Macrofaunal associations with seagrass epiphytes
Relative importance of trophic and structural characteristics


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Received 9 June 1998; received in revised form 28 May 1999; accepted 28 June 1999

Abstract

Attached epiphytes often make important contributions to total primary production in seagrass meadows. Additionally, they may increase the spatial complexity of seagrass habitats. Experiments conducted using artificial seagrass units (ASU) manipulated both epiphytic structure and epiphytic food resources. Previous work suggested that the increase in faunal density associated with epiphytes was related to increases in structure, but our results indicate that the primary impact of epiphytes lies in their trophic role. Data showed that epifaunal density was significantly greater in condition ASUs fouled with a live community of epiphytes (12 285 individuals m⁻²) compared to ASUs with artificially created epiphytic structure (5099 inds. m⁻²) and to control ASUs (5955 inds. m⁻²). This response to epiphytic trophic resources was significant for most herbivore/omnivore taxa, but not necessarily for filter feeding or predatory epifauna. However, densities of two predatory taxa (fish and mud crabs) were significantly greater where epiphytic biomass was higher, which may reflect their response to increased prey abundance. Additionally, ASUs conditioned with live epiphytes had greater taxa richness than other ASUs. Epiphytic structure appeared to play only a limited role in determining the density of most mobile epifauna, but epiphytic structure appeared to be important in augmenting the settlement of bivalves. By using ASUs we were able to control aspects of blade length and shoot density, but the pre-experiment conditioning of treatments fouled with live epiphytes may have played a role in determining absolute differences in macrofaunal density among ASU treatments. Overall, our work suggests that the trophic role of epiphytes can have a dramatic impact on associated epifaunal communities, although future investigations are needed to assess this relationship more fully. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Artificial seagrass unit; Epifauna; Epiphyte; Gulf of Mexico; Seagrass; Thalassia testudinum

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Pii: S0022-0981(99)00092-1
1. Introduction

Seagrass phototrophic epiphytes principally consist of cyanobacteria, diatoms, crustose and ephemeral algae (Ballantine and Humm, 1975; Thursby and Davis, 1984; Novak, 1984). Although a great deal of research has assessed seagrass primary production (Phillips, 1974; Wium-Andersen and Borum, 1984), epiphytic primary production has often been considered low and relatively unimportant due to their low biomass (Penhale, 1977; Borum et al., 1984). However, recent studies in seagrass meadows have shown that epiphytic primary production may often exceed seagrass production in both weight specific (g Carbon/g chlorophyll a, Pollard and Kogure, 1993) and total annual production (g Carbon m⁻² year⁻¹, Morgan and Kitting, 1984; Moncreiff et al., 1992). In these habitats the seagrasses themselves are not thought to be preferred food, due to their low nitrogen content (Koike et al., 1987), high cellulose content, and presence of phenolics (McMillan et al., 1980), and it has been suggested that seagrasses are usually only consumed to any significant extent by large vertebrate herbivores (Thayer et al., 1984, but see Camp et al., 1973; Valentine and Heck, 1991).

Many small herbivores, however, appear to rely upon associated algae for their nutritional needs (Kristensen, 1972, see Kikuchi and Peres, 1977; van Montfrans et al., 1984; Kitting, 1984; Nielsen and Lethbridge, 1989; Klumpp et al., 1992; Conlan, 1994; Alcoverro et al., 1996; Jernakoff et al., 1996; Jernakoff and Nielsen, 1997).

Seagrass epiphytes, both floral and faunal, can increase structural complexity (Heijs, 1987; Schneider and Mann, 1991b). However, little research has addressed the structural role of seagrass epiphytes (but see Hall and Bell, 1988). The increase in habitat heterogeneity associated with epiphytes may be important for passively settling organisms, those seeking refuge from predation and for organisms who preferentially select habitats with shelters that match their body size (Hacker and Steneck, 1990; Schneider and Mann, 1991a,b). Numerous studies involving seagrasses and supplemental structure (e.g., macroalgae) have shown that increases in habitat heterogeneity increase species richness and density of organisms (Lewis and Stoner, 1983; Stoner and Lewis, 1985; Edgar and Robertson, 1992; Martin-Smith, 1993). Because epiphytes provide additional structure to these habitats, it might be expected that the presence of epiphytes would also increase the density of organisms.

Epiphytes may change the nature of a seagrass community by providing food and habitat for associated fauna. However, understanding these interacting effects is difficult, because both presence of food and increases in structural complexity can increase the density of organisms. An experiment was designed to discriminate the relative trophic versus structural component of seagrass epiphytes using artificial seagrass units (ASU). By using ASUs, patch characteristics (e.g., area, shoot density, leaf length) were controlled, while manipulating the structure associated with epiphytes and epiphytic food resources. We reasoned that if organisms strictly utilize seagrasses as refugia, then the presence or absence of epiphytic food resources should not influence faunal density. However, if species are intimately tied to epiphytic food resources, then epiphyte presence and productivity should increase the abundance of these species. Below we examine the relative roles of epiphytic structure and trophic resources on seagrass, Thalassia testudinum, associated macrofauna.
2. Methods

Work was conducted during the summer of 1994 in St. Joseph Bay, Florida, USA (29°N, 85.5°W). St. Joseph Bay is a shallow semienclosed lagoon with little fresh water input and salinities generally range from 22‰ to 35‰ and temperatures from 8.5°C to 32°C (Bologna, 1998). Extensive seagrass meadows occupy the shallows (< 2 m) and are comprised of *T. testudinum*, *Halodule wrightii*, and *Syringodium filiforme*. *T. testudinum* is the dominant species and covers approximately 2300–2400 hectares in the bay (Savastano et al., 1984; Iverson and Bittaker, 1986). Research was conducted in an extensive (50 hectare), shallow sand-*T. testudinum* habitat mosaic (depth < 1.2 m Mean Low Water).

