



Padilla Bay

National Estuarine Research Reserve

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**TEMPORAL, DIEL, AND VERTICAL DISTRIBUTION
VARIATION OF EPIPHYTE GRAZERS IN A TEMPERATE
EELGRASS (*ZOSTERA MARINA* L.) SYSTEM**

Travis C. Shaw

May 1994

Publication No. 94-157

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A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Travis C. Shaw

May 1994

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TEMPORAL, DIEL, AND VERTICAL DISTRIBUTION VARIATION
OF EPIPHYTE GRAZERS IN A TEMPERATE EELGRASS
(*ZOSTERA MARINA* L.) SYSTEM

by

Travis C. Shaw

ABSTRACT. Temporal abundance and the vertical distribution of macroinvertebrate epiphyte grazers on eelgrass (*Zostera marina*) were examined over the diel cycle and through time. Discrete samples of eelgrass and associated epifauna were collected weekly from the apical, intermediate and basal fractions of *Zostera marina* during the day and at night. The abundance of individual grazer taxa fluctuated temporally during the study. In addition, three of four grazers exhibited a vertical diel migration. *Idotea resicata* and *Caprella californica* were more abundant on the apical fraction of the eelgrass shoot at night. However, the migration of caprellids occurred only during the period of maximum abundance. Similarly, the migration of the opisthobranch *Phyllaplysia taylori* from the basal to the intermediate eelgrass fractions occurred during the period of maximum abundance. The opisthobranch *Haminoea vesicula* was more abundant on the upper end of the eelgrass plant during day and night. The most numerous grazers, *Caprella californica* and *Phyllaplysia taylori*, reached peak abundance at different times and avoided direct competition. In addition, the epiphyte resource was partitioned vertically by the two opisthobranchs. Temporal and vertical distribution variation of grazers reflected niche separation and allowed wider use of the epiphyte resource.

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INTRODUCTION

The importance of eelgrass to production in nearshore marine waters is widely recognized. Measurements of net primary production in temperate North American eelgrass meadows range from 351 to 401 g C m⁻² yr⁻¹ (Thom, 1990; Penhale, 1977). In addition to the contribution to primary production, eelgrass meadows in the Pacific Northwest provide critical habitat for many organisms. Juvenile Dungeness crab (*Cancer magister*) use stands of *Zostera marina* as a refuge from predation (Armstrong et al., 1982). Eelgrass provides a substrate for the attachment of Pacific herring eggs (*Clupea harengus pallas*) (Thom, 1987). Eelgrass associated epiphyte grazers are utilized by outmigrating juvenile Chum salmon (*Oncorhynchus keta*) (Simentstad and Wissmar, 1985). In addition, grazing birds, including the black brant (*Branta bernicula*), are found in eelgrass meadows in large numbers during autumn and winter (Phillips, 1984).

Eelgrass meadows modify the abiotic environment. Dense meadows slow current velocities and stabilize sediments. These modifications influence the density and diversity of epifaunal populations. As a result, eelgrass systems have a major impact on secondary productivity relative to adjacent bare mudflats (Stoner, 1980).

The primary productivity of the eelgrass system is comprised of two components. The first component is the contribution made by the macrophyte. In the Pacific Northwest, the dominant eelgrass macrophyte is *Zostera marina*. The second major contributor to primary production is the epiphyte community. Epiphytes are a broad range of organisms that include both single celled and multicellular algae that

colonize the *Zostera* blades. The contribution of the epiphyte community to total primary production in Pacific Northwest eelgrass systems may be as high as 50% (Thom, 1990). In addition, epiphytes experience a higher rate of turnover than the macrophyte host. This combination of high productivity and turnover make epiphytes a major source of carbon to the food web of the eelgrass system.

The epiphyte community is dynamic. Species composition and biomass change seasonally (Borum et al., 1984). Community composition and biomass also change along a vertical gradient. The apical portions of older leaves hosts a thick diatom crust and a greater diversity of algae (Borum et al., 1984). In contrast, young basal leaf sections are colonized primarily by bacteria and the pennate diatom *Cocconeis* sp. (Sieburth and Thomas, 1973; Borum et al., 1984; Mazzella and Russo, 1989).

Epiphyte grazers are an important link between primary production of the eelgrass community and higher trophic levels. In addition, the grazing of surface epiphytes may influence the health and distribution of seagrasses (Caine, 1980; Howard, 1982; Robertson and Mann, 1982; Van Montfrans et al., 1982; Orth and Van Montfrans, 1984; Bronmark, 1985; Hootsmans and Vermaat, 1985; Howard and Short, 1986). *Zostera marina* heavily fouled by epiphytic growth exhibit a reduce rate of photosynthesis due to shading and competition for inorganic nutrients (Sand-Jensen, 1977). As a result, the removal of epiphytes may influence the vertical distribution of *Zostera marina*.

Epiphyte grazing may impact the host macrophyte by removing light competitors (Orth and Van Montfrans, 1984). Models based on the interaction of

epiphytic fouling and grazing under light saturation conditions predict reduced eelgrass production in the absence of grazers (Orth and Van Montfrans, 1984). As a result, grazer mediation of epiphytic fouling may affect light penetration to the leaf surface of the macrophyte and influence the depth at which *Zostera marina* can grow. This relationship between grazers and epiphytic fouling may be particularly important to the macrophyte in areas where environmental perturbation reduces light intensity in the water column.

Numerous studies have attempted to identify the important invertebrate grazers in Pacific Northwest eelgrass systems. Thom et al. (1991) found *Idotea ressecata* to be a major grazer in the intertidal meadows of Padilla Bay. The gastropod *Lacuna variegata* has been identified as the primary grazer in subtidal meadows north of Deception Pass (T. Nelson, personal communication). Caprellid amphipods have also been shown to graze substantial amounts of epiphyte biomass and have an impact on the growth of *Zostera marina* in the laboratory (Caine, 1980).

While the relative importance of individual grazers has been examined in relation to epiphyte grazing, how the epiphyte resource is utilized by the entire grazer community is unknown. The structure of the grazer community (species number, species composition, relative abundance) may influence the grazing pressure on the epiphyte community. This may be particularly important in locations where several taxa identified as primary grazers occur together.

The co-occurrence of several grazers implies some element of niche separation. Ecological niches can be separated several ways, including time of activity and

resource utilization. The abundance of epiphyte grazers in the *Zostera marina* ecosystem has been shown to vary seasonally (Thom et al., 1991). In sub-tropical seagrass meadows, the entire macroinvertebrate community structure changes significantly during the diel cycle (Greening and Livingston, 1982). Furthermore, members of the genus *Idotea* exhibit distinctive changes in activity over the diel cycle during different seasons (Horlyck, 1973).

Niche separation based on resource utilization can take the form of differential feeding. Mazzella and Russo (1989) documented the feeding habits of two epiphyte grazing gastropod species on the Mediterranean seagrass *Posidonia oceanica*. The spatial distribution of the gastropods along the seagrass blades corresponded to changes in the structure of the epiphyte community. As a result, the gastropod species coexisted along the blades of *Posidonia oceanica*. Faunal zonation has been documented along the shoots of *Zostera marina* (Caine, 1980) and may result from similar preferential feeding by epiphyte grazers.

This investigation explored the structure and vertical spatial distribution of the macroinvertebrate epiphyte grazer community along the blades of *Zostera marina* over the diel cycle and through the growing season. Community structure was examined in terms of species composition, species number and relative abundance on eelgrass shoots. In addition, niche separation between abundant grazer taxa was examined on the basis of resource utilization (vertical distribution) and time of activity (diel cycle and week).

Objectives

The specific objectives of this study were:

- I. Quantify species composition, species number, relative abundance and vertical distribution of macroinvertebrate epiphyte grazers in a temperate eelgrass (*Zostera marina* L.) meadow.
- II. Determine if changes in grazer spatial distribution and abundance occur over the diel cycle or through time.
- III. Attempt to determine if changes in grazer abundance and vertical distribution are correlated with changes in epiphyte biomass.

Statement of hypothesis

The structure of the macroinvertebrate epiphyte grazer community on *Zostera marina* will be dynamic. Abundance and vertical distribution of grazers will change over the diel cycle and with time. These changes in abundance and distribution will correlate with variation in epiphyte biomass and reflect ecological niche separation in the eelgrass meadow.

METHODS AND MATERIALS

Study site

This study was conducted at an intertidal site in Padilla Bay in northern Puget Sound. Padilla Bay is in Skagit County, Washington, and has been designated as a National Estuarine Research Reserve. Eelgrass meadows cover more than 3,200 ha of the bay and constitute one of the largest continuous seagrass beds on the west coast of the United States (Bulthuis, 1991). The study site was a circular area, 60 meters in diameter, off the east shore of March Point (Fig. 1). While the site is identified as intertidal on habitat maps of the bay, it remained inundated during the period of this investigation.

Zostera marina was the dominant macrophyte on the site with a mean density of 47.3 shoots m⁻² (n=24). The vertical height of the eelgrass plants averaged 143.6 cm (n=62) during the study. Water temperatures on the site ranged between 13° and 18°C (Appendix A). Salinity also varied during the study ranging from a low of 21 ppt to a high of 30 ppt (Appendix A). Temperature and salinity measurements were made with a YSI model 33 S-C-T meter.

Grazer sampling

Samples were collected weekly during July, August and September in 1993 (Table 1). Diurnal and nocturnal samples for each week were collected during the same 24 hour period. Collections were conducted only during flood tides with

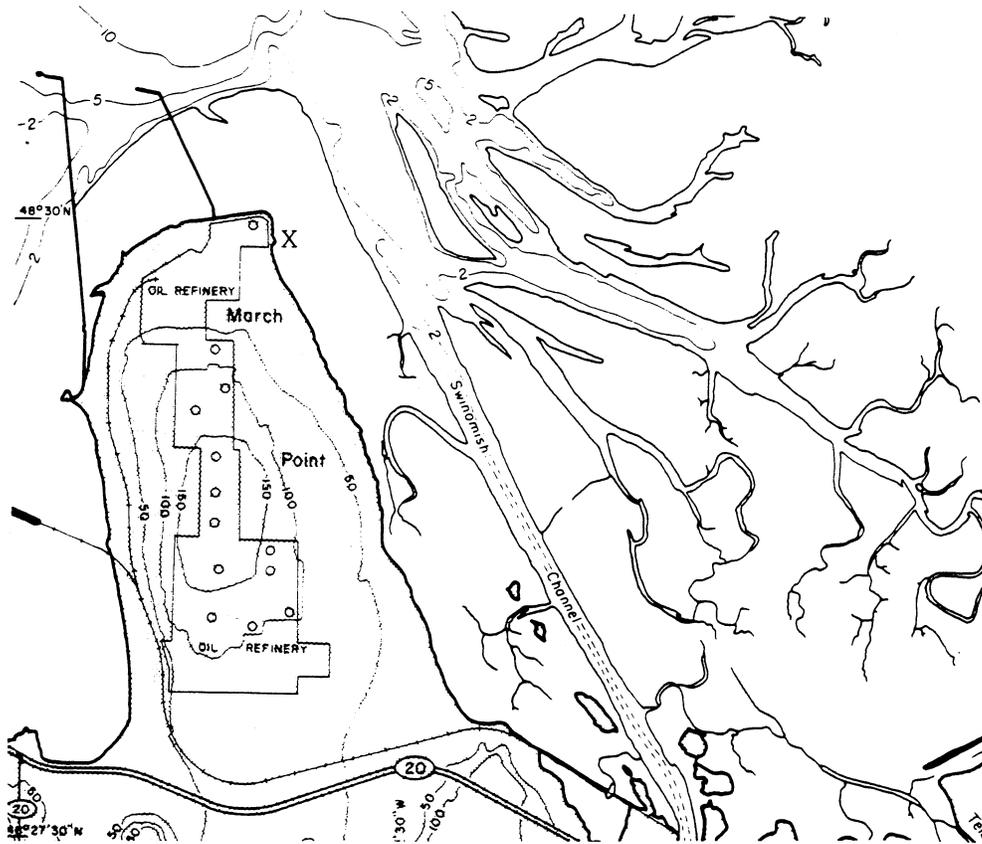


Figure 1. Map of the study area. The X marks the center of the circular study site.

