

**Variation in Epiphyte Assemblage within the Friday Harbor Laboratories *Zostera marina*
Meadow on San Juan Island, Washington**

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Abstract

Eelgrass (*Zostera marina*) is an important foundation species in the Salish Sea, providing habitat, stabilizing sediment, cleaning water, and sequestering carbon. These ecosystem services are under threat in the San Juan Islands, as seagrass wasting disease (SWD) and heat stress have contributed to major declines in intertidal eelgrass meadow extent in the past decade. Deeper meadows experience lower SWD levels and heat stress, suggesting that these sites may act as refugia amidst continued climate change. An additional stressor is epiphyte load (epiphyte species richness and abundance on an eelgrass leaf). The presence of epiphytes can be influenced by leaf age, but influence from depth has not been reported. The present study investigates the relationships between depth, leaf age, and epiphyte load for one subtidal eelgrass meadow at Friday Harbor Laboratories, San Juan Island, Washington. Eelgrass leaves (n=29) were collected across a belt transect via subtidal snorkel survey and analyzed for epiphyte abundance and species richness. Epiphyte abundance and species richness were found to be consistent across depth. This suggests that depth relationships, such as with disease, act independent of epiphyte load. There was also a significant relationship between leaf age and epiphyte abundance and species richness. The difference in epiphyte load between young and old leaves raises further questions about varying stress levels between leaves of different ages. These findings can be used to guide further efforts in understanding eelgrass community dynamics, as well as contextualizing environmental stressors most relevant to eelgrass meadows on a site-specific basis.

Introduction

Seagrasses are essential foundation species in coastal ecosystems across the globe. Seagrass meadows provide nursery habitat for many ecologically and economically important species (Unsworth & Cullen, 2010). In addition to providing habitat, seagrass meadows sequester carbon, improve water quality, and stabilize sediments (Fourqurean et al. 2012; de la Torre-Castro & Rönnbäck, 2004; Lamb et al., 2017). In the Salish Sea ecosystem of the Eastern Pacific, eelgrass (*Zostera marina*) fulfills the functions described above, protecting juvenile salmon and Dungeness crab, as well as stabilizing shoreline (Kenworthy et al., 2007; Semmens, 2009). However, these ecosystem services are now threatened since eelgrass meadows are in decline in the San Juan region of the Salish Sea (Christiaen et al., 2022). Intertidal meadow extent in this region has been greatly reduced, partially due to intense desiccation from heat waves during low tide (Aoki et al., 2022, 2023). Subtidal meadows, while relatively stable, are also experiencing shifts in spatial extent (Christiaen et al., 2022). Although the root cause of these changes has not been well established, one likely contributing factor is disease.

Seagrass wasting disease (SWD) is caused by the protist *Labyrinthula zosterae* and is characterized by reduced eelgrass growth rate and sugar storage capacity (Graham et al., 2021). Groner et al. (2016) found high variability between seagrass meadows in terms of disease prevalence (number of infected individuals) and severity (amount of infection per individual) in the Salish Sea. SWD symptoms are severity-dependent, and higher severity results from more efficient growth of *L. zosterae*. Eelgrass plants that are stressed are more susceptible to SWD infection, suggesting that meadows with high disease prevalence and severity may experience other non-disease stressors. The *in vitro* growth rates of *L. zosterae* increase with higher temperatures, suggesting that warmer sites may be more strongly affected by disease (Dawkins et al., 2018). Atmospheric temperature and sea surface temperature (SST) increases have been associated with declines in eelgrass and can be used to predict eelgrass presence (Aoki et al., 2022; Plaisted et al., 2022). Light availability also influences eelgrass health, with reduced light limiting the ability of eelgrass to photosynthesize and produce defensive phenols, resulting in higher SWD severity in eelgrass plants (Jakobsson-Thor et al., 2020).

The complex nature of these environments requires comprehensive study for the primary causes of decline to be identified. Deeper waters moderate temperature change, creating a more consistent and less stressful environment for eelgrass (Graham et al., 2023; Jakobsson-Thor et al., 2018). While depth may appear to be a refuge for eelgrass meadows, the benefits of depth may not be shared for every meadow. Eelgrass is also impacted through direct symbiotic interactions. Eelgrass epiphytes can further limit light availability (Sand-Jensen, 1977). Epiphyte loads (defined here as the species richness and abundance of epiphytes on an eelgrass leaf) can differ across spatial scales (Johnson et al., 2005), induce or reflect environmental stress (Groner et al., 2016), and influence the abundance of eelgrass-grazing amphipods in an area (Saunders et al., 2003). Each of these impacts has the potential to alter the vulnerability of eelgrass and thereby the transmissibility of SWD.