To assess the relative trophic and structural role of seagrass epiphytes, ASUs were constructed in the following manner: substrata were cut from 1.9 cm black Vexar™ mesh to which artificial seagrass shoots, made from either smooth or crimped green polyribbon (5 mm width), were tied. ASUs were circular with a radius of 124.5 mm (0.0487 m²). Shoots were constructed with four leaves shoot⁻¹ and tied at densities (~ 762 shoots m⁻², mean number leaves shoot⁻¹ = 4) and leaf heights (~ 30 cm) similar to those found in natural high density *T. testudinum* beds (cf., Iverson and Bittaker, 1986). Three experimental treatments were used: nonmanipulated ASUs (Control), ASUs with artificially created epiphytic structure (ART), and ASUs fouled with live *T. testudinum* epiphytes (NAT). Nonmanipulated ASUs had leaves constructed from smooth polyribbon. ART ASUs also had leaves constructed using smooth polyribbon, but these leaves were then “stripped” in a haphazard manner using a coarse wood rasp which raised the surface and created small filaments (< 5 mm), or separated small portions of the artificial leaf creating small filamentous coils (< 40 mm²). These filaments provided additional surface area (although not quantified), and mimicked “epiphytic” structure. NAT ASUs had leaves that were constructed of crimped polyribbon. The decision to use crimped ribbon resulted from previous attempts to establish a natural phototrophic fouling community on smooth polyribbon, which entailed long term deployment of ASUs that often resulted in severe faunal fouling (e.g. barnacles, oysters, tunicates, etc . . . ). It was found that crimped polyribbon allowed rapid colonization of algae while minimizing sessile faunal colonization. Measurements using digital vernier calipers showed that these two ribbons differed, in that each crimped ribbon was 0.12 mm thick compared to 0.1 mm for smooth, and had slight (0.01 mm depth) indentations alternating at 0.625 mm. This difference translated into an additional 22.6 cm² surface area for each ASU in the NAT treatments, which represented an increase of 0.5% greater surface area (4440 cm² for Control and ART ASUs vs. 4462 cm² for NAT ASU).

Based on this design, we attempted to evaluate the following predictions regarding the distribution of fauna among ASUs.

H₁: If epiphytic structure and food resources are unimportant in determining faunal distributions, there should be no significant differences in faunal density among ASUs varying in the amount of structure or food.
H$_2$: If epiphytic structure is of primary importance, faunal densities should be similar between NAT and ART ASUs, but greater than Control densities.

H$_3$: If epiphytic trophic resources are of primary importance, then herbivore density should be greater in NAT than in ART and Control ASUs, while nonherbivore density should be consistent among ASUs.

H$_4$: If total trophic resources are of primary importance, then density of herbivores and predators should be greater in NAT ASUs, but lower and similar in both ART and Control ASUs (i.e., if herbivore prey abundance is high, then predator abundance should be high also).

H$_5$: If epiphytic food and structure are both important, then faunal density should follow a gradient from high to low as follows: NAT (food and structure) $>$ ART (structure) $>$ Control.

Ten spatial replicates of experimental treatments were deployed randomly within a $3 \times 10$ matrix in an unvegetated region of the *T. testudinum* mosaic. ASUs were placed in the grid at a spacing interval of 5 m. Consequently, each ASU was 5 m from an adjacent ASU in the matrix, and greater than 7 m from existing *T. testudinum* beds. NAT ASUs were deployed in the field for 2 weeks before the start of the experiment. Before placement into the experimental array, NAT ASUs were rinsed and held in fresh water for 5 min to insure removal of all fauna (Edgar, 1992). All experimental ASUs were buried to cover the Vexar™ mesh and anchored using 16 gauge wire. Experimental ASUs were allowed to undergo colonization/recruitment for 4 weeks, at which point (July 27, 1994) ASU samples were collected using a gasoline powered suction sampler (cf. Orth and van Montfrans, 1987). ASUs were sampled sequentially in the matrix by visually locating each ASU and then vacuuming only the area occupied by the ASU until the mesh base was exposed. Two Control replicates were lost to storm events during the experiment.

Collected samples were sieved to retain organisms $>500 \mu m$, preserved in 10% formalin and stored in 50% isopropanol. Organisms were identified to lowest possible taxa, enumerated, and categorically placed into groups representing feeding mode (e.g., herbivore/omnivore, nonherbivore) and habitat requirements (e.g., epifaunal, infaunal, sessile). Identification and classification of taxa were accomplished using Gosner (1971, 1978), Bousfield (1973), Fauchald and Jumars (1979), Williams (1984), Robins and Ray (1986), Kensley and Schotte (1989), Andrews (1994), Rehder (1995), and Abbott and Morris (1995). Only motile taxa that were strictly associated with the seagrass leaves and canopy were used in analyses. Due to the time required to process samples comprised of high abundance and species diversity, most taxa were identified to the family level. Six replicate samples were taken in *T. testudinum* to compare species richness, density, and biomass between experimental treatments and natural seagrass beds. *T. testudinum* was sampled by randomly placing a polyvinyl chloride cylinder 1.2 m high and 0.073 m$^2$ in area (15.24 cm radius) within the bed. The cylinder was then drained using a suction sampler and all macrofauna were retained, preserved, and processed as above.