Table 1. Collection dates in 1993 for epiphyte grazer samples.

Week number	Date
#1	30 June
#2	7 July
#3	15 July
#4	22 July
#5	4 August
#6	11 August
#7	16 August
#8	26 August
#9	30 August
#10	9 September
#11	15 September
#12	21 September

nocturnal sampling beginning one hour after darkness. Water depth at the time of sampling ranged between 3-4 meters. An exception occurred during the sampling for week 2 (7 July) when the depth at the beginning of the diurnal collection was only one meter.

Samples were collected using a modified Virnstein motile epifaunal sampler (Fig. 2). This apparatus allowed for the sampling of discrete 30 cm sections of eelgrass shoots and any associated epifauna. Mesh bags equipped with velcro closures were fitted into the sampler to isolate the sample at the time the apparatus was placed around the eelgrass plant.

SCUBA divers were provided a sample address by an assistant in a boat anchored in the center of the circular site. The sample address provided direction (compass heading) and distance from the center of the site. Random number tables generated on the software program Minitab were used to select distance and direction. In addition, the sample address included a randomly selected eelgrass fraction to be sampled (basal, intermediate or apical).

The diver would swim to the randomly selected point in the study site and settle to the bottom. The sampler was then placed around the appropriate eelgrass fraction and clamped in place. Shears were then used to cut away the portion of the eelgrass plant remaining outside the sampler and the velcro closures were pressed together. The sampler was then returned to the boat and the mesh bag was placed in a cooler containing seawater and an enclosed ice pack. This process was repeated until three replicates for each eelgrass fraction were collected.



Figure 2. Photograph of the modified Virstein Motile Epifaunal Sampler.

Diurnal samples were sorted on the beach immediately after collection. Grazers were separated from the eelgrass and fixed in 10% formalin in filtered seawater. Nocturnal samples were transported to the 10°C coldroom at Western Washington University and sorted the next morning. Samples in the coldroom were provided with aeration. Fixed grazer samples were transferred to bottles containing 70% isopropanol in filtered seawater after 48 hours. With the grazers preserved, counting and identification could then be conducted at leisure.

Macrophyte and epiphyte sampling

Site characterization and biomass samples were collected on 19 July, 16 August, 30 August and 21 September. Densities of macrophyte shoots were determined at six randomly chosen points in the study site. A 25 x 25 cm frame was placed on the substrate and the number of shoots within the frame was recorded. The shoots were then cut at the sediment surface and placed in a plastic bag. The bags were transported to lab in a cooler and stored in a -20°C freezer.

Macrophyte and epiphyte biomass determination

Macrophyte samples were thawed in a shallow pan containing freshwater to remove some of the adhering sediment. Once thawed, the height of individual shoots were measured. The shoots were then fractioned into apical, intermediate and basal 30 cm portions. This was done to mimic the fractionation that occurred to plants sampled

in the grazer collections. The area of the vertical fractions was then estimated using length and width measurements of the individual leaves.

The epiphytic material from each eelgrass fraction was then scraped onto preweighed Whatman GF/C glass fiber filters. Visible animals such as amphipods and polychaetes were removed from the epiphyte samples, but no effort was made to remove smaller organisms such as copepods. The filters were then dried at 100°C for a minimum of 72 hours. Dried filters were then weighed on an analytical balance and the dry biomass of the epiphytic material was determined by difference of mass.

The clean eelgrass fractions were also dried at 100°C for a minimum of 72 hours. Eelgrass sections not used directly for epiphyte biomass determination were cleaned and dried. The dry biomass was determined by simply weighing the dried eelgrass shoots on an analytical balance.

Statistical analysis

Experimental design allowed analysis of variance for individual grazer data based on the model of a 2 x 3 x 12 factorial. The three main factors were diel cycle, vertical distribution and week. All three factors were crossed in the linear model of the design and week was the only random factor. The factor diel cycle contained two levels, day and night. Basal, intermediate and apical fractions of the eelgrass shoot represented the three levels of the factor for vertical distribution. The twelve weeks of the study contributed the levels for the factor week.

The specific model for this analysis was:

$$Y_{ijkl} = \mu + D_i + V_j + DV_{ij} + W_k + DW_{ik} + VW_{jk} + DVW_{ijk} + \epsilon_{(ijk)l}$$

Diel cycle = D_i	$i=1..2$
Vertical Dist. = V_j	$j=1..3$
Week = W_k	$k=1..12$
Replicates	$l=1..3$

The sampling protocol for each grazer taxa allowed for three replicates from each level of each factor resulting in a total sample size of 216 eelgrass fractions. In some instances, the density of eelgrass at the site made it impossible to sample a single eelgrass shoot. As a result, some samples actually represent the mean number of grazers from 2-4 eelgrass plants. Factorial analysis was conducted on the Number Cruncher Statistical System using the GLM ANOVA procedure. The relationships between means of significant factors and interactions were determined with a Student-Newman-Keuls multiple range test (Underwood, 1981; Zar, 1984).

Data used in analysis of variance tests was examined for homoscedasticity. Hartley's and Cochran's tests for homogeneity of variances were used because of their lack of sensitivity to deviations from normality (Underwood, 1981). Both tests indicated a slight heterogeneity of variance for all four grazer taxa. However, the same tests performed following a square root transformation were found to be homoscedastic.

Assumptions of normality were not tested. However, balanced design and large sample size make ANOVA particularly robust to departures from normality

(Underwood, 1981). Since samples were collected randomly, compliance with the assumption of sample independence was assured. Random sampling, lack of gross heteroscedasticity, design balance and large sample size provided confidence in the stated probability of committing a type I error ($\alpha=0.05$).

The significance of changes in epiphyte biomass were tested by a one-way analysis of variance. A two-way analysis had been planned to test for variation in epiphyte biomass among sampling dates and vertical fractions of the eelgrass shoots. Unfortunately, the loss of some samples made this approach impossible while maintaining compliance to the assumptions of ANOVA. Instead, the vertical distribution data were pooled and the analysis was conducted for sampling dates alone. The resulting analysis of variance tested for differences among four dates ($k=4$) with five replicates for each treatment ($n=20$).

Since grazers occurred together on the eelgrass sections, independent samples were not available to make parametric comparisons between grazer taxa. As a result, two non-parametric techniques were used to test the relationship between grazer taxa. First, Spearman's rank correlation was used to test for interspecific covariation (Ludwig and Reynolds, 1988). Secondly, Schluter's (1984) test for interspecific association was used after the data were converted into a presence and absence format. Chi-square 2 x 2 contingency tables were then constructed to determine the association between specific species pairs (Zar, 1984; Ludwig and Reynolds, 1988).

Both non-parametric tests were conducted with software provided by Ludwig and Reynolds (1988). Covariation between epiphyte biomass and the temporal abundance of grazer taxa was examined using simple linear correlation.

RESULTS

Caprella californica

The abundance of caprellid amphipods varied with time. Furthermore, differences in caprellid abundance over the diel cycle and among vertical fractions of the eelgrass shoot changed temporally (Fig. 3). At all three eelgrass heights, the mean number of caprellids peaked in late July and then declined (Fig. 3). Caprellid abundance followed a similar pattern in diurnal and nocturnal samples at the basal and intermediate fractions of the eelgrass plant. However, nighttime abundances of caprellids were much higher on the apical portions of the eelgrass shoots during the period of maximum abundance (Fig. 3).

Analysis of variance for the caprellid amphipod data confirmed these patterns. The main factor week was significant and accounted for 69% of the treatment effect (Table 2). Temporal abundance of caprellids was greatest during weeks 2-5 (Fig. 3). Mean abundance of caprellids, averaged over the three vertical fractions and the diel cycle, was significantly greater during weeks 3 and 4 (Table 3). Weeks 2 and 5 contained significantly more caprellids than week 8-12 but were not significantly different from weeks 1,6 and 7 (Table 3).

The interaction between all three main factors was also significant and contributed 14% to the treatment effect (Table 2). Significant interaction of the main factors (diel cycle, vertical distribution and week) indicates that these factors are not independent. In this case, lack of independence implies that the effect of diel cycle and

