisthobranch eyes are believed to be too simple in structure to form an image; however, it is believed that certain species of ascoglossans can discriminate between colors of light. In a study done by Weaver and Clark (1981), four out of the five species tested did exhibit a significant

color preference. The most probable explanation of these preferences is their function in locating food sources in conjunction with predator avoidance strategies.

The majority of the numbers of *Phyllaplysia* tested did demonstrate a significant preference for green (Table 4). This supports the theory that they can distinguish, if not between colors, at least between intensities of light. In the range of intensities, the brown and black were of low intensity, the green color was of a medium intensity, and the red and white represented the higher intensities. From my results, it appears that *Phyllaplysia* avoids the higher intensities. Red and white were avoided by more subjects than any of the other colors, and only one subject demonstrated a preference for white. The medium intensity, green, was preferred, and the lower intensities, brown and black, although avoided by more, were also preferred by a fair number of subjects. After green, brown was the second most preferred color (Figure 3).

Because of the simplicity of my study on color choice, too many variables exist to make my results conclusive. *Phyllaplysia* could choose eelgrass for reasons other than its color. The main attraction is probably the epiphytes, the food source of *Phyllaplysia*, growing on the grass. However, this does not explain why in the field they were never found on macroalgae located within the eelgrass beds and often in direct contact with the blades of eelgrass. This macroalgae was probably colonized by similar epiphytes as colonized the eelgrass. In a study done on the distribution of epiphytic diatoms by Main and McIntire (1974), those species of diatoms found on *Zostera* did not differ significantly from the diatom species fouling species of macroalgae such as *Polysiphonia*, *Ulva*, and *Enteromorpha*.

I believe that *Phyllaplysia*'s choice of eelgrass as their only substrate is a result of a combination of factors, one of which is color. Perhaps *Phyllaplysia* do crawl on other substrates, but their survival rate is greatly reduced on these other substrates since they would not be camouflaged. In addition to differential survivorship, texture of the substrate, and the characteristic phenolics of eelgrass could also be contributing factors to the presence of *Phyllaplysia* on *Zostera marina*.

The Epiphytic Community and *Phyllaplysia* Distribution

Epiphytes can alter the habitat of *Phyllaplysia*. A heavy growth of epiphytes on *Zostera* forms a much different environment than does a sparse epiphytic community. In comparing the biomass of the epiphytes among the three sites, the most significant differences emerged between March Point and Kirby Beach (Fig. 4). During the majority of the sampling, the eelgrass at March Point had the heaviest fouling of algae and diatoms. Epiphytic biomass was lighter at both Kirby Beach and Bayview, and differences in biomass were not as obvious.

The epiphytic community is also important to *Phyllaplysia* as a source of food. Simple contrasts of the sites showed much variation among the three sites with respect to the percent organic content of the epiphytes. Many of the samples differed significantly in each of the three contrasts. Generally, Kirby Beach had the highest percentage of epiphytic organic material on eelgrass, and March Point had the lowest percentage (Fig. 5). Throughout the sampling period, the higher the biomass of the epiphytes, the lower the percent of organic material. Because larger epiphytic loads have a tendency to trap more inorganic debris, this would cause the percent of organic material to decrease. This,

however, does not necessarily mean that the actual organic content of the epiphytes decreases with an increase in epiphytic biomass.

A better estimate of the availability of food in the epiphytic community is the chlorophyll content. Chlorophyll a, b, and c were generally highest at March Point and lowest at Kirby Beach (Fig. 6). Diatoms are composed primarily of chlorophyll a and c (Smith, 1955; Reid, 1965). The consistency of the ratio of chlorophyll a to chlorophyll c indicates that the diatom community did not change much over time (Fig. 7). The low values of this ratio show that the level of chlorophyll c, with respect to chlorophyll a, remained fairly high. The ratio of chlorophyll a to b was generally higher than that of chlorophyll a to c indicating that levels of chlorophyll b were, on average, lower than levels of chlorophyll c. Chlorophyll b is found primarily in green algae and higher plants. Some of the chlorophyll b could be contributed by cells of *Zostera* accidentally scraped off during removal of epiphytes. Therefore, the level of chlorophyll b, although the lowest, is probably exaggerated with reference to the content in the epiphytes due to the method of removal. The fluctuation of the ratio of chlorophyll a to b also shows that the flora containing chlorophyll b are more transient.