Individuals of a given taxon were grouped, dried to constant weight at 80°C (42–96
h), ashed for 8 h at 500\(^\circ\)C and reweighed to determine ash free dry weight (AFDW) per individual. Experiments were analyzed using one-way analysis of variance (ANOVA) with treatment as an independent categorical factor and density, biomass, and species richness as separate dependent variables. Square-root transformations of count data were completed before analysis. Multiple comparisons among treatments used Fishers Least Significant Difference ($\alpha = 0.05$) to determine significant differences.

Upon termination of the experiment, but before sampling began, six leaves from two replicates of each treatment were collected to assess degree of epiphytic fouling. Leaves were transported to the laboratory where epiphyte composition, percent cover, and biomass (AFDW) were estimated. Epiphyte functional groups (sensu Steneck and Dethier, 1994) and percent cover were estimated using a point-intercept grid (square = 4 mm \(\times\) 4 mm). Leaves were then scraped with a razor blade and the epiphytic algal component was dried at 80\(^\circ\)C for 72 h and ashed at 500\(^\circ\)C for 6 h. This eliminated weight bias associated with calcium carbonate from coralline algae. Epiphyte load was then calculated as \(\mu\)g AFDW cm\(^{-2}\) surface area, to standardize for leaf area sampled. Epiphyte percent cover and biomass were also estimated from 137 \textit{T. testudinum} leaves gathered from nearby, large continuous grass beds on July 18, 1994 (K. Heck, unpub. data) and compared with experimental treatments. Square-root transformed epiphyte load was analyzed using a one-way ANOVA with treatment as the independent categorical variable. Mean biomass among treatments was compared using a Scheffe \(F\)-test ($\alpha = 0.05$).

Structural components of epiphytes (sensu Hacker and Steneck, 1990) were compared between ART leaves and leaves collected from \textit{T. testudinum}. Leaves were submerged in water and observed from the edge. All structures emanating from the leaf surface were counted and identified according to the functional groups of Steneck and Dethier (1994). Encrusting coralline algae were excluded from structural component comparisons, as they did not protrude from the leaf surface. Epiphytic structure gathered from eight leaves of \textit{T. testudinum} consisted of three basic forms: individual straight filaments (hereafter referred to as “filament” or “filamentous”), branching filaments and tufted filaments. Branching and tufted filaments were grouped into a single category (hereafter referred to as “branched” or “branching”), because structurally, they occupied a spatial volume that could be utilized by small organisms. Figure 1 provides a schematic of epiphyte structural units present on \textit{T. testudinum} leaves. Filaments were counted and their length in mm was measured. Branches were counted and had their “area” [height from leaf surface (mm) \(\times\) maximum lateral extent (mm)] measured. Before deployment into the experiment, six ART leaves were also assessed in this manner and structural components of the roughened artificial grass leaves were measured. Structural units were then standardized on a per unit surface area basis for comparison. Specifically, the number of structural units per unit surface area (i.e., filaments and branches), percentage of branching versus filamentous structures, mean length of filaments and area of branches were analyzed using an unpaired \(t\)-test ($\alpha = 0.05$). Percent of structural units was transformed using arcsin before analysis. Branch area data were square-root transformed before analysis to eliminate heteroscedasticity.
3. Results

3.1. Epiphyte fouling

Naturally fouled experimental ASUs (NAT) that used crimped polyribbon had greater coverage and biomass of epiphytes (Table 1, Fig. 2). These data showed that greater than 85% of the surface area for NAT ASUs was covered by epiphytes (although not quantified, much of the free space for all experimental ASUs and *T. testudinum* was probably also covered by bacteria and diatoms, see Ballantine and Humm, 1975; Novak, 1984). Additionally, filamentous algae were more abundant on NAT ASUs than either Control or ART ASUs and samples from *T. testudinum*. This difference in epiphytic cover translated into significantly greater epiphyte biomass (μg AFDW cm⁻²) on NAT treatments than Control or ART treatments ($F_{3,5} = 8.08, P < 0.023$; Fig. 2). There was approximately twice the epiphytic biomass on NAT treatments ($60.98±2.98 \mu g \text{ AFDW cm}^{-2}, x±SD$) and samples from *T. testudinum* ($73.8±20.29$) than on Controls ($29.83±5.05$) or ART treatments ($35.69±1.78$). The high biomass associated with *T. testudinum* may have been a result of diatom biomass, since percent cover of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Clean</th>
<th>% Crustose coralline</th>
<th>% Filamentous</th>
<th>% Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.205</td>
<td>36.67</td>
<td>6.01</td>
<td>0.115</td>
</tr>
<tr>
<td>ART</td>
<td>44.885</td>
<td>35.45</td>
<td>19.38</td>
<td>0.285</td>
</tr>
<tr>
<td>NAT</td>
<td>14.385</td>
<td>56.375</td>
<td>28.955</td>
<td>0.275</td>
</tr>
<tr>
<td><em>T. testudinum</em></td>
<td>56.205</td>
<td>40.06</td>
<td>2.35</td>
<td>1.385</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic representation of epiphytic structural units. Length, height, and lateral extent are expressed in mm.
filamentous algae was low, but also may reflect the potential addition of *T. testudinum* biomass in this estimate. Unlike artificial blades, the scraping procedure used to assess epiphyte biomass on *T. testudinum* may have removed some live grass tissue, thus biasing the biomass data. Since this was not possible for artificial blades, this may account for the differences between low epiphyte percent cover and high biomass seen in *T. testudinum* data.