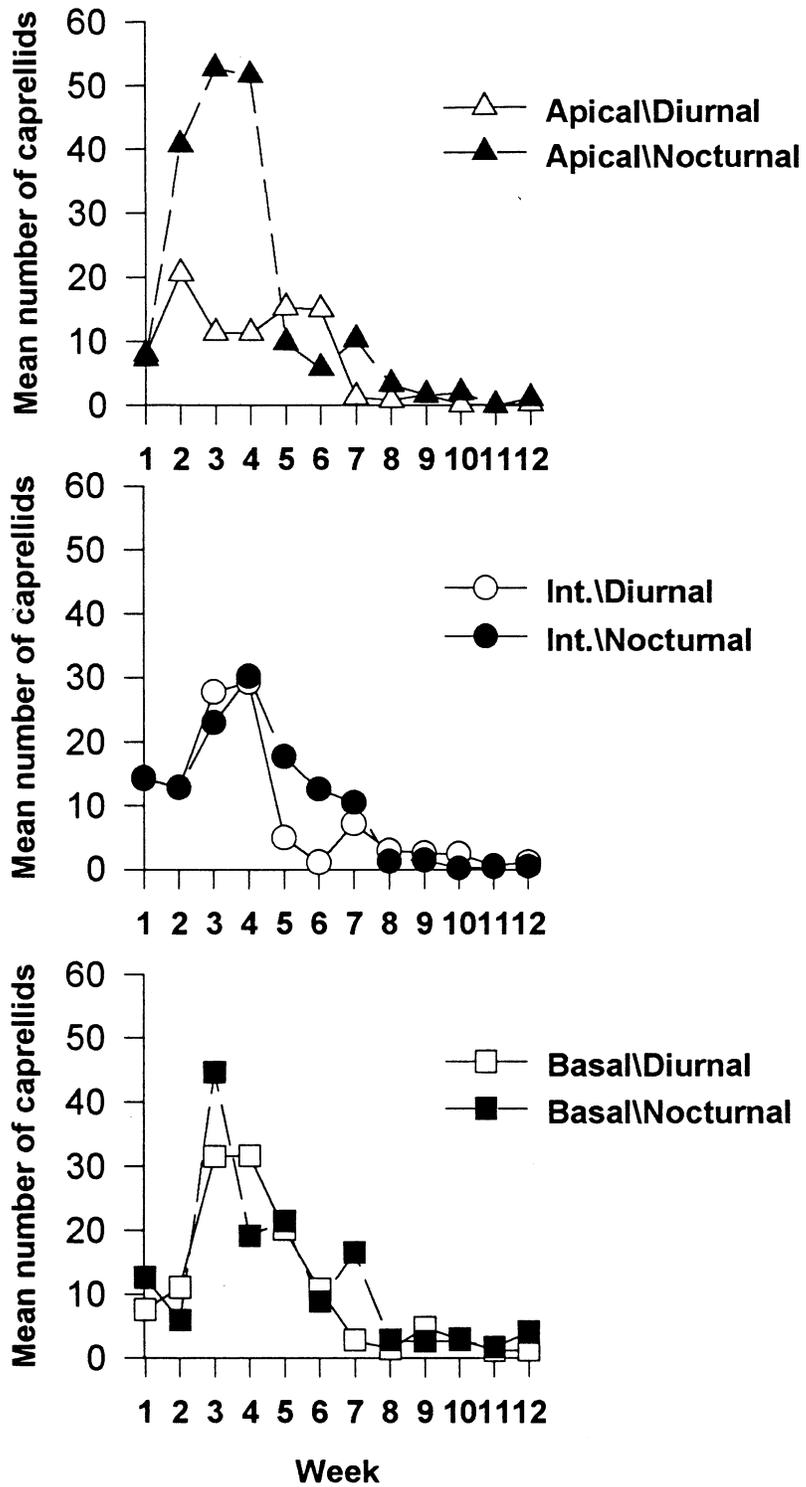


Figure 3. Temporal abundance of caprellid amphipods over the diel cycle on basal, intermediate and apical fractions of eelgrass shoots. (weeks 1-4=July, weeks 5-8=Aug.; weeks 8-12=Sept.) (n=216)

Table 2. Analysis of variance for caprellid amphipods. Treatment effect represents the percentage of the sum of squares each factor or interaction contributes to treatment sum of squares. ($\alpha=0.05$)

Source	df	F	P	% trt. effect
Diel	1	5.84	0.034	2.3%
Vert. Dist.	2	0.53	0.593	0.4%
Week	11	16.82	<0.000	69.0%
Diel x Vert. Dist.	2	1.41	0.265	1.8%
Diel x Week	11	1.05	0.410	4.3%
Vert. Dist. x Week	22	1.01	0.451	8.3%
D x V x W	22	1.70	0.035	13.9%
Error	144			
Total	215			

Table 3. Results of the Student-Newman-Keuls multiple range test of the main factor week for caprellid amphipods. Means connected by an underline are not significantly different from each other. ($\alpha=0.05$)

Means:											
0.6	1.4	1.8	2.1	2.5	8.1	9.0	10.7	14.9	17.3	28.9	31.8
Week:											
11	12	10	8	9	7	6	1	5	2	<u>4</u>	<u>3</u>

week on caprellid abundance varies with levels of vertical distribution. The interaction of the three main factors is demonstrated at the apical fraction of the eelgrass shoots during the period of maximum abundance. Mean caprellid abundance was greater on the apical fraction of the eelgrass shoot at night than during the day (Fig. 3). However, differences among nocturnal and diurnal means only occurred during the period when temporal abundance of caprellids was greatest. Specifically, the mean number of caprellids collected on the apical eelgrass fractions at night were significantly greater than means for any other combination of diel cycle and height during weeks 2-4 (Fig. 3). Statistical significance was determined with a multiple range test of all means. Increased abundance of caprellids on the apical eelgrass fraction at night during the period of maximum abundance probably caused the significance of the diel cycle factor (Table 2). Nocturnal and diurnal means followed similar patterns on the basal and intermediate fractions of the eelgrass shoots (Fig. 3).

Lack of significance for the vertical distribution factor is not surprising given the power of the experimental design. Calculation of the power for this factor after the study was complete estimated the probability of committing a type II error at 65%. In contrast, the power for detecting a significant difference within the interaction of diel cycle and vertical distribution was a more robust 82%.

Idotea ressecata

The vertical distribution of *Idotea ressecata* varied with time and over the diel cycle. In addition, the abundance of isopods changed with time during the study.

Temporal abundance of isopods peaked early in the study then remained unchanged (Fig. 4). The vertical distribution of isopods also changed between day and night, particularly at the apical fraction of the *Zostera* turions (Fig. 4).

This interaction between diel cycle and vertical distribution was significant and accounted for 8.4% of the total treatment effect (Table 4). A significant interaction implies that these factors are not independent. Isopod abundance at different height levels of eelgrass varies with the time of day (Fig. 5). Results of the multiple range test found the mean number of isopods collected on the apical fractions of eelgrass shoots was significantly greater at night than during the day.

Vertical distribution of *Idotea* was also varied with time. In fact, the interaction between vertical distribution and week was significant and accounted for the majority of the treatment effect (Table 4). This interaction is evident when weeks early in the study are contrasted with samples collected at the end of the study. The mean number of isopods collected in the apical and intermediate samples during week 2 were greater than the basal mean (Fig. 6). All other comparisons between vertical fractions within each week were not significant in the multiple range test. This change in vertical distribution corresponds to shifts in temporal abundance of *Idotea*. Within the main factor week, the mean number of isopods collected during week 2 was significantly greater than weeks 1 and 8-11 (Table 5).

Power of the experimental design to detect differences in non-significant fixed main factors was low. The estimated power for the test of significance for the factor diel cycle was 0.35. Similarly, the power for the vertical distribution factor was 0.30.

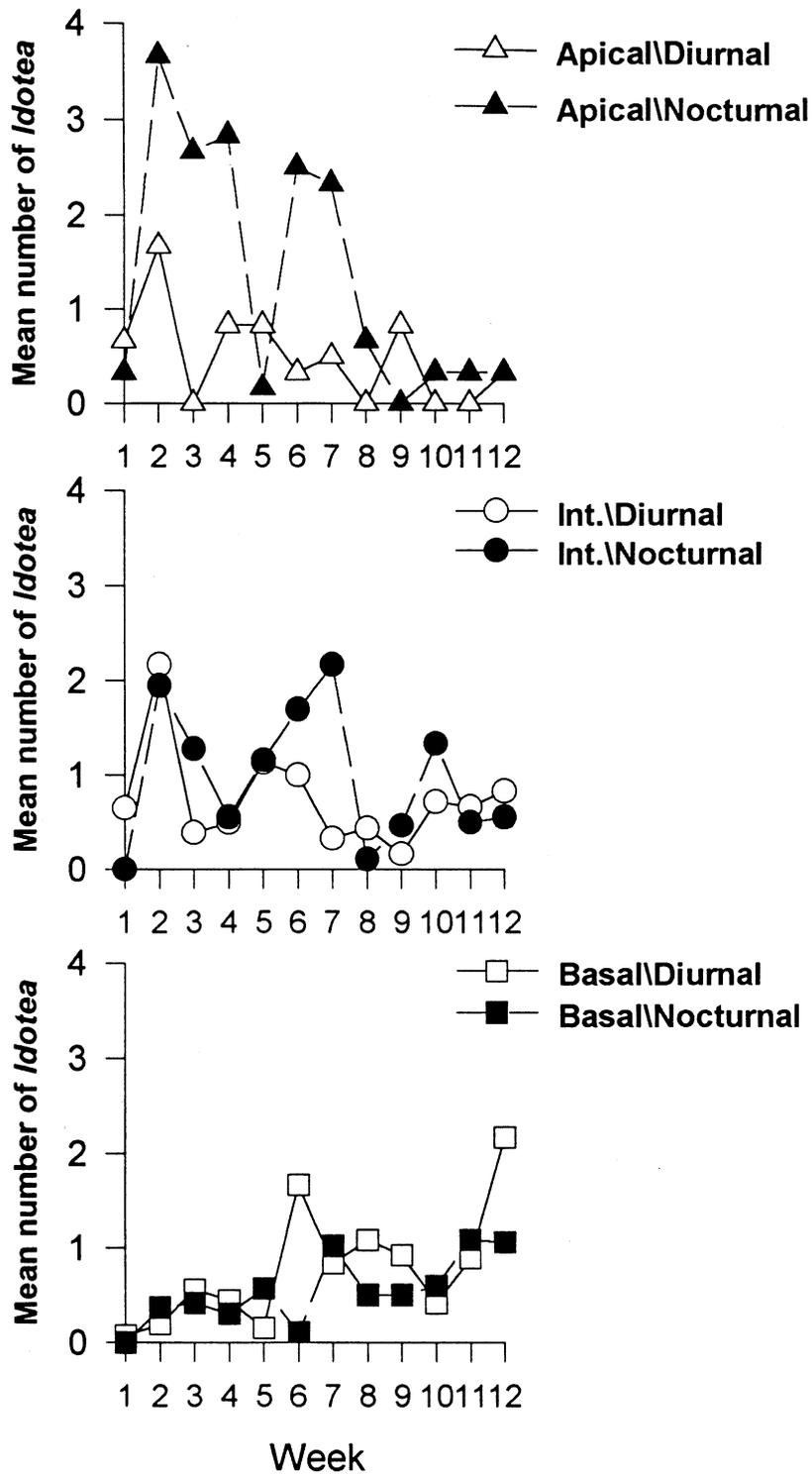


Figure 4. Temporal abundance of *Idotea resecata* over the diel cycle on basal, intermediate and apical fractions of eelgrass shoots. (weeks 1-4=July; weeks 4-8=Aug.; weeks 9-12=Sept.) (n=216)

Table 4. Analysis of variance for *Idotea ressecata*. Treatment effect represents the percentage of the sum of squares each factor or interaction contributed to treatment sum of squares. ($\alpha=0.05$)

Source	df	F	P	% trt. effect
Diel	1	2.81	0.122	3.3%
Vert. Dist.	2	0.72	0.496	2.1%
Week	11	2.90	0.012	23.8%
Diel x Vert. Dist.	2	5.44	0.002	8.4%
Diel x Week	11	1.55	0.118	12.8%
Vert. Dist. x Week	22	1.98	0.009	32.5%
D x V x W	22	1.04	0.421	17.1%
Error	144			
Total	215			