The variability of eelgrass epiphyte assemblages across spatial scales (leaf age and depth) is not well understood in the San Juan Islands. To understand whether a relationship exists between epiphyte assemblage and spatial parameters, the present study investigates four hypotheses: 1) Deep eelgrass leaves will have higher epiphyte abundance relative to shallow leaves, 2) Deep eelgrass leaves will have higher epiphyte species richness relative to shallow leaves, 3) Old eelgrass leaves will have higher epiphyte abundance compared to young leaves, 4) Old eelgrass leaves will have higher epiphyte species richness compared to young leaves. Investigating these relationships will help to contextualize other depth associations surrounding eelgrass and *L. zosterae*, allowing us to prioritize sites for conservation, inform restoration, and preserve the ecosystem services that people benefit from.

Methods

Sample collection

Eelgrass was collected via two subtidal snorkel surveys at the Friday Harbor Laboratories (FHL) subtidal meadow (48.545593, -123.013271). The first survey took place on 4/27/2024, and the second on 5/24/2024. A dive safety float was prepared prior to the surveys. This safety float had a rope attached to it with a lead weight at the bottom. The rope was measured using a meterstick and marked from the bottom up with tape for each 20 cm to estimate depth for each sample depth. A belt transect, which allows for sampling perpendicular to the transect line (Grant, 2004), was established using latitude/longitude coordinates. This created a reproducible survey area which may be used by future researchers who wish to investigate eelgrass communities at this site. (Figure 1).



Figure 1. Established belt transect for the surveys, starting from shore. Light pink areas represent survey locations along the transect.

The snorkeler entered the water to observe the distribution of the meadow at the site. An onshore starting point for the transect was subjectively selected in reference to subtidal portions of the meadows that had a consistent coverage of eelgrass across depth (Gayaldo, 2002). This subjective selection allowed for complete sample collections across the depths without missing samples due to a lack of plants along the transect. The latitude/longitude of this starting position was recorded using Google Maps on a smartphone and temporarily marked using a bucket. While shore-support held the larger portion of the measuring tape, the snorkeler swam 45 m offshore across the meadow while holding the end of the measuring tape as well as the dive float.

Once reaching 45 m offshore the latitude/longitude of the position was estimated, establishing the end of the belt transect. Back along this transect, the float was used to measure depth to 1.4 m where shoots were surveyed. The snorkeler then placed a 0.25 m² quadrat on the center of the transect to select eelgrass shoots to sample. The youngest leaves (innermost) were collected from one shoot in the top right and one shoot in the bottom left corner of the quadrat to ensure a randomization of sampling. When no shoots were present in these corners, the next closest shoot was surveyed. The next quadrat was placed approximately 0.5 m to the left, where two more leaves were sampled in the same pattern. This was repeated for a quadrat ~1.0 m to the left and ~1.0 m to the right of the transect center. A total of eight leaves were collected for this

depth. A photo of each quadrat was taken using a GoPro Hero 12 Black camera (GoPro Inc., San Mateo, California) prior to sampling. On the first day (April 27, 2024), the youngest leaf was separated from the rest of the shoot near the sheath and placed in a mesh bag.

Rite-in-the-rain paper labelled with the survey depth was placed in the mesh bag. The mesh bag was then placed in a bucket of seawater from the site to keep the leaves hydrated. Another collection of eight leaves was repeated for a second depth at 0.8 m, once again labelled and placed in the bucket of seawater. The survey team took the bucket immediately to Lab 3 at FHL once all 16 leaves were collected and placed the mesh bags in flowing sea tables for later analysis. On 5/24/2024, this survey was conducted again across the transect at depths of 0.5 m and 1.0 m to sample the oldest leaves. The oldest leaf on an eelgrass shoot is the outermost leaf. Similar abundance and species of epiphytes are found between the oldest leaf and the entire shoot in seagrass, so an estimation of epiphyte load of an entire shoot can be extrapolated from analyzing the oldest leaf (Kendrick & Lavery, 2001). This allowed us to record epiphytes for a whole shoot without removal of the whole plant, reducing the environmental disturbance of the survey.