### 3.2. Structural comparison of artificial epiphytes

When the epiphytic structural components were analyzed, *T. testudinum* leaves contained more structural units per unit area than ART treatments. ART ASUs had fewer filamentous and branching units compared to epiphytes on *T. testudinum*, but mean filament length and branch area on ART ASUs were greater (Table 2). This was due, in part, to the abundance of small (1 mm × 1 mm) algal tufts on *T. testudinum* leaves (see Fig. 1). The opposing differences in branch density and branch area produced similar mean branching area per unit surface area of blade (Table 2). Although differences in density of epiphyte structural units existed between initial ART ASUs and *T. testudinum*, the similarities in density of filaments cm⁻² (which comprised 70–80% of structural units) and mean branch area cm⁻² suggest that epiphytic structure associated with ART ASUs may have mimicked overall available secondary structure of *T. testudinum* epiphytes. Unfortunately, epiphytic structure associated with live epiphytes on NAT
Table 2
Comparison of epiphytic structural units between epiphytes on *T. testudinum* and artificially created epiphytes associated with ART ASUs; values represent means±SD

<table>
<thead>
<tr>
<th>Structural component</th>
<th><em>T. testudinum</em> epiphytes</th>
<th>Artificial epiphytes (ART)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. structural units cm⁻²</td>
<td>1.28±1.25</td>
<td>0.677±0.24</td>
</tr>
<tr>
<td>No. filaments cm⁻²</td>
<td>0.779±0.833</td>
<td>0.558±0.226</td>
</tr>
<tr>
<td>No. branches cm⁻²</td>
<td>0.498±0.461</td>
<td>0.119±0.033</td>
</tr>
<tr>
<td>Percent filamentous</td>
<td>0.813±0.062</td>
<td>0.68±0.251</td>
</tr>
<tr>
<td>Percent branching</td>
<td>0.187±0.062</td>
<td>0.32±0.251</td>
</tr>
<tr>
<td>Mean filament length (mm)</td>
<td>1.386±0.707</td>
<td>2.439±0.644*</td>
</tr>
<tr>
<td>Mean branch area (mm²)</td>
<td>1.72±1.33</td>
<td>18.95±17.5*</td>
</tr>
<tr>
<td>Mean branch area per unit surface area (mm² cm⁻²)</td>
<td>1.153±1.148</td>
<td>2.54±2.787</td>
</tr>
</tbody>
</table>

* Indicates significant differences between means (α = 0.05).

ASUs was not assessed upon termination of the experiment, because these leaves were used to estimate percent cover and algal biomass. This was also the case for ART and Control ASUs. Based on the data from percent cover and algal biomass, results suggest that epiphytic structure may have been similar between NAT ASUs and *T. testudinum*, but definitive evidence is lacking. However, the high percent cover of filamentous algae associated with NAT ASUs suggests that epiphyte structural complexity may have been highest in this treatment. Although all possible comparisons of epiphyte structure among *T. testudinum*, and ASUs were not completed, the similarity in structural units and mean branch area between ART ASU and *T. testudinum* (Table 2) and similarity in biomass between NAT ASUs and *T. testudinum* (Table 1, Fig. 2) suggests that overall epiphytic structure among these treatments was similar.

3.3. Community response

A total of 186 taxa was identified from samples, including representatives from 45 orders in nine phyla. However, only 76 taxa, identified as potential canopy fauna, were used for comparative analyses. Taxa richness was greater in NAT ASUs and *T. testudinum* compared to Control and ART ASUs (*F*₃,₃₀ = 29.4, *P* < 0.0001; Fig. 3a). NAT ASUs had significantly greater faunal densities (*F*₃,₃₀ = 14.89, *P* < 0.0001) than the other experimental treatments or samples gathered from *T. testudinum*. When taxa were analyzed based on feeding mode (Fig. 3b), the results showed that significantly greater densities of herbivores/omnivores occurred in NAT ASUs when compared to other experimental treatments and samples collected from *T. testudinum* (*F*₃,₃₀ = 13.41, *P* < 0.0001). Additionally, densities of nongrazing organisms also were significantly greater in NAT compared to other experimental treatments (*F*₃,₃₀ = 10.44, *P* < 0.0001). The greater density associated with NAT ASUs directly translated into significantly greater faunal biomass compared to other ASUs and *T. testudinum* (*F*₃,₃₀ = 9.27, *P* < 0.0002). The faunal community, however, did not respond to the increase in habitat heterogeneity associated with ART ASUs. In fact, overall faunal density was greater in Control than ART ASUs (Fig. 3b) and both treatments contained similar mean taxa richness (Control = 23.75 taxa, ART = 22.2; Fig. 3a).
Fig. 3. Community response to experimental epiphyte treatments. (a) Comparison of taxa richness among experimental treatments and *T. testudinum*. Differing letters above treatments indicate significant differences among means (α = 0.05). (b) Total herbivore and nonherbivore density among ASUs and *T. testudinum*. Values represent standardized densities (number m⁻² ± one standard deviation). Differing letters above treatments indicate significant differences in means (α = 0.05). Herbivore distinctions are denoted with a letter, while nonherbivore distinctions are denoted with a letter prime (i.e., a').
3.4. Faunal response

Four major taxonomic groups were identified by relative abundance and biomass in the suite of 76 canopy fauna, pooled for analysis, and include: Peracarida (74.3% total identified), Decapoda (17%), Gastropoda (the “group” Gastropoda also includes members of the Polyplacophora, because of their functional similarities, 4.7%), and Osteichthyes (0.3%). The density of the dominant members of each group are presented in Table 3 for comparison. In most cases, taxa density was greatest in NAT ASUs.