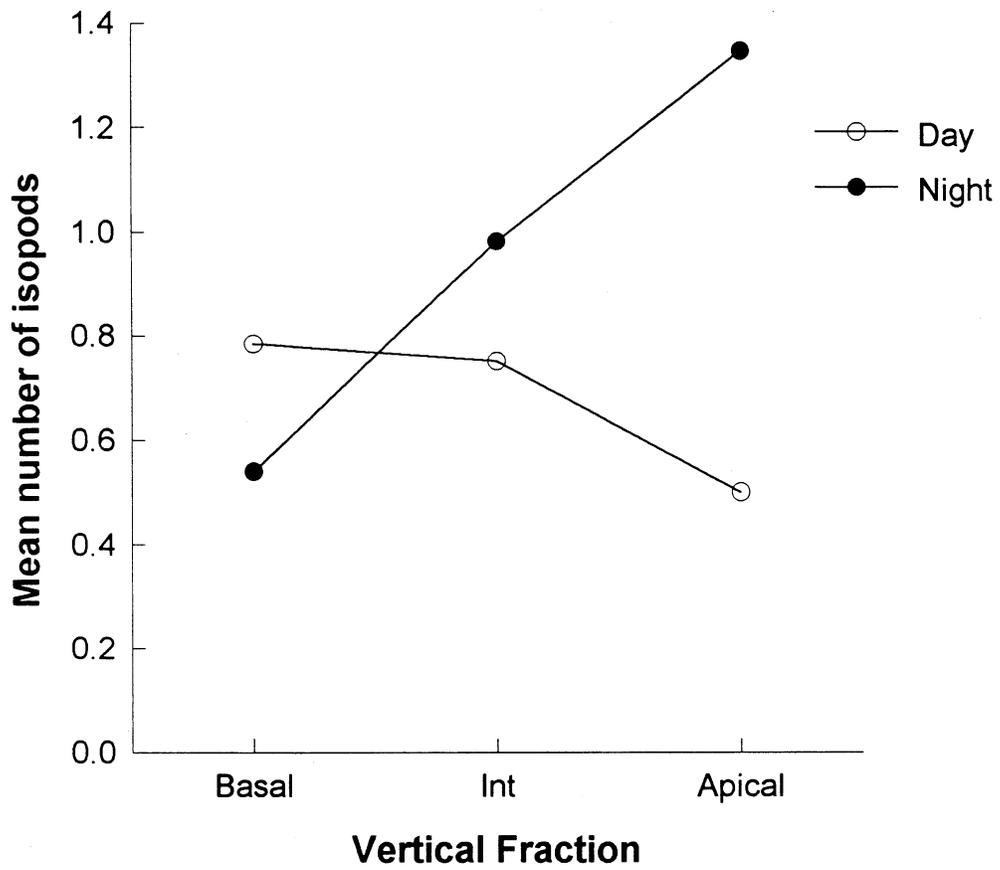


Figure 5. Vertical distribution of *Idotea resecata* over the diel cycle.

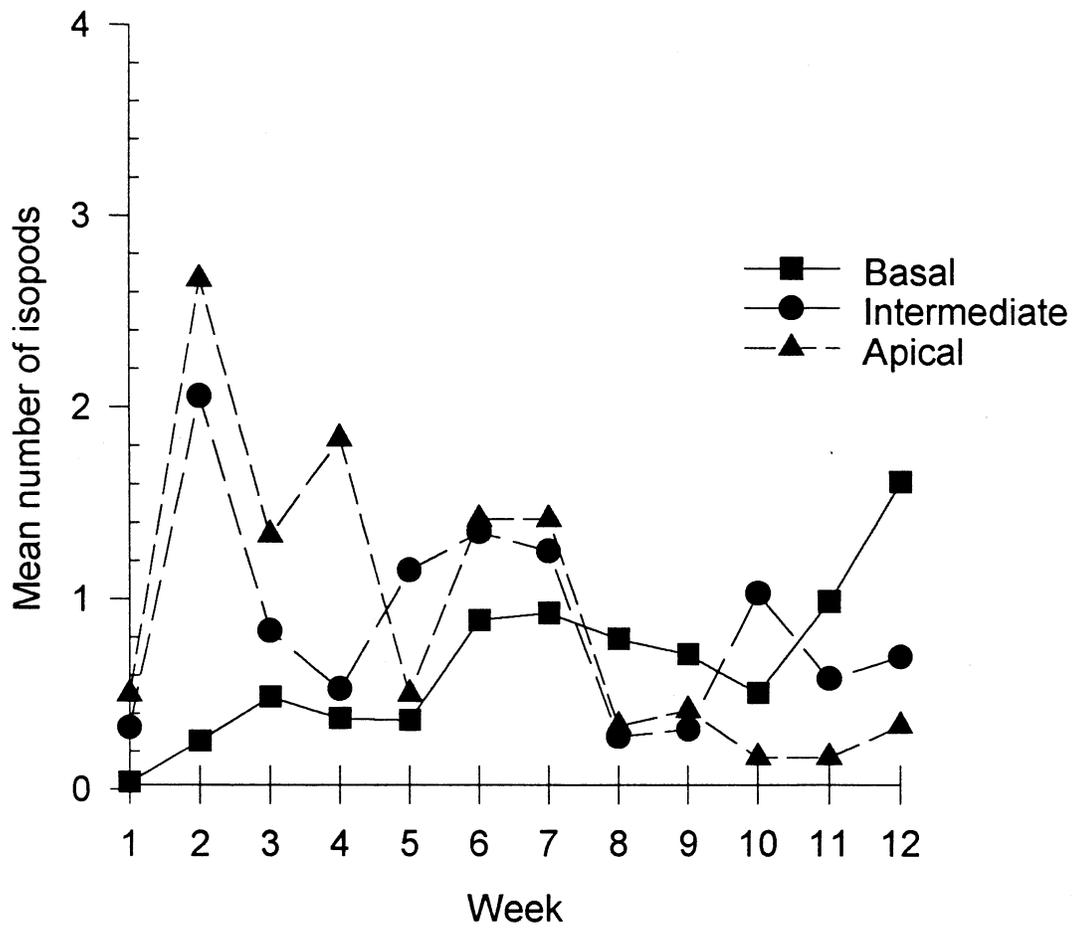


Figure 6. Temporal abundance of *Idotea ressecata* on the basal, intermediate and apical fractions of eelgrass shoots.

Table 5. Results of the Student-Newman-Keuls multiple range test of the main factor week for *Idotea resecata*. Means connected by an underline are not significantly different from each other. ($\alpha=0.05$)

Means:

0.29 0.47 0.48 0.57 0.58 0.67 0.88 0.88 0.91 1.2 1.2 1.7

Week:

1 8 9 10 11 5 12 3 4 7 6 2

Phyllaplysia taylori

The vertical distribution of *Phyllaplysia taylori* varied over the diel cycle. Changes in the vertical distribution of *Phyllaplysia* between basal and intermediate fractions of the eelgrass shoot at night corresponded with fluctuations in temporal abundance (Fig. 7). The mean number of *Phyllaplysia* sampled on the basal 30 cm of eelgrass turions declined at night but increased at the intermediate portion during August (Fig. 7). *Phyllaplysia* was rarely found on the apical end of *Zostera marina* turions. In addition, *Phyllaplysia* was almost completely absent from the eelgrass meadow in July (Fig. 7).

The impact of the interaction of the three main factors (diel cycle, vertical distribution and week) is illustrated when contrasted with the results of the main factors alone. The main factors vertical distribution and week were significant with week accounting for 32% of the treatment effect (Table 6). Averaged over all twelve weeks of the study, significantly more sea hares were collected on the basal portion of the eelgrass shoot. However, the interaction between diel cycle and vertical distribution was also significant (Table 6). At night, the abundance of *Phyllaplysia* appears to decrease at the basal fraction of the eelgrass shoot while increasing at the intermediate eelgrass fraction (Fig. 8). Despite the significance of this interaction in the analysis of variance, the multiple range test was unable to detect differences between nocturnal and diurnal means at any of the vertical eelgrass fractions. Estimated power of experimental design to detect differences over the diel cycle alone was <0.30.

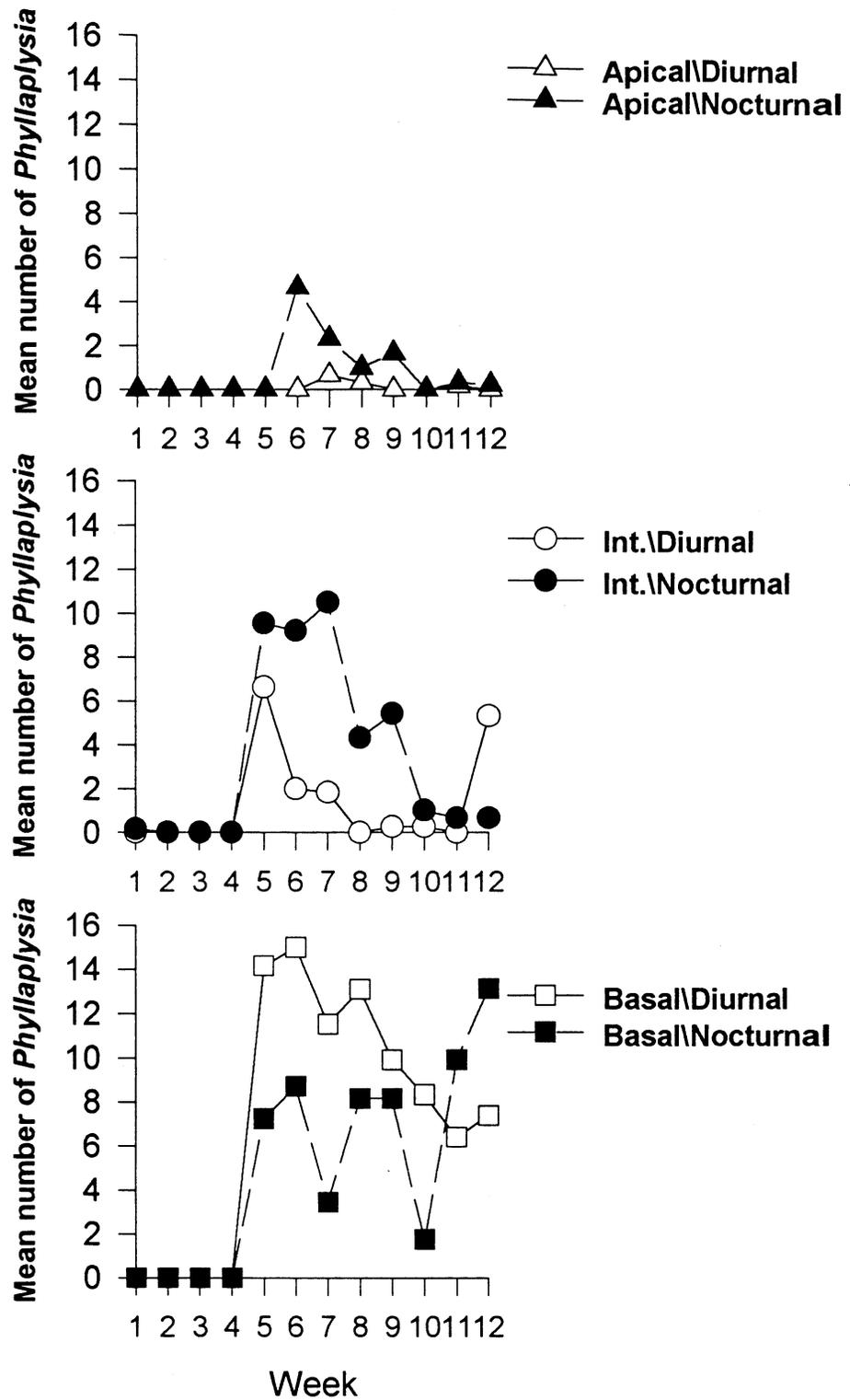


Figure 7. Temporal abundance of *Phyllaplysia taylori* over the diel cycle on the basal, intermediate and apical fractions of the eelgrass shoot. (weeks 1-4=July; weeks 5-8=Aug.; weeks 9-12=Sept.) (n=216)

Table 6. Analysis of variance for *Phyllaplysia taylori*. Treatment effect represents the percentage of the sum of squares each factor or interaction contributed to treatment sum of squares. ($\alpha=0.05$)