The life cycle of *Phyllaplysia* appears to have adapted to take full advantage of the food supply. The diet of opisthobranchs controls both their growth rate and number of generations per year (Miller, 1962). *Phyllaplysia* completes two overlapping waves of reproduction each year. Beeman (1970) classified these two cycles as the winter crop and the summer crop. In Elkhorn Slough, the winter crop typically hatches in September. This crop experiences rapid growth through January. In April or May, once maximum size is reached, eggs are deposited and the individuals then die. These eggs then hatch in

late May or June, giving rise to the summer crop. The individuals in this crop grow much faster and reach maximum size in September or October, and the cycle is repeated. The winter crop tends to have fewer, but larger individuals and a typical life span of 7-9 months. The summer crop as a whole is larger, containing many more individuals; however, the individuals do not reach the typical sizes of the winter crop and only live on average 3-4 months.

The same cycles of reproduction were apparent in my sampling of Padilla Bay. When I first started sampling in March, the few individuals that were collected were quite large. Egg cases were noticed throughout May and the first weeks of June. Finally, in July and August, large numbers of *Phyllaplysia* were collected. The sampling ended in early September, presumably before the second crop of eggs were laid as no egg cases were found after June.

The consistency of the ratio of chlorophyll a to chlorophyll c indicates that the diatoms, the primary food source of *Phyllaplysia*, were consistently present in the epiphytic community. During the spring and summer months, the epiphytes become much more abundant. The ratio of chlorophyll a to c graphed over time indicates that diatoms are found in consistently higher abundance in the epiphytic community than either green algae or higher plants from April through early September. It is during this time that the more numerous summer crop of *Phyllaplysia* is present. The following September, when epiphytic biomass has declined but is still present, the winter crop, composed of fewer individuals, hatches. These individuals grow slowly over a 7-month period and lay eggs whose hatching coincides with increased epiphyte biomass.

Associated Species of Macrofauna and *Phyllaplysia* Distribution

A hierarchical cluster analysis was used to analyze the similarities in species composition and densities among the samples taken from the three sites. Cluster analysis is one of the less sophisticated methods of multi-variate statistical analysis. However, it is recommended by Field (1971) as a type of initial analysis. Cluster analysis effectively sorts large amounts of data objectively and can define causal relationships in data. It also can help define community structure, identify characteristic species of the communities, and ascertain seasonal community dynamics (Whitlatch, 1977).

In cluster analysis, the Bray-Curtis dissimilarity index is frequently used by ecologists and rarely gives spurious results (Ludwig and Reynolds, 1988). Field (1971) also agrees that this distance measure is applicable to most types of ecological data, including species counts. And, since Bray-Curtis is not affected by large numbers of zeros in the data matrix it is equally effective for clustering heterogeneous data matrices. However, because this measurement has the tendency to weight species according to their abundance, it can be greatly influenced by an outstandingly abundant species (Clifford and Stephenson, 1975). To correct this problem, Field (1971) recommends log-transforming all species counts before calculating the Bray-Curtis coefficient.

The best type of clustering technique is also debated by many ecologists. There are three clustering methods widely used in ecology, single-linkage, complete-linkage, and group-average. Because group-average is considered space conserving, it introduces relatively little distortion from the original similarity matrix when compared with the other two techniques (Ludwig and Reynolds, 1988). Likewise, other ecologists have found the group-average clustering method to yield the more instructive classification of

samples (Whitlatch, 1977). Therefore, this method was employed in the clustering of my samples.

In the dendrogram resulting from clustering the 35 samples at a distance of 0.4, the samples tend to be grouped primarily by site and secondarily by season (Fig. 9). Within each group, the samples which belong to the group come from the same site. Additionally, the groups are generally composed of consecutive samples thereby representing an early, mid, and late season. This indicates that the samples taken from the same site are more similar to each other than to those taken from a different site, and that within each site there is temporal variation of many of the species of macrofauna. This supports the research conducted by T. Shaw (1994) who also found significant temporal variation of certain macrofauna during his study of Padilla Bay.

At the level which the nine groups were divided, the variation between sampling areas is greater than sampling and temporal variation within a sampling area, and the sites do not appear to be similar to each other. From a broader division, using a distance of 0.5 to cluster similar samples, some similarities between sites can be established. The majority of samples taken from March Point are linked more closely with the first nine samples taken from Kirby Beach than with any sample from Bayview. Therefore, more similarities seem to exist between March Point and Kirby Beach with respect to the macrofaunal community, while Bayview supports a community of macrofauna more distinctive from the other two sites.