Table 3
Taxa density comparison among ASU treatments and T. testudinum; values presented are mean (number m⁻²) ± SD

<table>
<thead>
<tr>
<th>ASU Treatment</th>
<th>Trophic mode</th>
<th>P</th>
<th>Control</th>
<th>ART</th>
<th>NAT</th>
<th>T. testudinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastropoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitrella lunata</td>
<td>h</td>
<td>*</td>
<td>115.5±79.1</td>
<td>152.0±231.5</td>
<td>342.2±156.2</td>
<td>43.4±42.8</td>
</tr>
<tr>
<td>Crepidula spp.</td>
<td>f</td>
<td>ns</td>
<td>59.0±41.7</td>
<td>37.0±27.0</td>
<td>53.4±33.8</td>
<td>159.8±167.9</td>
</tr>
<tr>
<td>Modulae modularis</td>
<td>h **</td>
<td>7.7±10.6</td>
<td>6.2±9.9</td>
<td>50.2±46.5</td>
<td>11.4±10.3</td>
<td></td>
</tr>
<tr>
<td>Chitonidae</td>
<td>h ***</td>
<td>2.6±7.3</td>
<td>0.0±0.0</td>
<td>47.2±33.6</td>
<td>13.7±22.9</td>
<td></td>
</tr>
<tr>
<td>Diastoma varium</td>
<td>h</td>
<td>ms</td>
<td>0.0±0.0</td>
<td>6.2±9.9</td>
<td>13.7±14.4</td>
<td>11.4±13.5</td>
</tr>
<tr>
<td>Cerithium maculatum</td>
<td>h</td>
<td>ms</td>
<td>0.0±0.0</td>
<td>8.2±14.4</td>
<td>9.1±19.8</td>
<td>4.6±11.2</td>
</tr>
<tr>
<td>Rissoina bryerea</td>
<td>h</td>
<td>ns</td>
<td>2.6±7.3</td>
<td>8.2±14.4</td>
<td>6.8±10.6</td>
<td>9.1±16.6</td>
</tr>
<tr>
<td>Mitrella ocellata</td>
<td>h</td>
<td>ns</td>
<td>2.6±7.3</td>
<td>4.1±8.7</td>
<td>4.6±8.7</td>
<td>0±0</td>
</tr>
<tr>
<td>Eptiponinae</td>
<td>ep</td>
<td>ms</td>
<td>2.6±7.3</td>
<td>0.0±0.0</td>
<td>6.2±9.9</td>
<td>16.0±16.0</td>
</tr>
<tr>
<td>Peracarida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aonidae</td>
<td>h</td>
<td>***</td>
<td>2464.1±1401.6</td>
<td>1441.5±624.5</td>
<td>6094±2810.3</td>
<td>1141.6±581</td>
</tr>
<tr>
<td>Cymadusa spp.</td>
<td>h</td>
<td>***</td>
<td>782.9±402.0</td>
<td>932.2±404.5</td>
<td>175.7±157.9</td>
<td>66.2±43.7</td>
</tr>
<tr>
<td>Amphithoidae</td>
<td>h</td>
<td>ms</td>
<td>369.6±265.9</td>
<td>199.0±120.9</td>
<td>397.0±309.3</td>
<td>4.6±7.1</td>
</tr>
<tr>
<td>Sphaeromatidae</td>
<td>h *</td>
<td>202.8±157.3</td>
<td>154.0±107.0</td>
<td>447.2±371.9</td>
<td>114.2±51.7</td>
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</tr>
<tr>
<td>Caprellidae</td>
<td>h</td>
<td>ms</td>
<td>143.7±80.7</td>
<td>197.1±117.4</td>
<td>246.4±207.2</td>
<td>10.0±5.4</td>
</tr>
<tr>
<td>Melitidae</td>
<td>h</td>
<td>***</td>
<td>59.0±77.2</td>
<td>49.3±46.6</td>
<td>413.0±198.4</td>
<td>184.9±117.4</td>
</tr>
<tr>
<td>Oedicerotidae</td>
<td>h</td>
<td>*</td>
<td>77.0±36.0</td>
<td>69.8±50.5</td>
<td>212.2±268.5</td>
<td>41.1±36.8</td>
</tr>
<tr>
<td>Pleustidae</td>
<td>h</td>
<td>ms</td>
<td>97.5±33.9</td>
<td>61.6±38.7</td>
<td>164.3±92.4</td>
<td>315.1±180.5</td>
</tr>
<tr>
<td>Liljeborgiidae</td>
<td>h</td>
<td>***</td>
<td>10.3±5.5</td>
<td>8.2±10.6</td>
<td>228.2±189.2</td>
<td>0±0</td>
</tr>
<tr>
<td>Lystianasidae</td>
<td>p</td>
<td>***</td>
<td>2.6±7.3</td>
<td>4.1±13.0</td>
<td>134.6±155.1</td>
<td>0±0</td>
</tr>
<tr>
<td>Erichthonea spp.</td>
<td>h</td>
<td>ns</td>
<td>10.3±11.0</td>
<td>4.1±13.0</td>
<td>82.1±88.2</td>
<td>4.6±7.1</td>
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<td>Decapoda</td>
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<tr>
<td>Caridea</td>
<td>h</td>
<td>ns</td>
<td>764.9±405.0</td>
<td>932.2±814.4</td>
<td>1104.3±673</td>
<td>246.6±90.0</td>
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<tr>
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<td>h</td>
<td>*</td>
<td>290.0±76.4</td>
<td>205.4±78.6</td>
<td>470.2±312.5</td>
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<td>ms</td>
<td>35.9±30.6</td>
<td>24.6±28.7</td>
<td>38.8±40.8</td>
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<tr>
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<td>***</td>
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<td>2.1±6.5</td>
<td>70.7±86.7</td>
<td>11.4±28</td>
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<tr>
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<td>***</td>
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<td>6.2±9.9</td>
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<td>11.4±16</td>
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<tr>
<td>Gobionoma bone</td>
<td>p</td>
<td>ms</td>
<td>7.7±10.6</td>
<td>22.6±40.4</td>
<td>29.7±36.3</td>
<td>6.8±11.5</td>
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<td>Synaptidum spp.</td>
<td>p</td>
<td>ns</td>
<td>2.6±7.3</td>
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<td>4.1±8.7</td>
<td>0±0</td>
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<tr>
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<td>ns</td>
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<td>0.0±0.0</td>
<td>9.1±10.6</td>
<td>0±0</td>
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<tr>
<td>Opsanus spp.</td>
<td>p</td>
<td>ns</td>
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<td>0.0±0.0</td>
<td>4.6±8.7</td>
<td>0±0</td>
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</tbody>
</table>