Source	df	F	P	% trt. effect
Diel	1	0.58	0.463	<.1%
Vert. Dist.	2	15.56	<0.000	29.1%
Week	11	12.59	0.049	31.6%
Diel x Vert. Dist.	2	3.53	<0.000	4.1%
Diel x Week	11	0.63	0.805	1.6%
Vert. Dist. x Week	22	4.10	<0.000	20.6%
D x V x W	22	2.56	<0.000	12.8%
Error	144			
Total	215			

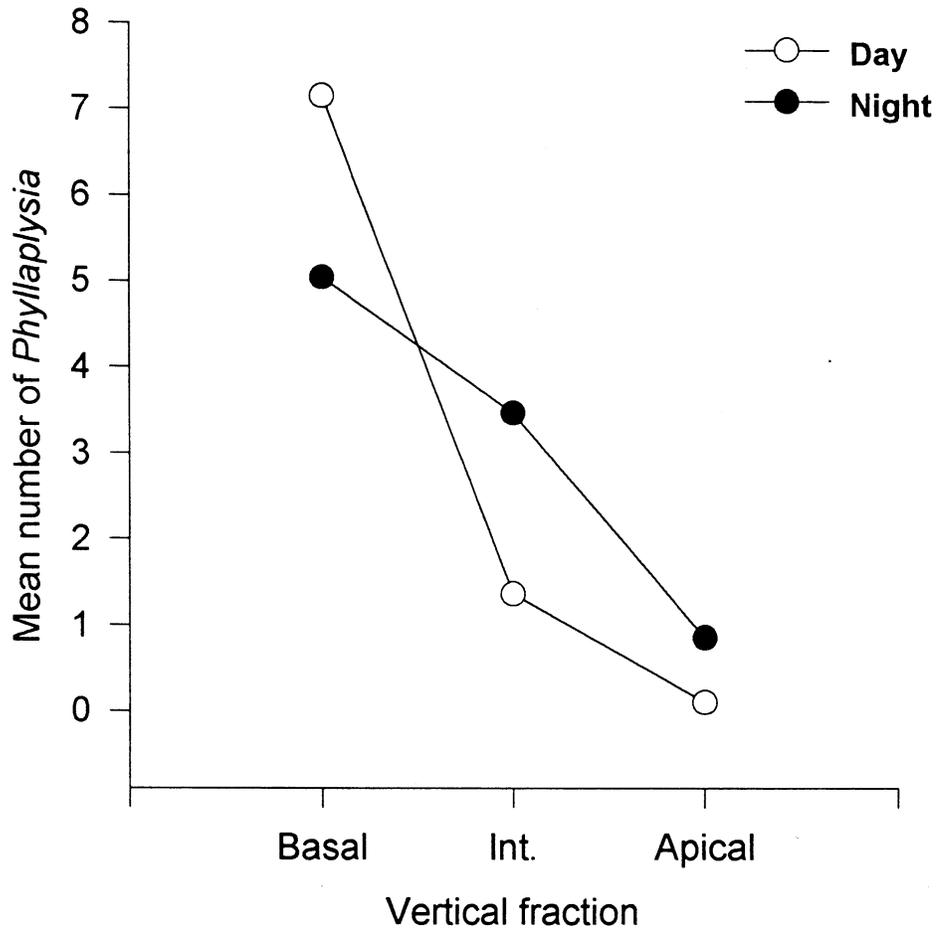


Figure 8. Vertical distribution of *Phyllaplysia taylori* over the diel cycle.

While the first order interactions suggest *Phyllaplysia* was more abundant on the basal portion of the *Zostera* turions, the higher order interaction reveals an additional pattern. The mean number of *Phyllaplysia* was zero or not significantly different from zero at all levels of vertical distribution at both day and night during weeks 1-4 (Fig. 7). A dramatic increase in the abundance of *Phyllaplysia* took place beginning in week 5 (Fig. 7). In August and early September, *Phyllaplysia* was more abundant on the basal fractions of eelgrass plants than the intermediate or apical fractions during the day. At night, the mean number of *Phyllaplysia* on the basal fractions decreases but increases on the intermediate eelgrass fractions (Fig. 7). The multiple range test for all means confirmed this pattern. For weeks 5-10, the mean number of *Phyllaplysia* on the basal eelgrass fractions were significantly greater during the day than at night each week. Conversely, the mean number of *Phyllaplysia* collected on the intermediate eelgrass fractions at night were significantly greater than daytime collections.

The period when *Phyllaplysia* demonstrates a diel shift in abundance between basal and intermediate fractions corresponds with the period of maximum abundance. When the main factor week is considered alone; the mean number of *Phyllaplysia* was significantly greater during weeks 5-7 than weeks 1-4 and 10 (Table 7).

Table 7. Results of the Student-Newman-Keuls multiple range test of the main factor week for *Phyllaplysia taylori*. Means connected by an underline are not significantly different from each other. ($\alpha=0.05$)

Means:											
0.0	0.0	0.0	0.03	1.89	2.91	4.24	4.45	4.49	5.05	6.27	6.60
Week:											
4	2	3	1	10	11	9	12	8	7	5	6

Haminoea vesicula

The temporal abundance of *Haminoea vesicula* varied and there were differences in vertical distribution. *Haminoea* appeared to be more abundant in July and August than September (Fig. 9). Furthermore, more of these opisthobranchs were collected on the apical and intermediate eelgrass fractions than the basal fraction (Fig. 9). Analysis of variance of the data collected for *Haminoea vesicula* reveals two significant main effects (Table 8). The factor week was significant and contributed 30% to the deviation of the treatment effect. Vertical distribution was also significant and accounted for 15% of the total treatment sum of squares (Table 8). Specifically, significantly more *Haminoea* were sampled at the apical and intermediate fractions than in basal fractions (Fig. 10).

While the ANOVA found significant difference in the factor week, the Student-Newman-Keuls multiple range test did not discern any significant differences between means (Table 9). Despite this inability to make a statement regarding statistical significance, the highest means occur during week 2, 3, 4 and 6 (Fig. 11). The estimated power of the performed test to detect differences in the main factor diel cycle was < 0.30 . Likewise, the test for the interaction of the fixed factors diel cycle and vertical distribution had a power < 0.30 .

Association Analysis

The variance ratio (VR) calculated from presence and absence data was greater than 1 (VR=1.18) (Table 10). The significance of this statistic was tested with

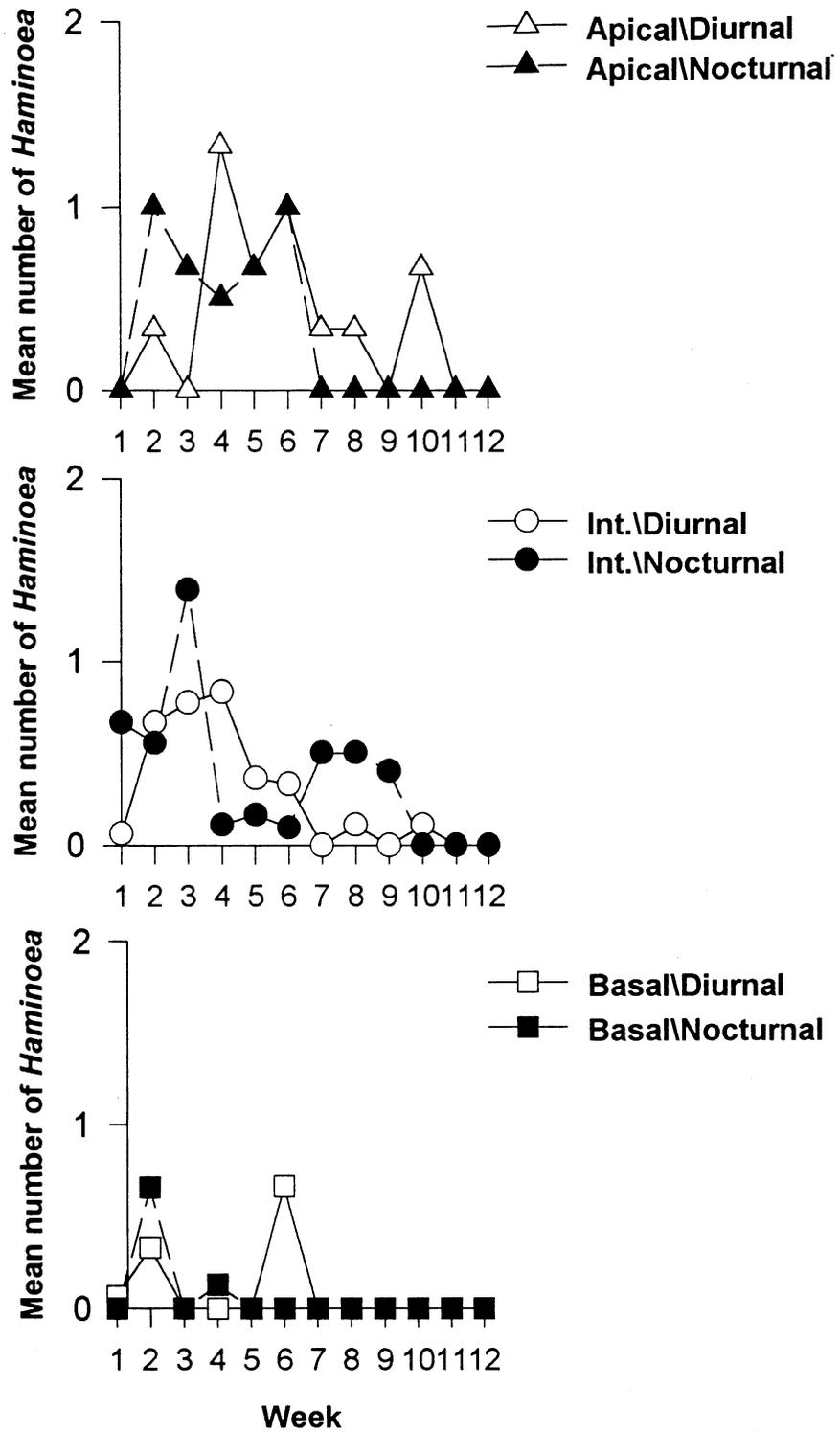


Figure 9. Temporal abundance of *Haminoea vesicula* over the diel cycle on the basal, intermediate and apical fractions of eelgrass shoots. (weeks 1-4=July; weeks 5-8=Aug.; weeks 9-12=Sept.) (n=216)

Table 8. Analysis of variance for *Haminoea vesicula*. Contribution to treatment effect represents the percentage the sum of squares for each factor or interaction contributed to treatment sum of squares. ($\alpha=0.05$)

Source	df	F	P	% trt. effect
Diel	1	0.05	0.819	<0.1%
Vert. Dist.	2	5.55	0.011	14.6%
Week	11	2.62	0.004	29.9%
Diel x Vert. Dist.	2	0.91	0.416	1.2%
Diel x Week	11	0.93	0.515	10.6%
Vert. Dist. x Week	22	1.27	0.202	29.0%
D x V x W	22	0.64	0.887	14.7%
Error	144			
Total	215			