Correlations between *Phyllaplysia* and other macrofauna. *Phyllaplysia* was found abundantly at March Point, Bayview had a smaller population of *Phyllaplysia*, and numbers collected from Kirby Beach were negligible. Therefore, only the populations of *Phyllaplysia* at March Point and Bayview were analyzed for correlations. At each site, different species showed positive and negative correlations with *Phyllaplysia* (Table 16). The positive correlations between *Phyllaplysia* and some of the species at each of the sites may be a result of the similar responses of these grazers to an environmental factor other than epiphytic biomass. The differences in the species of grazers which showed negative correlations could be a result of the different patterns of density of *Phyllaplysia* at the two sites. The peak density of *Phyllaplysia* at March Point was much higher and continually increased over three sampling dates. At Bayview, there was only a modest peak in density at one sampling date. The high numbers of *Phyllaplysia* at March Point could have reached levels high enough to inhibit other species and in this way account for the greater number of negative correlations with other species of macrofauna. At Bayview, no species were negatively correlated with *Phyllaplysia* suggesting that numbers were not high enough for negative interactions to become evident.

Many dominant species such as *Phyllaplysia* inhabit a specific niche in their communities, whether it is separated by time, location, or food source. One of the strongest positive correlations was found between *Phyllaplysia* and a nereid polychaete (Fig. 13). *Phyllaplysia* is a herbivore, feeding primarily on diatoms. The anatomy of its radula and stomach teeth are extremely effective in grinding the silicon cases of diatoms (Beeman, 1969). Little is known about the diets of polychaetes, but Orth and Van Montfrans (1984) believe that any species which exhibits a surface deposit feeding mode

will likely ingest some of the epiphytic growth. So, although both of these organisms occupy the same habitat during similar times, their source of food may not overlap.

Temporal Variation of Epiphytes and Macrofauna

Previous studies have demonstrated the seasonal variation in epiphytic communities (Thom *et al.*, 1991; Williams and Ruckelshaus, 1993). The significant differences by date in the biomass of the epiphytes collected during my sampling support this conclusion. The epiphytic load is generally highest during the spring and summer in seagrass systems (Thom *et al.*, 1991). In my study, the biomass of the epiphytes had a major peak during July and a smaller peak in August. This occurred simultaneously at all three sites. Nutrient levels in the water column are generally higher in the winter than in the summer (Muller-Parker and Peele, 1998). However, epiphytic biomass does not seem to be limited by nitrogen concentrations (Williams and Ruckelshaus, 1993). Irradiance has been determined to be the primary controlling factor in eelgrass systems in central Puget Sound (Thom and Albright, 1990). During the spring and summer, light is not limiting, therefore, these observed accumulations of biomass are expected. During the autumn and winter months, the decrease in epiphytic biomass is attributed to the reduced availability of light. However, when light is not limiting, the regulation of plant biomass is attributed to grazers (Thom *et al.*, 1991; Cushing, 1962). In my study this can explain the fluctuations in epiphyte biomass. The similar temporal fluctuations in chlorophyll content and organic content are also a result of the changes in biomass.

Grazers have a large influence on vegetative growth (Southward, 1962), including the epiphytic growth on eelgrass. The peak densities of many of the dominant

macrofauna corresponded with a decline in epiphytic biomass. The presence of *Phyllaplysia* followed this pattern. At the two sites where *Phyllaplysia* was found, decreases in epiphytic biomass correspond with the peak densities of this species (Figs. 18 and 20). This trend is most striking at March Point where the rapid decline in epiphyte biomass during August occurs during the period of highest numbers of *Phyllaplysia*. The nereid polychaete also peaks during this time, and some of the decline in biomass is probably a result of this species. However, because the diet of *Phyllaplysia*, unlike that of the nereid polychaete, is primarily composed of epiphytic diatoms, the majority of the biomass is most likely consumed by *Phyllaplysia*. At Kirby Beach, where numbers of *Phyllaplysia* were negligible, the species richness was significantly higher, and other species occupied the role of grazer during the time period when *Phyllaplysia* peaked at the other sites (Fig 19).

Three of the more abundant grazers did not follow this trend. *Phylloplana viridis*, a flatworm, peaked in August at March Point and Kirby Beach. The food source of this species is not known, but, as with polychaetes, they probably do ingest some of the epiphytic growth. However, their peak density cannot be related to any decreases in epiphytic biomass. Similarly, *Caprella californica*, a well-documented epiphyte grazer on eelgrass, shows a peak in density at the three sites which almost always corresponded with high epiphytic biomass. Some of the branching species of epiphytes which add considerable biomass also function as a refuge for caprellids. Within this type of epiphytic growth, they are highly concealed. Perhaps, they peak during this time because they are harder to detect by predators. The population of the second main species of polychaete, a hesionid, fluctuates at all three sites. It is therefore difficult to relate its

density to any patterns in epiphytic biomass, and, as with the nereid, the diet of this polychaete is not known.