Significant differences among means indicated by P, where * P<0.05, ** P<0.01, *** P<0.001, ns = no significant difference. Results were calculated using ASU data only. Trophic mode abbreviations: f = filter feeder, h = herbivore/omnivore, ep = ectoparasitic, p = predator.
Fig. 4. Density comparisons among ASUs and *T. testudinum* for the four major taxonomic groups. Values represent standardized densities (number m$^{-2}$ ± one standard deviation) Differing letters above treatments indicate significant differences in means ($\alpha = 0.05$, square-root transformed count data). Herbivore distinctions (a–c) are denoted with a letter, while nonherbivore distinctions are denoted with a letter prime (i.e., a'). (a) Peracarida herbivore and nonherbivore density. Note, Y axis is logarithmic. (b) Decapoda density. (c) Gastropoda density. (d) Fish density.
(exception: nonherbivore Gastropoda densities were greater in *T. testudinum* and Control ASUs). The density of both herbivorous and nonherbivorous epifaunal Peracarida was significantly greater in NAT compared to other ASUs and samples gathered in *T. testudinum* \( (F_{3,30} = 12.7, P < 0.001) \) herbivores, \( F = 12.8, P < 0.001 \) nonherbivores; Fig. 4a). Additionally, when the most abundant Peracarida families were analyzed separately among ASU treatments, most showed greater densities in NAT ASUs when compared to Control and ART ASUs (Table 3). Only *Cymadusa* spp. (Amphithoidae) showed significantly greater densities in Control and ART ASUs when compared to NAT ASUs \( (F_{2,25} = 14.7, P < 0.0001) \). However, no Peracarida showed significant responses to changes in epiphyte structure associated with ART ASUs (i.e., ART = NAT ⇒ Control).

Decapoda densities varied significantly between ASU treatments and samples collected from *T. testudinum*. Specifically, decapod herbivore density was greater in ASUs than *T. testudinum* \( (F_{3,30} = 5.2, P < 0.005) \), but density did not differ significantly among ASUs (Fig. 4b). However, both Paguroidea and Majidae showed significantly greater densities in NAT than in ART or Control ASUs (Table 3). Additionally, nonherbivore Decapoda density was significantly greater in NAT ASUs compared to other ASUs and *T. testudinum* \( (F = 10.5, P < 0.0001) \) and this response was primarily due to Xanthidae, but not Portunidae (see Table 3).

Herbivorous Gastropoda, primarily *Mitrella lunata*, *Modulus modulus*, and Chitonidae, were significantly greater in NAT ASUs than in other ASUs and *T. testudinum* \( (F_{3,30} = 7.17, P < 0.001) \). However, nonherbivorous gastropod density was greater in samples collected in *T. testudinum* than in ASUs \( (F_{3,30} = 3.19, P < 0.05) \), although densities did not differ significantly among ASU treatments (Fig. 4c, Table 3). Fish density was also significantly greater in NAT ASUs than in Control ASUs or *T. testudinum* \( (F = 3.2, P < 0.036; \text{Fig. 4d}) \).

Although bivalves were not considered in the above analyses because they are not motile epifauna, they did provide a post-hoc opportunity to assess the structural impacts of epiphytes on recruiting organisms. Specifically, Mytilidae and *Argopecten irradians* are known to use seagrass leaves as initial settlement sites (Bayne, 1964; Eckman, 1987; Newell et al., 1991), and their density distributions were analyzed among treatments. Bivalves settled in significantly higher densities in NAT ASUs \( (2704.3 ± 1451.8) \) and *T. testudinum* \( (1054.8 ± 626.7) \) compared to ART \( (457.9 ± 553.2) \) and Control \( (182.2 ± 100.9) \) ASUs \( (F_{3,30} = 16.75, P < 0.0001) \).

4. Discussion

Faunal density was clearly greater in NAT compared to other ASUs (Fig. 3b) and the hypothesis of no differences among treatments was rejected. The attempt to mimic secondary epiphytic structure provides interesting insights into the effects of increased habitat heterogeneity on mobile seagrass fauna. Comparisons of faunal density between Control and ART ASUs found that the community as a whole did not vary significantly with increased heterogeneity associated with epiphytic structure on ART ASUs, and in fact, Control ASUs often had greater densities of many taxa (see Table 3). Only Caridea
and Caprellidae showed greater densities in ART over Control ASUs (albeit nonsignificant), and they appear to be the only taxa collected in this study that may be associated with both the structure and trophic resources of epiphytes. These results suggest that the structure of seagrass blades may be adequate refuge from predation for macrofauna and that minor increases in structure associated with epiphytes provide no additional refuge value. Therefore, either taxa did not respond to the experimental manipulations, the level of taxonomy could not distinguish faunal epiphytic structural preferences, or epiphytic structure plays only a limited role for larger mobile seagrass fauna (e.g., vs. meiofauna, Hall and Bell, 1988).