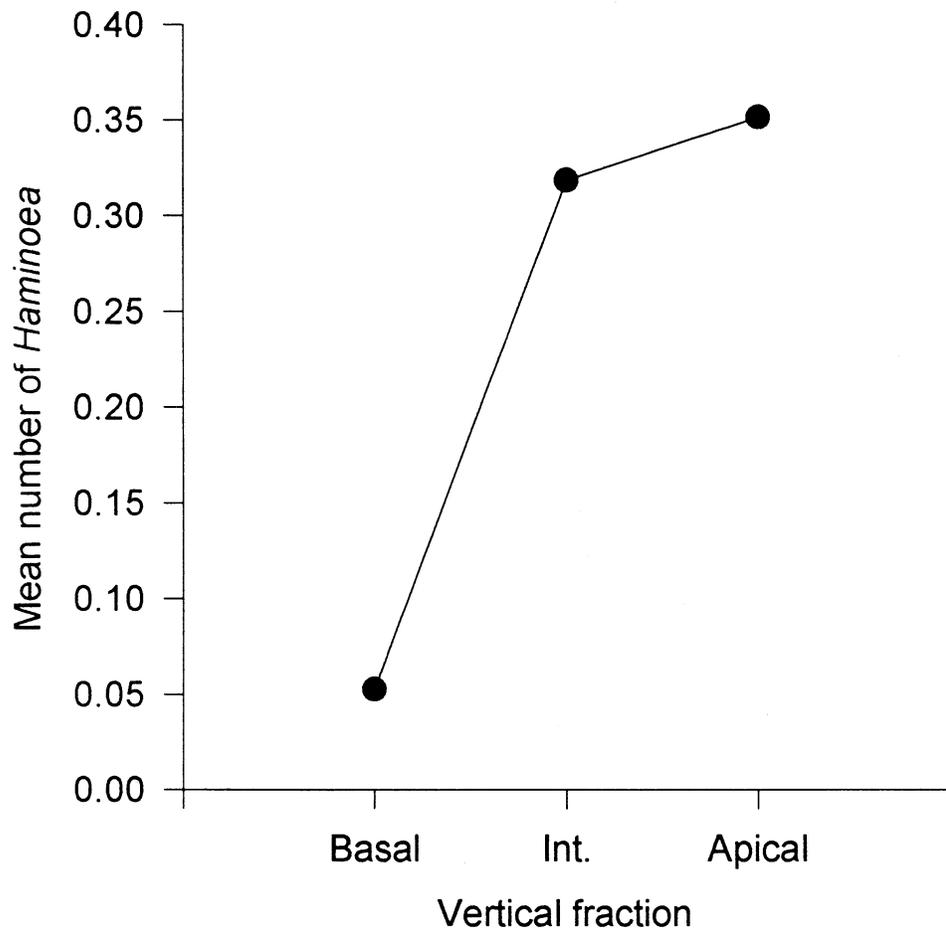


Figure 10. Vertical distribution of *Haminoea vesicula* on eelgrass shoots.

Table 9. Results of the Student-Newman-Keuls multiple range test of the factor week for *Haminoea vesicula*. Means connected by an underline are not significantly different from each other. ($\alpha=0.05$)

Means:											
0	0	0.07	0.13	0.13	0.14	0.16	0.31	0.47	0.48	0.49	0.52
Week:											
11	12	9	10	1	7	8	5	3	4	2	6

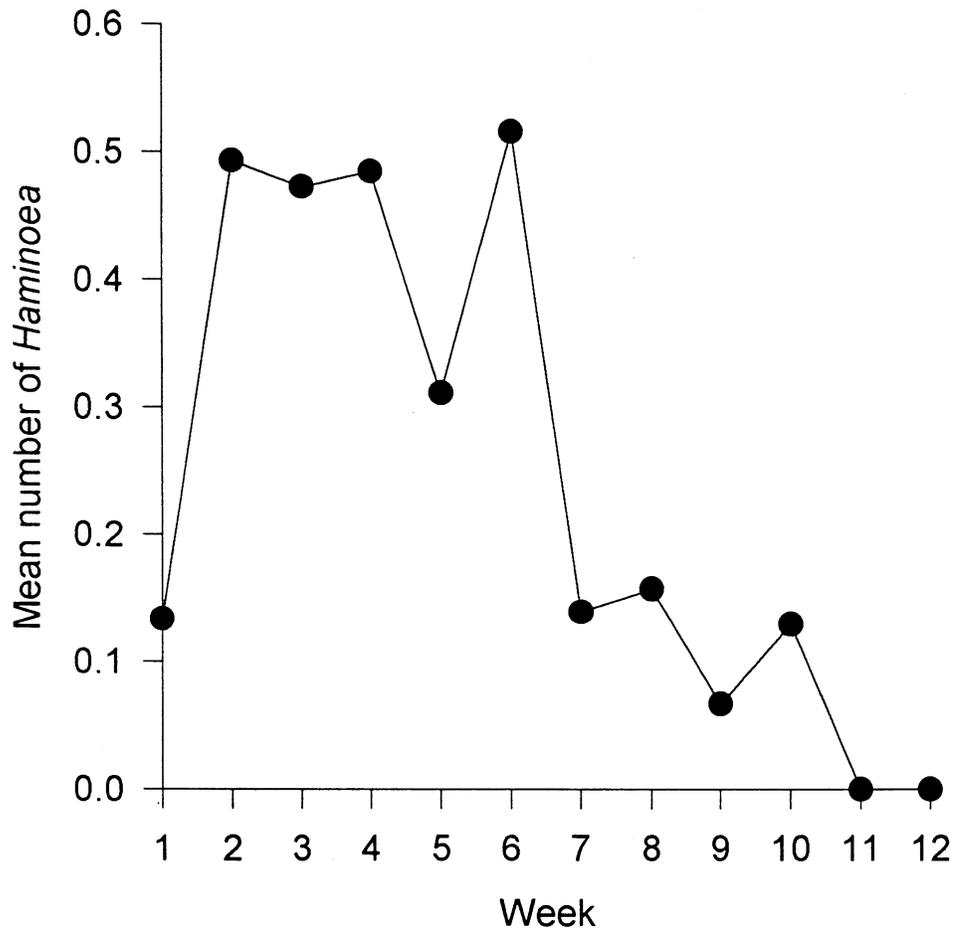


Figure 11. Temporal abundance of *Haminoea vesicula*. (weeks 1-4=July; weeks 5-8=Aug.; weeks 9-12=Sept.)

Table 10. Association analysis for all four grazer taxa based on presence and absence in sampling units. The variance ratio (VR) was significantly greater than one if the test statistic (W) was outside the 90% probability limits of the Chi-square distribution at 200 df ($168_{0.05} < W < 234_{0.95}$). Species pairs were tested for independence with 2 x 2 contingency tables. Independence is rejected if the corrected Chi-square statistic for each comparison is greater than 3.84 at 1 df. (* denotes significant associations; n/s identifies non-significant associations) (1 = *Caprella californica*; 2 = *Idotea resicata*; 3 = *Phyllaplysia taylori*; 4 = *Haminoea vesicula*)

Species		Association Indices			
Pair	Assoc.	χ^2	Ochiai	Dice	Jaccard
1 vs 2	+	5.780*	0.779	0.767	0.622
1 vs 3	n/s	0.755	0.653	0.619	0.448
1 vs 4	n/s	2.907	0.506	0.416	0.263
2 vs 3	+	5.834*	0.608	0.601	0.429
2 vs 4	n/s	0.279	0.415	0.374	0.230
3 vs 4	-	5.355*	0.235	0.224	0.126

Schluter's W test statistic. The value of W ($W=255.57$) lies outside the 90% probability limits provided by the Chi-square distribution ($168.0_{0.05} < W < 234.0_{0.95}$). Since VR is significantly greater than one, the null hypothesis of no association is rejected. As a result, a positive association is suggested among all four grazer taxa.

Pairwise comparisons of presence and absence between different taxa were analyzed using Chi-square 2×2 contingency tables. The null hypothesis of independence was rejected for three pairs of grazers. The pairwise comparison between caprellids and isopods resulted in a Chi-square value of 5.78 which was greater than the critical value of 3.84 (Table 10). Similarly, isopods and *Phyllaplysia* were not independent. The same was true for *Phyllaplysia* and *Haminoea* (Table 10).

Association indices provide a qualitative measure for the strength of the association. The highest association indices are provided for the association between caprellids and isopods (Table 10). The association between *Idotea* with *Phyllaplysia* appears slightly weaker as indicated by lower values for all three indices (Table 10). One characteristic of these association indices is a lower value returned for negative associations. As a result, the association of *Haminoea* with *Phyllaplysia* appears negative based on the values of the association indices (Table 10).

Correlation analysis indicated several species pairs covaried during the study. Caprellid amphipods were positively correlated with *Idotea resecata* and *Haminoea vesicula* (Table 11). In contrast, caprellids and *Phyllaplysia taylori* were negatively correlated. Both opisthobranch species were also negatively correlated (Table 11).

Table 11. Analysis of covariation between grazer taxa based on ranked data. (Correlation coefficients marked by * are significantly different from 0 at the 5% probability level.)

Spearman's Rank Correlation				
	<i>Caprella</i>	<i>Idotea</i>	<i>Phyllaplysia</i>	<i>Haminoea</i>
<i>Caprella</i>	1.00	0.225*	-0.183*	0.203*
<i>Idotea</i>		1.00	0.138	0.086
<i>Phyllaplysia</i>			1.00	-0.188*
<i>Haminoea</i>				1.00

Relative Abundance

Relative abundance reflected the temporal changes in individual grazer abundance but did not reflect any difference over the diel cycle. Caprellids dominated the grazer community during the first four weeks of the study when they made up 90% or greater of the sampled community during both day and night (Figure 12). After week 4, *Phyllaplysia* appeared on the eelgrass shoots. *Phyllaplysia* gradually increased in abundance during the remaining eight weeks of the study and increased in relative abundance. Like caprellids, there was no difference in relative abundance between day and night samples (Figure 12).

The relative abundance of *Idotea* increased throughout the 12 weeks of the study. However, the percentage of *Idotea* never exceeded 20% and did not change appreciably over the diel cycle (Figure 12). *Haminoea vesicula* remained at relative low abundance and rarely exceeded 5% of the sampled community.

Epiphyte biomass

Epiphyte biomass varied significantly during the study. The mean dry weight of epiphytes per cm² along the eelgrass shoots was significantly greater on July 19 ($\bar{X}=0.893 \mu\text{g dry wt. cm}^{-2}$; $P=0.03$) than all other sampled dates (Table 12). Biomass samples taken August 16, August 30 and September 21 were not significantly different from each other.

The abundance of most grazer taxa and epiphyte biomass covaried. Only the temporal abundance of isopods did not correlate with epiphyte biomass (Table 13).