Yearly Variation in Populations of *Phyllaplysia*

At the onset of this study, after doing preliminary sampling in Padilla Bay, I had hoped to determine why *Phyllaplysia* were not found at March Point and why they were so abundant at Kirby Beach and Bayview. Based on the data collected during 1997 when *Phyllaplysia* were not found at March Point, I hypothesized that the epiphytic community, with respect to either biomass or species content, was not conducive to the life cycle of *Phyllaplysia* at this site. Kirby Beach and Bayview seemed to have similar epiphytic communities, and the densities of *Phyllaplysia* were also more similar at these two sites. However, sampling in 1998 disproved this initial hypothesis because *Phyllaplysia* were found abundantly at March Point and not at Kirby Beach although I was able to detect no apparent differences in epiphytes. The analysis of the second factor which I thought might influence the distribution of *Phyllaplysia*, the macrofaunal community, also gave inconclusive results. The cluster analysis primarily indicated that differences are greater among the sites than within the sites over time. A second analysis indicated that the species composition of March Point and Kirby Beach were the most similar. *Phyllaplysia* was most abundant at March Point and rarely ever collected from Kirby Beach. Therefore, it is apparent that these three sites are hard to characterize when looking for similarities among them, and that a year or two of sampling is insufficient to ascertain ecosystem characteristics.

Guesses can only be made as to the causes of the disappearance of *Phyllaplysia* from Kirby Beach and the reappearance at March Point in 1998 (Fig. 11). Chambers (1934) hypothesized that the sudden disappearance of a colony of nudibranchs could be caused by environmental factors such as a reduction in food supply or an increase in predators. Since *Phyllaplysia* is not particularly selective in its diet, food was probably not the cause of its absence from Kirby Beach. This leaves predation. Some species of fish prey on *Phyllaplysia*, but since my sampling did not encompass free-living fauna, increases of this type of predator were not measured. I also observed a species of nudibranch, *Hermisenda crassicornis*, preying on *Phyllaplysia*. This *Hermisenda* was found most abundantly at Kirby Beach; however, it is debatable as to whether it was numerous enough to affect the population of *Phyllaplysia* so dramatically.

March Point was repopulated with *Phyllaplysia* in 1998. Chambers (1934) predicts that a single, fertilized individual could establish a colony. This is especially likely if the veligers do not undergo a free-swimming veliger stage. Since *Phyllaplysia* does not have a planktonic larvae stage but directly develops on the same substrata on which the eggs were laid this could explain the recovery of the population of *Phyllaplysia* at March Point.

Significance of Study

Eelgrass systems are an important resource of coastal areas in the Pacific Northwest. A diverse assemblage of organisms relies on these systems for food, shelter, and as a nursery for larvae and juveniles. The biological, structural, and economical significance of these systems are a result of the biodiversity inherent in eelgrass

meadows. Maintaining a diverse community is dependent on the health of the eelgrass. Ultimately, grazers have a very important role in maintaining the health of these plants.

In Padilla Bay, *Phyllaplysia taylori* is one of the more prevalent species of grazers on the epiphytic growth which fouls *Zostera*. It, as well as other species of grazers, can be used as a gauge of the health of eelgrass ecosystems. As Thom (1987) indicates in his analysis of the importance of northwest estuaries, any gaps we have in our understanding of the biological aspects of these systems can severely limit the confidence we have in management decisions affecting the conservation of estuaries. By gaining a better understanding of the behavior of *Phyllaplysia* and more knowledge of its distribution and temporal variation, it will be easier to monitor the health of eelgrass meadows and conserve the integrity of these valuable resources.