Sample processing

Physical labels listing leaf number and side number were made. Photos of both sides of each leaf were taken, including the physical labels (Figure 2). Leaf length (mm) was measured from the base of the leaf to the tip. Leaf width (mm) was measured halfway up this leaf length. Both sides of a leaf were analyzed under a dissecting scope to determine epiphyte species presence and abundance. A macroalgal identification key (Gabrielson & Lindstrom, 2018) along with online databases such as AlgaeBase (Guiry, M.D. & Guiry, G.M., 2024) and BOLD Systems (Ratnasingham & Hebert, 2007) was used to identify epiphytes to the lowest possible taxon. The relative abundance (ranked percent cover using an abundance coefficient; Table 1) of epiphyte taxa on a leaf was used in place of the actual percent coverage of epiphyte taxa on a leaf, following methods outlined by Kendrick and Lavery (2001). Following observation under the dissecting scope, epiphytes were scraped off the entire leaf using a glass slide and analyzed under a compound scope to identify additional taxonomic groups. Species richness (the sum of different species identified on a leaf) was recorded. Once analysis for a leaf concluded, the leaf was placed in a discard bucket. This procedure was repeated for each collected leaf. Following data collection, specimens were reintroduced into the Salish Sea from the Friday Harbor Laboratories dock.

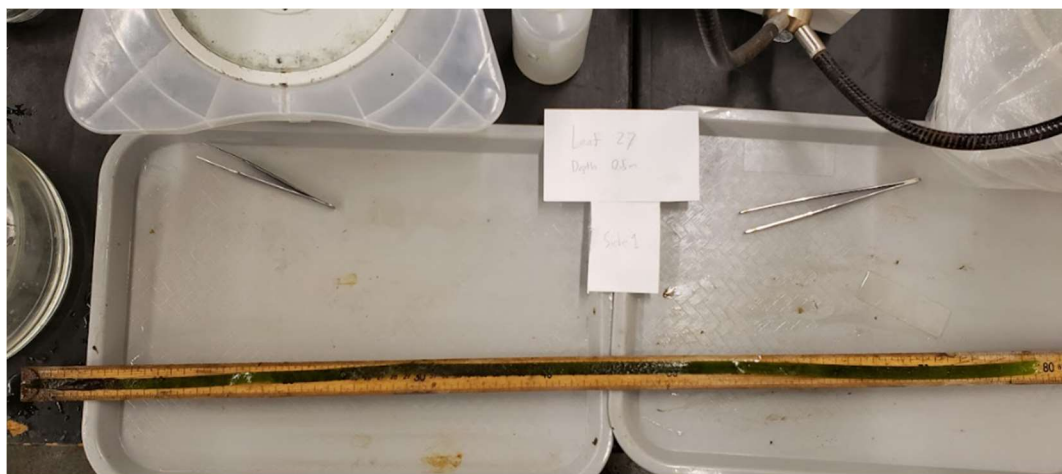


Figure 2. Photo of leaf seven with physical label and meterstick for reference.

Table 1. Abundance coefficients used to describe the relative abundance of epiphyte species on a leaf (Kendrick & Lavery, 2001).

Abundance Coefficient	Percent Cover
1	1%
2	2-9% (5%)
3	10-29% (20%)
4	30-54% (40%)
5	55-79% (70%)
6	80-100% (90%)

Data Analysis

Data analysis was performed through R (R Core Team, 2022). Analysis of similarities (ANOSIM) tests were used to analyze depth and leaf age relationships with epiphyte abundance using the vegan package (Oksanen et al., 2022), following the methods of Kendrick and Lavery (2001). Analysis of variance (ANOVA) tests were used to analyze depth and leaf age relationships with epiphyte species richness. Field-recorded depths were set relative to the meters mean lower low water (m MLLW) for the time for each site by subtracting the m MLLW value from the recorded depth. m MLLW data was gathered from NOAA Tides and Currents for Friday Harbor (CO-OPS, 2024).

Results

Across both surveys, a total of 29 leaves were collected. The belt transect began at 48.5454027, -123.0131043 and ended at 48.5449943, -123.0130291 (Figure 1). The quadrat method was used for the -1.8 m MLLW collections on 4/27 and not used for the rest of the sampling depths (Table 2). Low visibility for the shallow samples made the use of the quadrat ineffective. To maintain consistency, the snorkeler sampled 1 leaf each from 2 shoots in the same location, then repeated the sampling for two shoots ~0.5 m away from the previous sample. Depth data was consolidated into the following categories: “Deep” samples were taken at -1.5 and -1.8 m MLLW (Table 2). “Shallow” samples were taken at -1.1 and -1.2 m MLLW (Table 2).