Crustaceans and gastropods often constitute the greatest density of seagrass epifauna (Fig. 4a–c), and discerning the cause of their distributional patterns is essential in understanding how secondary production is regulated in seagrass habitats (Howard, 1981; Kitting, 1984; Valentine and Heck, 1993). When seagrass epiphytes were abundant on NAT ASUs, we found that the densities of small grazers (primarily crustaceans and gastropods) were significantly greater (based on 55 identified/suspected herbivore/omnivore taxa, Fig. 3b, Table 3). If herbivores were merely attracted to increases in surface area or structure, then both ART and NAT ASUs should have had similar densities; or minimally, ART ASUs should have had greater densities than Control ASUs. That was clearly not the case for herbivores as a whole, or within taxonomic groupings, which suggests that trophic resources may be relatively more important for herbivores than the structure provided by epiphytes. However, nonherbivore density was also significantly greater in NAT compared to Control and ART ASUs (Fig. 3b). The most abundant identified nonherbivorous epifauna were either filter feeding or parasitic (Gastropoda) or predatory in nature (Peracarida, Decapoda, and Osteichthyes). When the differences in density among ASUs were assessed for separate taxonomic groups, two relatively distinct responses occurred: no response or elevated densities in NAT ASUs. Nonherbivore Gastropoda showed no significant differences in density among ASUs (Fig. 4c). This response was primarily due to the similarity in Crepidula spp. density among ASUs (Table 3). Crepidula spp. are filter feeders often associated with surfaces. Consequently, it might be expected that they would not be influenced by either structure or epiphytic trophic resources and densities were consistent with the predictions that nonherbivore taxa should show no preference among ASUs.

Densities of predatory Peracarida, Decapoda, and Osteichthyes either showed no significant density differences among ASUs (e.g., Portunidae, Table 3) consistent with predictions of no preference, or showed elevated densities in NAT compared to Control and ART ASUs. In general, if predators were associated with increases in epiphytic secondary structure, then their densities should have been similar between ART and NAT ASUs. Since most did not show a relationship with increasing structure associated with ART ASUs, we contend that their densities probably reflect patterns of prey abundance (Fretwell and Lucas, 1970) and not increases in epiphyte structure or leaf surface area (Edgar and Robertson, 1992). Consequently, both herbivores and predators may find trophic resources in NAT ASUs. The one glaring exception to this pattern was the density of Cymadusa spp. (Amphipoda). Cymadusa spp. showed significantly greater densities in Control and ART than in NAT ASUs (Table 3). Edgar (1983) found that small Cymadusa spp. were competitively displaced to less preferred habitats under high
amphipod densities. Given the high densities of Peracarida in NAT ASUs (> 8500 m⁻²), *Cymadusa* spp. may have been competitively displaced from NAT ASUs by larger or more competitive Peracarida. Therefore, faunal densities in ASUs may be affected by trophic resource availability, competitive interactions, predation potential as well as refuge requirements.

Given the rapid colonization of patches (Virnstein and Curran, 1986) and the high turnover rates for fauna in seagrass habitats (Howard, 1985; Edgar, 1992), increased density in NAT ASUs suggests that the presence of algal food resources affects the immigration to emigration ratios for mobile herbivores. If organisms are exposing themselves to predation risk by crossing open sediment to colonize new habitats, they must either be seeking essential resources (i.e., food, mates, or habitat) or they are being competitively excluded from preferred habitats. If they are excluded from natural habitats under high density conditions, then why should epifaunal density be less in seagrass beds than in artificial seagrass habitats (Fig. 3a)? Rather, Virnstein and Curran (1986) proposed a nearest refuge hypothesis where mobile fauna must seek refuge when conditions favor visual predators. Therefore, ASUs in unvegetated regions become islands of refuge in inhospitable habitat and may concentrate individuals over a larger area. This certainly can explain the differences in density between ASUs and *T. testudinum* and this response is similar to results from other studies (Sogard, 1989; Bologna, 1998). The presented results suggest that when epifauna leave large continuous seagrass habitats they may seek the primary structure of artificial grass to escape being eaten. However, because Control and ART ASUs provided little nourishment for herbivores (see Fig. 2, Table 1), these same organisms must again leave to forage if they are to survive. Therefore, ASU herbivore density should be determined by the immigration (seeking food or refuge) to emigration (seeking food) ratio (see Edgar and Robertson, 1992). These data suggest that when food is available, herbivore density should increase because immigration rates would remain constant while the relative emigration rate decreases until resources are depleted (i.e., if there is food and shelter, why leave?).

In addition to the primary trophic and structural aspects of epiphytes, other secondary aspects may impact the recruitment/colonization of organisms with pelagic larvae. Epiphyte presence may increase adhesive properties of surfaces and may reduce flow to create areas where larval settlement may be enhanced. Results show that the presence of epiphytes significantly increased bivalve settlement on experimental NAT ASUs and that the secondary structure of artificially created epiphytes (ART ASUs) increased bivalve settlement density by a factor of approximately two compared to Control ASUs. It is recognized that conditioned substrates (i.e., ones covered with epiphytes or organic macromolecules) increase larval settlement (Tritar et al., 1992; Weiczorek and Todd, 1997). Additionally, mechanisms that may be operating on this level include effects of bioroughness and changes in microscale turbulent flow (Koch, 1994). If flow around blades with secondary structure is altered, then microenvironments may exist where larval swimming velocities exceed flow velocity and larvae could choose to settle, or return to the water column for dispersal. On the other hand, if larvae are being transported passively, then the increases in structure may reduce flow and enhance larval delivery. Although we did not evaluate these possibilities, other studies have shown that
these forces are integral to settlement and recruitment of bivalves and polychaetes (Snelgrove et al., 1993).