Literature Cited

- Beeman, R.D. 1963. Variation and synonymy of *Phyllaplysia* in the Northeastern Pacific (Mollusca: Opisthobranchia). *Veliger* 6: 43-47.
- Beeman, R.D. 1968. The Order Anaspidea. *Veliger* 3 (suppl.): 87-102.
- Beeman, R.D. 1969. An autoradiographic demonstration of stomach tooth renewal in *Phyllaplysia taylori*. *Biol. Bull.* 136:141-146.
- Beeman, R.D. 1970. An ecological study of *Phyllaplysia taylori* Dall, 1900 (Gastropoda: Opisthobranchia) with an emphasis on its reproduction. *Vie et Milieu (A) Biol. Mar.* 21:189-211.
- Borum, J., H. Kaas, and S. Wium-Andersen. 1984. Biomass variation and autotrophic production of an epiphyte-macrophyte community in a coastal Danish area: II. Epiphyte species composition, biomass and production. *Ophelia* 23: 165-179.
- Bridges, C.B. 1975. Larval development of *Phyllaplysia taylori* with a discussion of development in the Anaspidea (Opisthobranchiata: Anaspidea). *Ophelia* 14:161-184.
- Bronmark, C. 1985. Interactions between macrophytes, epiphytes and herbivores: an experimental approach. *Oikos* 45: 26-30.
- Brusca, R.C. and G.J. Brusca. 1990. *Invertebrates*. Sinauer Associates, Inc., Sunderland, Mass.
- Bulthuis, D.A. 1991. Distribution of habitats and summer standing crop of seagrasses and macroalgae in Padilla Bay, Washington, 1989. Washington State Department of Ecology, Padilla Bay National Estuarine Research Reserve Technical Report No. 2, Mount Vernon, Washington, 35 pp.
- Bulthuis, D.A. 1993. Review of water quality data in the Padilla Bay / Bay View watershed. Washington State Department of Ecology, Padilla Bay National Estuarine Research Reserve Technical Report No. 10, Mount Vernon, Washington, 72 pp.
- Caine, E.A. 1980. Ecology of two littoral species of caprellid amphipods (Crustacea) from Washington, USA. *Mar. Biol.* 56:327-355.
- Chambers, L.A. 1934. Studies on the organs of reproduction in the nudibranchiate mollusks, with special reference to *Embletonia fuscata* Gould. *Bull. Amer. Mus. Nat. Hist.* 66:599-641.

- Clifford, H.T. and W. Stephenson. 1975. An introduction to numerical classification. Academic Press, Inc., NY, San Francisco, and London.
- Cushing, D.H. 1962. The work of grazing in the sea, pp. 207-226 In D.J. Crisp (ed.), Grazing in terrestrial and marine environments. Brit. Ecol. Symposium No.4, Blackwell, Oxford.
- Edmunds, M. 1987. Color in opisthobranchs. Amer. Malac. Bull. 5: 185-196.
- Field, J.G. 1971. A numerical analysis of changes in the soft-bottom fauna along a transect across False Bay, South Africa. J. Exp. Mar. Biol. Ecol. 7: 215-253.
- Frings, H. and C. Frings. 1965. Chemosensory bases of food-finding and feeding in *Aplysia juliana* (Mollusca, Opisthobranchia). Biol. Bull. 128: 211-217.
- Hadfield, M.G. 1963. The biology of nudibranch larvae. Oikos 14: 85-95.
- Hughes, R.N. and M.L.H. Thomas. 1971. Classification and ordination of benthic samples from Bedeque Bay, an estuary in Prince Edward Island, Canada. Mar. Biol. 10:227-235.
- Isaacs, A., J. Daintith, and E. Martin (Eds.) 1996. Concise science dictionary (3rd ed). Oxford Univ. Press.
- Kikuchi, T. 1980. Faunal relationships in temperate seagrass beds. p. 153-172 In R.C. Phillips and C.P. McRoy, eds. Handbook of seagrass biology: an ecosystem perspective. Garland STPM, NY and London.
- Ludwig, J.A. and J.F. Reynolds. 1988. Stastical ecology: a primer on methods and computing. John Wiley and Sons, Inc., New York.
- MacFarland, F.M. 1966. Studies of opisthobranchiate mollusks of the Pacific Coast of North America. Mem. Calif. Acad. Sci. vol 6. The Academy, San Francisco.
- Main, S.P. and C.D. McIntire. 1974. The distribution of epiphytic diatoms in Yaquina Estuary, Oregon (U.S.A.). Bot. Mar.17: 88-99.
- McRoy, C.P. and C. McMillan. 1977. Production ecology and physiology of seagrases. p.53-88 In C.P. McRoy and C. Helfferich, eds. Seagrass ecosystems: a scientific perspective. M. Dekker, NY.
- Miller, M.C. 1962. Annual cycles of some Manx nudibranchs, with a discussion of the problem of migration. J. Anim. Ecol. 31: 545-569.