Table 2. Summarized collection data for the two eelgrass surveys, including sample size and m MLLW adjusted depth values.

Date	Leaf age	Time (PST)	Depth (m)	Depth (m MLLW)	Leaves collected
4/27/2024	Young	13:30	0.8	-1.2	5
		14:00	1.4	-1.8	8
5/24/2024	Old	12:20	0.5	-1.1	8
		12:40	1.0	-1.5	8

Diatom abundance could not be accurately measured down to lower taxa, so the class Bacillariophyta is used to broadly describe diatom abundance on each leaf. Tube-dwelling diatoms were treated as a distinct group.

Depth vs. Epiphyte Abundance

There was no significant relationship between depth and epiphyte abundance across young and old leaves (ANOSIM, $R = 0.1804$, $p > 0.05$). We fail to reject our null hypothesis that depth has no relationship with epiphyte abundance. The low R value suggests that there is substantial overlap between the deep and shallow leaves in reference to abundance. This can be observed in Figure 3, as there is consistent overlap between deep and shallow groups. Our high p value confirms that this relationship is not statistically significant.

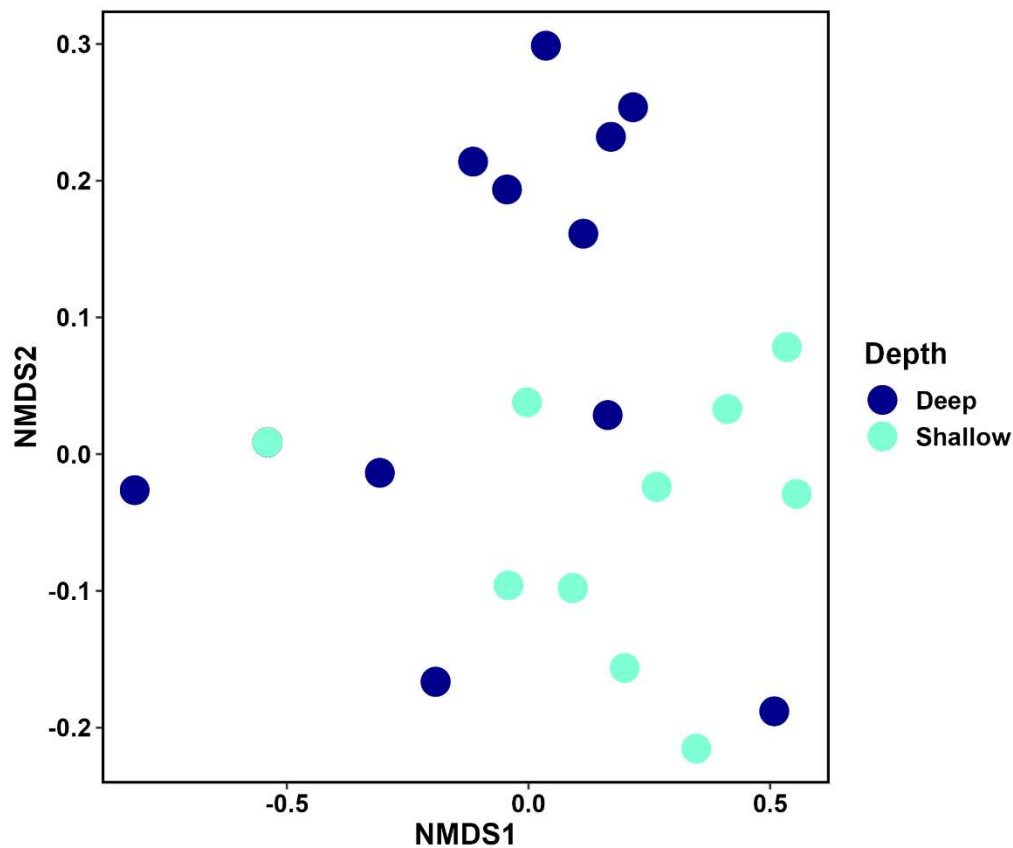


Figure 3. Non-metric MultiDimensional Scaling (NMDS) plot visualizing the relationship between depth and epiphyte abundance. The high level of overlap between deep and shallow categories reinforced the lack of significance for our sample.

Depth vs. Epiphyte Species Richness

There was no significant relationship between depth and species richness (ANOVA, $p > 0.05$). We fail to reject our null hypothesis that depth has no relationship with species richness. On average, deep leaves had an epiphyte species richness of two per leaf while shallow leaves had an epiphyte species richness of three per leaf (Figure 4).