4.1. Potential effects of experimental techniques

Because the goal of this experiment was to assess the trophic versus structural role of seagrass epiphytes, experimental ASUs needed to be fouled with epiphytic algae before use. As previous attempts using smooth polyribbon to create ASUs with a natural fouling community entailed long term deployments that often resulted in severe faunal colonization (e.g. oysters, barnacles, ascidians), the trade-off between using the crimped ribbon that allowed rapid floral colonization, while minimizing faunal fouling, and using identical smooth polyribbon in all experimental treatments was deemed acceptable. The creation of ASUs for experimentation was designed to tease apart the relative structural and trophic role of epiphytes and there is a clear relationship between Control and ART ASU treatments, because they were constructed of the same material and were placed in the field at the same time. Consequently, they differ only in presence of secondary structure mimicking epiphytes. However, the NAT ASUs differ from both Control and ART ASUs in several ways. First, NAT ASUs were constructed using a different ribbon, resulting in differences in initial surface area; second, NAT ASUs were placed in the field 2 weeks prior to the initiation of the experiments; and lastly, NAT ASUs were rinsed in fresh water to remove epifauna prior to initiation of the experiment. Each of these differences therefore, may have contributed to the differences in faunal density among treatments.

ASUs controlled leaf length (i.e., ASU canopy height), therefore, a small increase in surface area (0.5%) associated with crimped polyribbon occurred. Even though increases in leaf surface area have been associated with greater species richness and faunal density (Stoner, 1983; Lewis, 1984), we do not believe that a 0.5% increase in surfaced area would result in a 100% increase in faunal density (Fig. 3b). Differences in leaf morphology have been shown to influence epifaunal density (Schneider and Mann, 1991b; Kenyon et al., 1997) and this may have played a role in determining minor density differences among ASU treatments, but we believe that the differences in faunal densities and species richness are more likely attributable to the significantly greater epiphytic biomass associated with NAT ASUs (Fig. 2), and not to the minor differences between construction materials of artificial leaves.

The possibility exists that preconditioning may have impacted the results, since no controls for ASU conditioning were included in the experimental design (e.g., smooth polyribbon conditioned for 2 weeks, crimped polyribbon not conditioned). Edgar (1991) found that faunal recruitment (both larvae and adults) to artificial algae accelerated during the second, 2 week period of experimentation, which corresponded to the development of biofilms. Additionally, density reached an asymptote between 1 and 2 months after placement. Our experiment was conducted for 4 weeks, but NAT ASUs were in the field for a total of 6 weeks (2 weeks conditioning and 4 weeks experiment). Therefore, density of macrofauna may have been reaching an asymptote for NAT ASUs, while Control and ART ASUs were continuing to accumulate individuals. We believe that the conditioning process alone had minimal effects on epifaunal colonization,
because algal biomass was present on all ASUs (Fig. 2) and the presence of these algae represent the development of biofilms on ASUs. Additionally, if experiments were conducted for a greater length of time, fouling of epiphytes on all ASUs would have limited our ability to discern the structural versus trophic role of epiphytes. Although this may confound absolute interpretation of our results, we contend that the trophic role of epiphytes is clearly more important than their structure alone. Lastly, treatment of NAT ASUs in the water bath may have biased our results. Edgar (1992) showed that some amphipod taxa showed a positive response to a water bath treatment, dramatically increasing total epifaunal density. Consequently, the response of Peracarida taxa to NAT ASUs may be due, in part, to the exposure of NAT ASUs to the water bath treatments and not differences in food or structure. Recognizing these limitations, the data show that preconditioned NAT ASUs had the greatest density of organisms and the density differences among ASUs may be due in part to ASU construction materials, preconditioning and the water bath, and length of the experiment. However, we believe that the response of herbivorous taxa (Fig. 3b) clearly indicate a positive response to the presence of epiphytic food resources, while the absence of response to secondary structure associated with ART ASUs indicates that epiphytic structure may play only a limited role in determining macrofaunal densities in some seagrass communities.

5. Conclusions

In summary, this research has shown that mobile seagrass fauna respond positively to the presence of trophic resources, but not necessarily to increases in secondary structure. Although the first result may be intuitive, the lack of faunal response to epiphytic structure in the experiment was not expected, since numerous studies have shown positive faunal responses with increasing habitat heterogeneity (Stoner and Lewis, 1985) or changes in epiphytic presence (Hall and Bell, 1988; Edgar and Robertson, 1992). This suggests that epiphytic physical structure alone may have had little influence in determining the overall density patterns of mobile seagrass fauna in ASUs, although it is probable that epiphytic structure did play a role in determining the density of some fauna (Table 3). However, the striking density difference between NAT ASUs and other ASU treatments and T. testudinum provides evidence that trophic resources are extremely important. Consequently, future research should continue to address the trophic role of epiphytes and their importance in determining faunal density and secondary production.

Acknowledgements

We would like to thank J. Valentine, J. Cowan, R. Orth, G. Edgar, R. Steneck, and an anonymous reviewer for their careful review and suggestions for the betterment of this manuscript. We would also like to thank Y. Gonzales for assistance in the field. Funding and support for this research was provided by the Mississippi–Alabama Sea Grant Consortium (NA16RG0155), a Lerner–Gray Fellowship, the University of South
Alabama Department of Marine Science and the Dauphin Island Sea Lab. This is contribution #312 to the Dauphin Island Sea Lab.

References