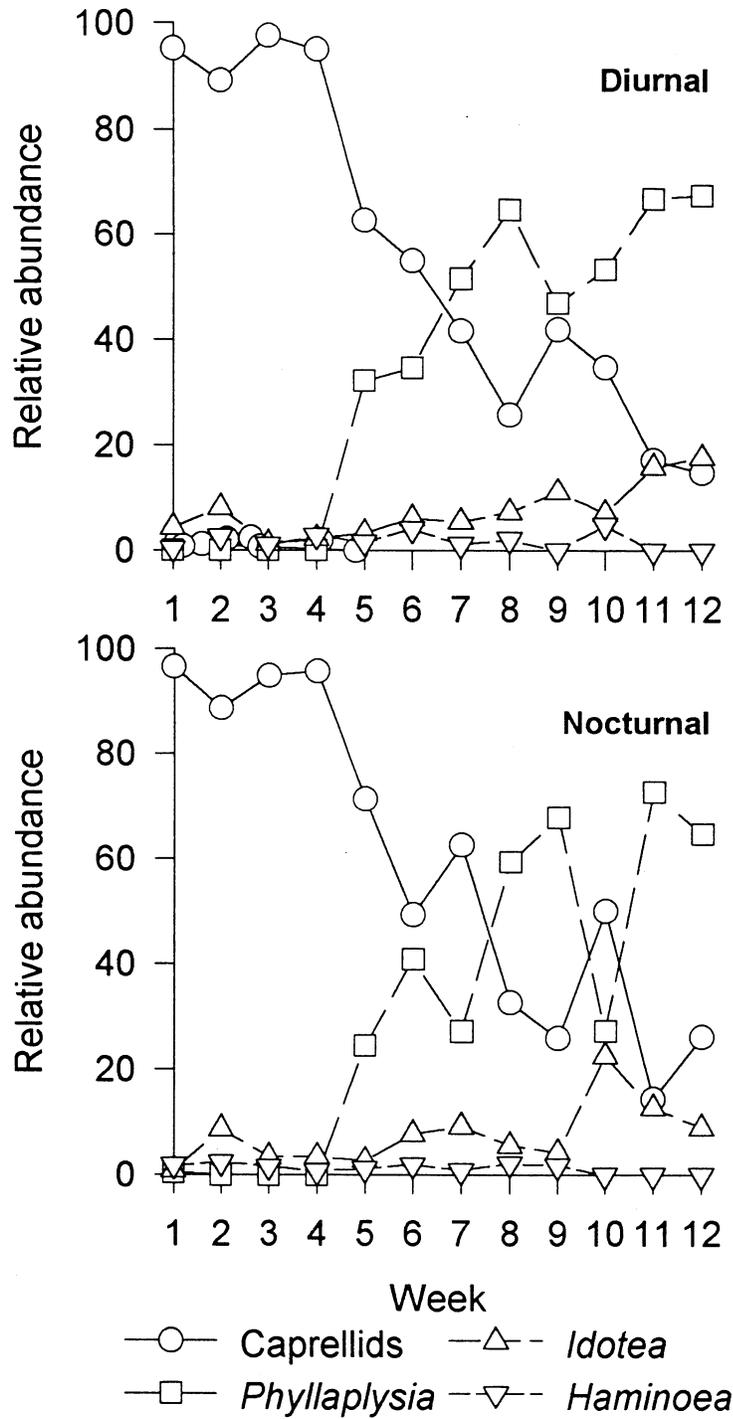


Figure 12. Relative abundance of epiphyte grazers along the sampled portion of the elgrass plant over the diel cycle. (weeks 1-4=July; weeks 5-8=Aug.; weeks 9-12=Sept.)

Table 12. Results of analysis of variance and Student-Newman-Keuls multiple range test for epiphyte biomass. Means connected by an underline in the MRT are not significantly different from each other. Mean values are expressed in $\mu\text{g dry wt. cm}^{-2}$. ($\alpha=0.05$)

Source	df	F	P
Date	3	3.83	0.03
Error	16		
Total	19		

Multiple Range Test:

Means:			
0.275	0.342	0.427	0.893
Date:			
August 30	August 16	September 21	July 19

Table 13. Analysis of covariation between epiphyte grazers and epiphyte biomass using simple linear correlation. (* denotes significance at the 5% probability level)

	<i>Caprella</i>	<i>Idotea</i>	<i>Phyllaplysia</i>	<i>Haminoea</i>
Epiphyte Biomass	0.606*	0.134	-0.619*	0.587*

The strongest correlation was between epiphyte biomass and *Phyllaplysia*. Abundance of *Phyllaplysia* tended to increase when epiphyte biomass decreased resulting in a negative correlation (Table 13). In contrast, the abundance of caprellids and *Haminoea vesicula* tended to decrease along with declines in epiphyte biomass.

DISCUSSION

Diel migration

Three of the four grazer taxa monitored in this study demonstrated a diel vertical migration. However, in all three cases, the pattern of diel migration varied over the duration of the study. *Idotea ressecata* was more abundant on the apical fraction of the eelgrass shoots at night averaged over all twelve weeks of the study. The mean number of isopods on the apical fraction was significantly greater at night than during the day (Fig. 5). However, the spatial pattern of abundance changes at the end of the study. The mean number of *I. ressecata* was significantly greater on the apical fraction early in the study than at the end of the study (Fig. 6). *Phyllaplysia taylori* was more abundant on the basal portion of the eelgrass plants. However, at night during the weeks of maximum abundance, significantly more *P. taylori* were collected on the intermediate fraction of the eelgrass shoots than during the day (Fig. 7). Similarly, caprellids underwent a diel vertical migration only when abundance was greatest. In July, significantly more caprellids were collected on the apical fraction of the eelgrass shoot at night than during the day (Fig. 3). The vertical distribution of *Haminoea vesicula* did not change over the diel cycle (Table 8).

The taxa exhibiting diel vertical migration contain representatives of different phyla. Why do such widely separated taxa demonstrate this similar behavior? The lack of change in community structure over the diel cycle suggests that these migrations are

not driven by interspecific competition. One possible explanation may lie in the importance of predation in the eelgrass system. Dietary shifts of predatory fish in Australian eelgrass communities are correlated with diel vertical migrations of zooplankton and benthic amphipods (Robertson and Howard, 1978). Evidence for predation as an engine for diel migration also comes from Caine's (1989) work with caprellid amphipods.

Many species of caprellids are generalist feeders utilizing several feeding modes (Caine, 1977). Caprellids browse on algae, scrape periphyton from a substrate and filter feed. Laboratory studies indicate that *Caprella californica* filter feeds in addition to scraping periphyton (Caine, 1977). Filter feeding caprellids fling their antennae into the water column to catch particles on the setae. This behavior has been shown to make caprellids vulnerable to predation by fish (Caine, 1989). Since many fish are visual predators, filter feeding caprellids would be less likely to be preyed on at night. Furthermore, water movement is increased at the apical end of the eelgrass shoot. Caprellids may specifically seek out this location for nighttime feeding.

Idotea resecata is found in the guts of eelgrass dwelling fish (Simenstad et al., 1979; Dinnel et al., 1990). Like caprellids, isopods avoided the apical end of the eelgrass plant during daylight to presumably avoid visual predators. The lower portion of the eelgrass turions form a more dense and complex habitat which provides a refuge from predation (Stoner, 1980; Leber, 1985).

A literature review did not uncover any work concerning potential predators of *Phyllaplysia taylori*. However, the coloration of the opisthobranch provides

camouflage. Diel migration combined with camouflage suggests that *Phyllaplysia* is exhibiting anti-predation strategies. Further work needs to be done to clarify *Phyllaplysia's* position in the food web of the eelgrass system.

Community structure

While individual taxa underwent a diel migration, the community structure of the sampled community changed temporally, not over the diel cycle (Fig. 12). The relative abundance of the sampled community reflected temporal changes in the abundance of individual taxa. During the first weeks of the study, caprellids were most abundant and made up 90% or more of the community (Fig. 12). Caprellid abundance declined dramatically after week 4 which corresponded to an increase in abundance of *Phyllaplysia*. These differences in temporal abundance are reflected by changes in the composition of the sampled grazer community (Fig. 12).

The wide swings in grazer abundance may be linked to changes in epiphyte community biomass and structure. The epiphyte community is very dynamic. In this study, a significant decline in epiphyte biomass was detected (Table 12). Change in epiphyte biomass was correlated with variation in grazer abundance (Table 13). Edgar (1990) used exclusion cages and light filtering screens in seagrass meadows to show that faunal abundance fluctuated with epiphyte biomass. In addition, grazers can have a substantial impact on epiphyte biomass in the lab (Caine, 1980; Thom et al., 1991; Williams and Ruckelshaus, 1993). While the functional relationship between grazer

abundance and epiphyte biomass was not tested in this study, the significant correlation coefficient between grazers and epiphytes suggests a relationship may exist.

Predation may influence temporal abundance patterns. When Shiner perch (*Cymatogaster aggregata*) migrate into the eelgrass meadow, caprellids make up 80% of their diet (Caine, 1991). However, when caprellid abundance declines, shiner perch shift to other prey species. Caprellid abundance in Caine's (1991) study, both on eelgrass and in the guts of Shiner perch, peaked in early June. This pattern of peak abundance of caprellids is only 30-40 days behind the temporal pattern quantified in this investigation (Fig. 3). Weather conditions or site differences may have contributed to the temporal differences measured by the two studies. The spring and early summer of 1993 were unusually cool and wet. In contrast, the region was experiencing a drought at the time of Caine's (1991) study.

The decline of caprellid amphipods may be related to the dramatic appearance of *Phyllaplysia taylori*. The life cycle of *Phyllaplysia* includes two annual reproductive waves (Beeman, 1970). One of these waves results in the hatching of trocophore larva in early summer. The individual sea hares collected early in August during this study were small indicating recent settlement. The decrease in caprellid abundance may have allowed for the settlement of *Phyllaplysia* larva. It might also be possible that the activity of caprellid grazing changed the surface of the eelgrass blade and enhanced settlement.

Association analysis

Association analysis is subject to a range of ecological interpretations. These interpretations can range from competition to mutualism based on the underlying basis for the association (Schluter, 1984). Caprellids and *Idotea* were positively associated but may not be in direct competition. Both crustaceans exhibited maximum abundance early in the study (Fig. 3 and 4). Caprellids and isopods also migrated to the apical end of the eelgrass plant at night. In addition, the abundance of caprellids and *Idotea* was positively correlated (Table 11). However, of the two, only caprellids were significantly correlated with epiphyte biomass (Table 13). This suggests that isopod and caprellid abundance and distribution respond similarly to factors other than epiphyte biomass.

Idotea and *Phyllaplysia* were also positively associated (Table 10). This net association probably resulted from the shift in isopod vertical distribution through time. More isopods were collected on the intermediate and apical fractions of the eelgrass turions early in the study than the apical fractions late in the study (Fig. 6). The association between *Phyllaplysia* and *Idotea* may be characterized by a similar spatial distribution. Both grazers occurred on the basal portion of eelgrass shoots during the later part of the study. Since the abundance of both taxa was not changing significantly during this period, competition is not an issue. However, both species may be responding to changes in the epiphyte community. Unfortunately, biomass sampling was not complete enough to test for significant differences in epiphyte

biomass between any of the eelgrass fractions. However, the epiphyte community does undergo dynamic change through time (Borum et al., 1984).

Phyllaplysia and *Haminoea* were negatively associated (Table 10). The negative association resulted from differences in spatial distribution. Significantly more *Haminoea* were collected on the apical and intermediate fraction of the eelgrass shoots (Fig. 10). Conversely, significantly more *Phyllaplysia* were sampled on the basal portion of *Zostera* turions (Fig. 8). The abundance of these two opisthobranchs negatively covaried (Table 11). *Haminoea* and *Phyllaplysia* also covaried differently with changes in epiphyte biomass (Table 13). Spatial distribution differences combined with an inverse response to changes in epiphyte biomass suggests that the two opisthobranchs have different resource requirements. Similar types of differential use relationships that result in spatial separation is exhibited by other seagrass gastropods (Mazzella and Russo, 1989).