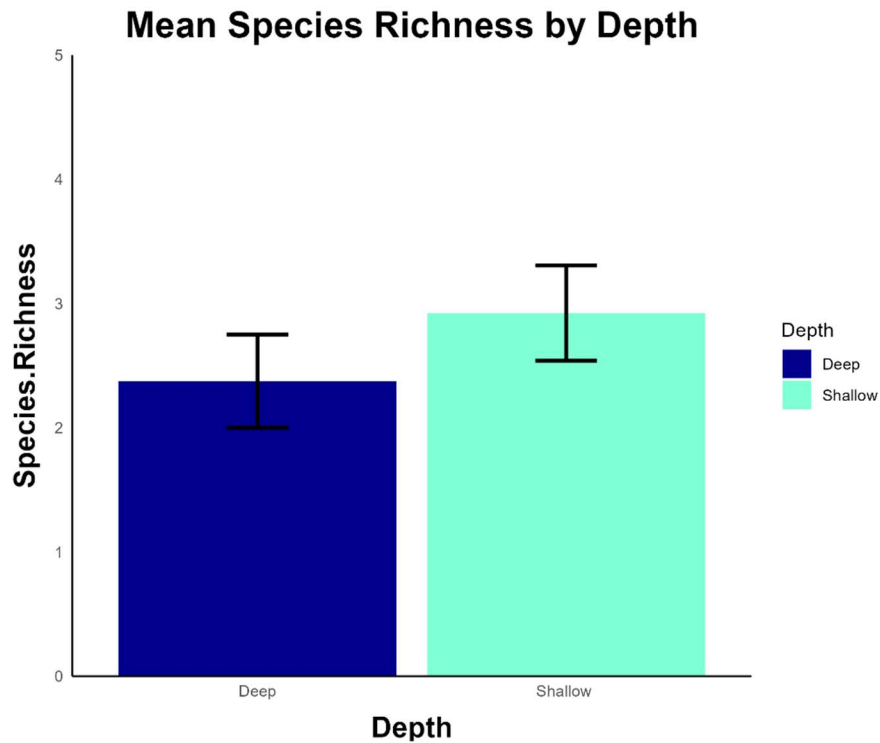


Figure 4. Mean species richness of epiphytes plotted against depth. Deep leaves averaged 2.4 species per leaf while shallow leaves averaged 2.9 species per leaf. Error bars represent standard error.

Leaf Age vs. Epiphyte Abundance

There was a significant relationship between leaf age and epiphyte abundance (ANOSIM, $R = 0.4941$, $p < 0.001$). The R value suggests that there is little overlap between the old and young leaf groups in reference to epiphyte abundance, which can be seen visually in Figure 5. Our low p value confirms that this relationship is statistically significant.