- Muller-Parker, G. and E.R. Peele. 1998. Seasonal control of phytoplankton growth by anthropogenic nutrient loading in Padilla Bay National Estuarine Research Reserve. NOAA Technical Report Series OCRM/DMEM: Contract #NA470R088.
- Nichols, F.H. 1970. Benthic polychaete assemblages and their relationship to the sediment in Port Madison, Washington. *Mar. Biol.* 6: 48-57.
- Orth, R.J. and J. van Montfrans. 1984. Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: a review. *Aquat. Bot.* 18: 43-69.
- Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York.
- Phillips, R.C. 1984. The ecology of eelgrass meadows in the Pacific Northwest: a community profile. U.S. Fish and Wildlife Service, FWS/OBS-84/24.
- Reid, G.K. 1965. Ecology of inland waters and estuaries. 4th ed. Reinhold Publ. Corp., NY.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.
- Ruesink, J.L. 1998. Diatom epiphytes on *Odonthalia floccosa*: the importance of extent and timing. *J. Phycol.* 34: 29-38.
- Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. *Aquat. Bot.* 3: 55-63.
- Shaw, M.C. 1995. Diel predator-prey interactions between shiner perch and caprellid amphipods relative to caprellid distribution upon *Zostera*. M.S. Thesis, Western Washington University.
- Shaw, T. 1994. Temporal, diel, and vertical distribution variation of epiphyte grazers in a temperate eelgrass (*Zostera marina* L.) system. Master's Thesis. Western Washington University, Bellingham, Washington. Padilla Bay National Estuarine Research Reserve Reprint No. 21, Reprinted October, 1994.
- Smith, G.M. 1955. Cryptogamic botany, Vol 1: Algae and fungi. McGraw-Hill Book Company Inc., NY.
- Southward, A.J. 1962. Limpet grazing and the control of vegetation on rocky shores. p.265-274 In D.J. Crisp, ed. Grazing in terrestrial and marine environments. *Brit. Ecol. Symposium* No.4, Blackwell, Oxford.

- Thom, R.M. 1987. The biological importance of Pacific Northwest estuaries. *Northw. Environ. J.* 3: 21-42.
- Thom, R.M. and R.G. Albright. 1990. Dynamics of benthic vegetation standing-stock, irradiance, and water properties in central Puget Sound. *Mar. Biol.* 104:129-141.
- Thom, R.M., B. Miller, and M. Kennedy. 1991. Temporal patterns of grazers and vegetation in a temperate seagrass system. Univ. Washington, Fish. Res. Inst. FRE-UW-9112. Seattle.
- Thompson, T.E. 1962. Grazing and the life cycles of British nudibranchs. p.275-297 In D.J. Crisp, ed. *Grazing in terrestrial and marine environments*. Brit. Ecol. Symposium No.4, Blackwell, Oxford.
- Weaver, S. and K.B. Clark. 1981. Light intensity and color preferences of five Ascoglossan (=Sacoglossan) molluscs (Gastropoda: Opisthobranchia): a comparison of chloroplast-symbiotic and aposymbiotic species. *Mar. Behav. Physiol.* 7: 297-306.
- Whitlatch, R.B. 1977. Seasonal changes in the community structure of the macrobenthos inhabiting the intertidal sand and mud flats of Barnstable Harbor, Massachusetts. *Biol. Bull.* 152: 275-294.
- Whittam, T.S. and D. Siegel-Causey. 1981. Species incidence functions and Alaskan seabird colonies. *J. Biogeogr.* 8:421-425.
- Williams, S.L. and M.H. Ruckelshaus. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology* 74:904-918.
- Winer, B.J. 1971. *Statistical principles in experimental design*. McGraw-Hill, New York and London.
- Wood, E.J.F. 1972. Substratum: unicellular plants. p.1271-1276 In Otto Kinne, ed. *Marine ecology. A comprehensive, integrated treatise on life in oceans and coastal waters*. Vol 1, Environmental factors, Part 3. Wiley, NY.
- Wood, E.J.F., W.E. Odum, and J.C. Zieman. 1969. Influence of sea grasses on the productivity of coastal lagoons. p. 495-502 In A.A. Castaneres and F.B. Phleger, eds. *Lagunas Costeras, Simposio. Mem. Symp. Intern. Lagunas Costeras*. UNAM-UNESCO, Nov. 28-30, 1967.
- Zapata, O. and C. McMillan. 1979. Phenolic acids in seagrasses. *Aquat. Bot.* 7: 307-317.
- Zar, J.H. 1999. *Biostatistical analysis* (4th ed). Prentice Hall, New Jersey.