Covariation analysis indicated that caprellid amphipods and *Phyllaplysia taylori* were negatively correlated (Table 11). However, association analysis failed to detect a significant negative association between these grazers. Association analysis uses presence and absence data to test the null hypothesis that the occurrence of one grazer is independent of another within sampling units. While the abundance of caprellids and *Phyllaplysia* covaried, their frequency of occurrence in sampling units was independent. This may result from differing resource requirements or the differential response to some other factors.

Niche separation

Two types of separation allow wider use of the epiphyte resource. The first is a temporal separation that is exemplified by the two most numerous taxa. Caprellids were most abundant during the first four weeks of the study. Caprellid abundance began to decline at the end of July. In contrast, *Phyllaplysia taylori* was largely absent from the eelgrass meadow until early August. Week 5 was the beginning of a dramatic increase in the abundance of *Phyllaplysia* (Fig. 13). The decline in caprellid abundance was correlated with the decline in epiphyte biomass.

The second type of separation was spatial and was exemplified by *Phyllaplysia* and *Haminoea*. *Phyllaplysia* was significantly more abundant on the basal portion of the eelgrass shoots. In fact, *Phyllaplysia* was rarely found on the apical portion of eelgrass shoots. *Haminoea*, in contrast, was found on the apical and intermediate fractions (Fig. 14). This type of spatial separation is similar to the type of vertical separation of gastropods on the seagrass *Posidonia oceanica* documented by Mazzella and Russo (1989). In that case, vertical distribution of the mollusks was related to changes in the epiphyte community along the leaves of the seagrass and the feeding habits of the gastropods.

Conclusions

The macroinvertebrate epiphyte grazer community in the *Zostera marina* is very dynamic. The abundance of individual grazer taxa fluctuated temporally during the study. Furthermore, three of the four grazers exhibited a diel vertical migration.

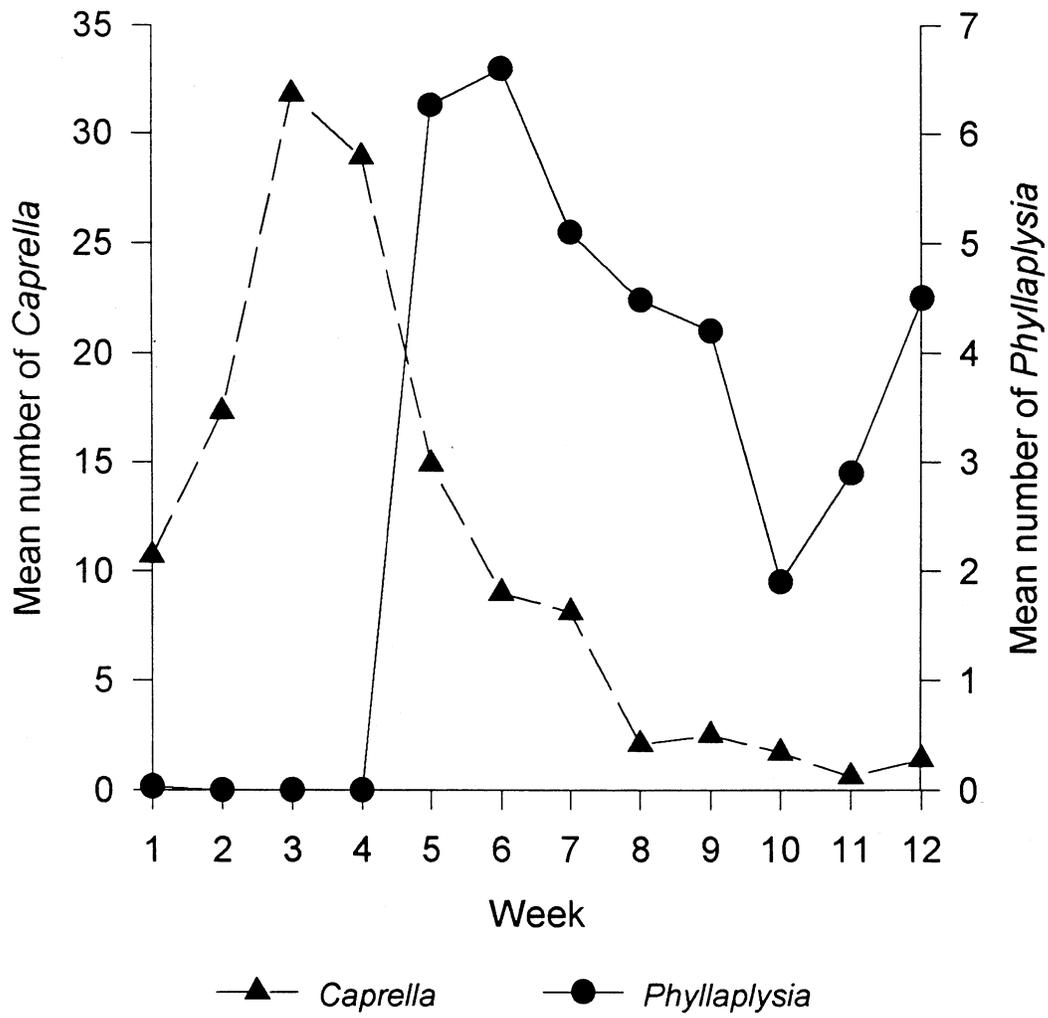


Figure 13. Temporal abundance of *Caprella californica* and *Phyllaplysia taylori*. (weeks 1-4=July; weeks 5-8=Aug.; weeks 9-12=Sept.)

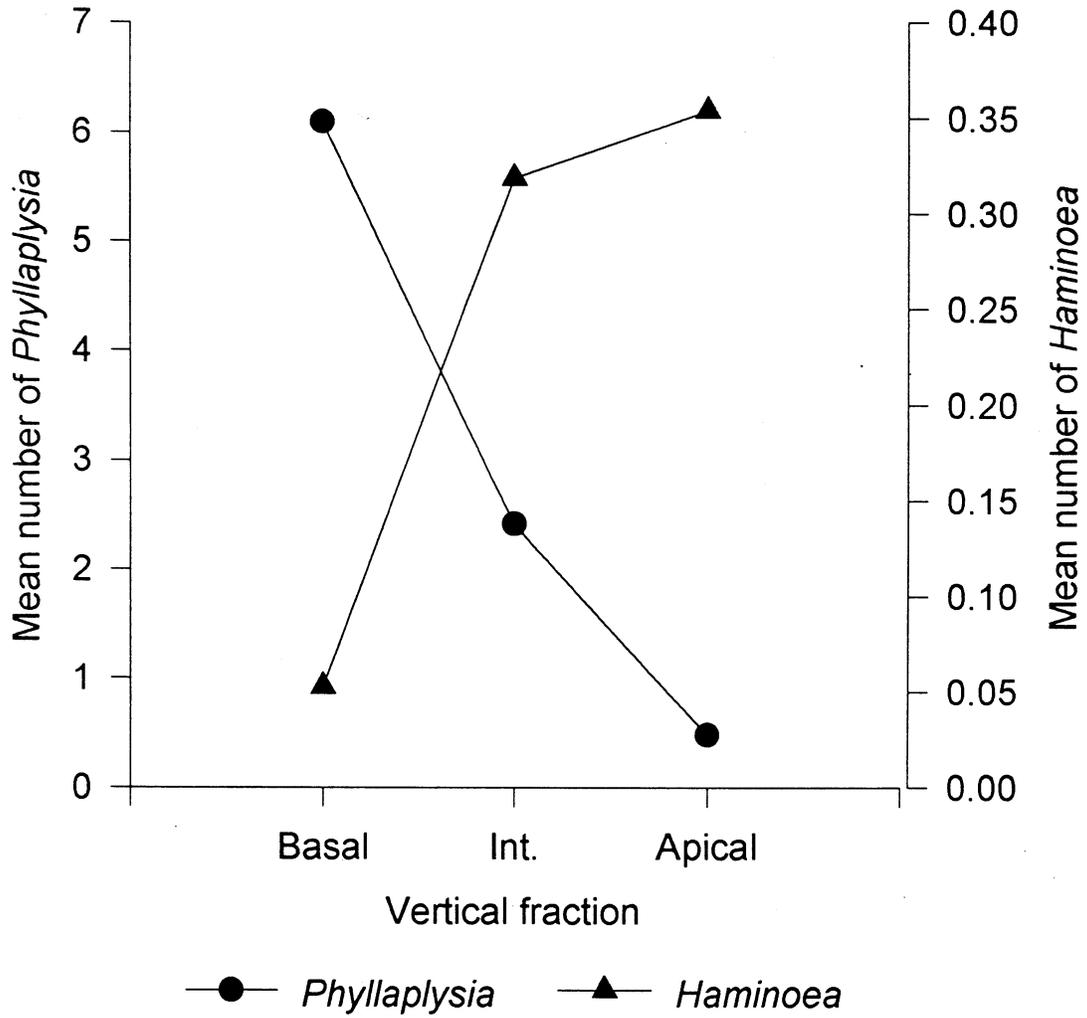


Figure 14. Vertical distribution of *Phyllaplysia taylori* and *Haminoea vesicula*.

However, the migration of caprellids and *Phyllaplysia taylori* occurred only during periods of maximum abundance.

While the vertical distribution of three of the grazer taxa examined changed over the diel cycle, the relative abundance of the sampled community did not. However, the relative abundance of the sampled community did change with time. Changes in relative abundance reflected variation in the temporal abundance of the individual grazer taxa.

Pairwise comparisons of independence revealed several taxa were associated. Caprellids and the isopod *Idotea resicata* were positively associated and responded to factors in the environment in a similar manner. Isopods and *Phyllaplysia taylori* were also positively associated and may have been responding similarly to variation in the epiphyte community. The two opisthobranch species were negatively associated and were separated in time and space.

Variations in the temporal abundance and vertical distribution of grazers allows greater utilization of the epiphyte resource. The grazers in this study partitioned their use of epiphyte biomass through time and along a vertical axis. The numerically dominant grazers reached peak abundance at different times and avoided direct competition. In addition, the epiphyte resource was partitioned vertically by the two opisthobranch taxa which may reflect differential resource utilization.

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APPENDIX A. Water temperature and salinity on grazer sampling dates during the summer of 1993.

Date	Salinity (ppt)				Temperature (°C)			
	Day		Night		Day		Night	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
6/30	22.5		22.5		15.5	15.0	14.0	13.0
7/7	21.0		23.5	23.5	17.0		15.0	14.0
7/14	22.0	21.5			15.0	14.0		
7/22	24.0		29.0	28.5	15.0		12.0	12.0
8/4	23.0	23.0	21.5	23.0	18.5	18.0	17.0	15.0
8/11	20.0	22.0	26.0	24.5	18.0	16.0	16.0	16.0
8/16	22.0	28.5	26.5	27.0	14.0	13.5	13.5	13.5
8/26	22.0	22.0	26.0	24.5	15.0	14.0	13.5	14.0
8/30	21.0	22.0	21.5	22.5	17.0	14.0	17.0	16.0
9/7	21.0	21.0	21.5	21.0	18.0	16.0	15.0	13.0
9/15	21.1	23.0	30.5	30.0	15.0	12.9	14.5	14.2
9/21	22.0	27.0	28.5	22.0	14.0	13.0	12.5	12.5