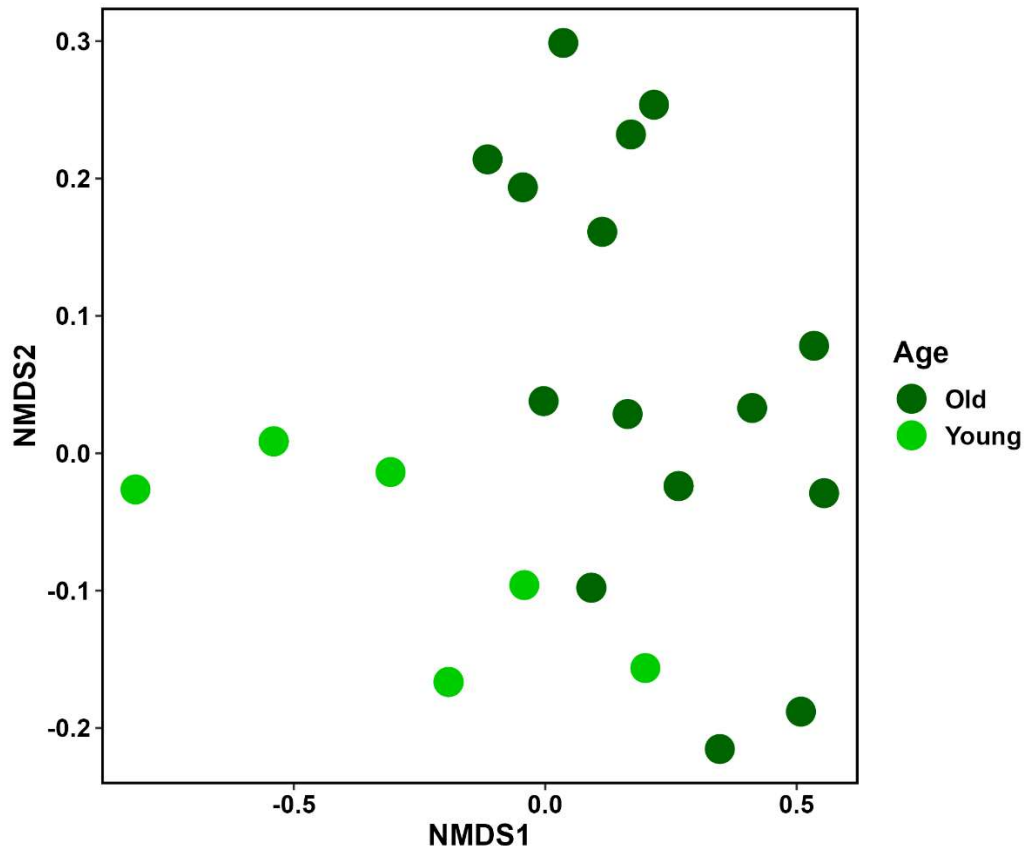


Figure 5. NMDS plot visualizing the relationship between leaf age and epiphyte abundance. There is observable spacing between a majority of the old and young groups, suggesting a relationship between age and epiphyte abundance.

Leaf Age vs. Epiphyte Species Richness

There was a significant relationship between leaf age and species richness (ANOVA, $p < 0.0001$). We reject our null hypothesis that leaf age has no relationship with species richness. On average, older leaves had a species richness of four while younger leaves had a species richness of one (Figure 6). Seven total epiphyte species were found growing on old leaves, where only two species were found across all young leaves (Table 3).

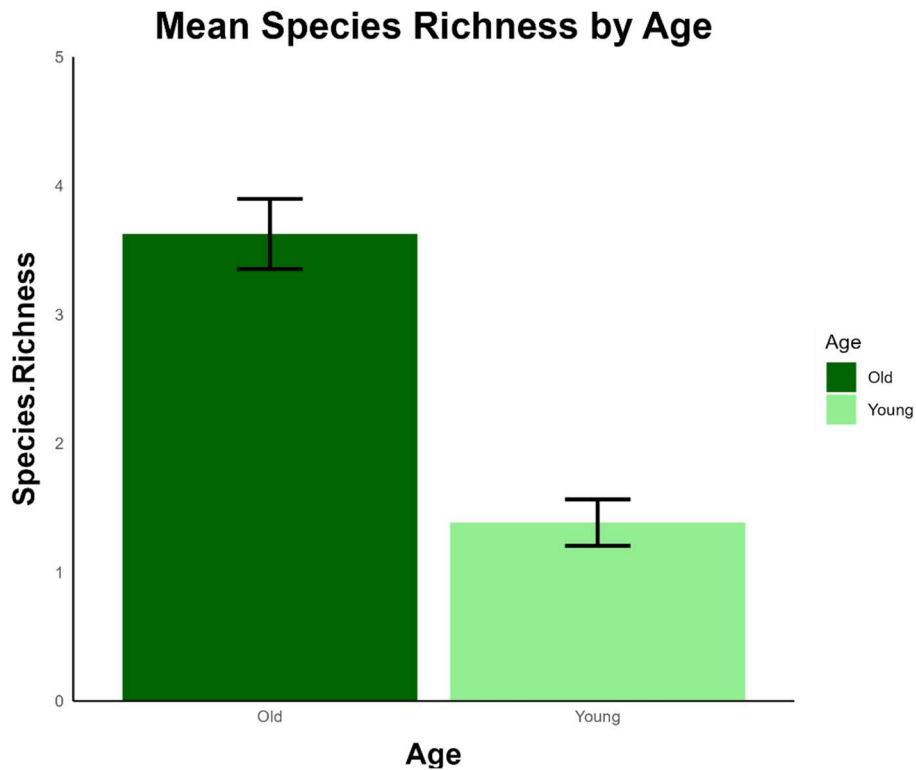


Figure 6. Mean species richness of epiphytes plotted against leaf age. Older leaves averaged at 3.6 species per leaf while younger leaves averaged at 1.4 species per leaf. Error bars represent standard error.

Table 3. Record of epiphyte taxa found growing on each leaf age.

Old Leaves	Young Leaves
Bacillariophyta	Bacillariophyta
Tube-dwelling Diatoms	Tube-dwelling Diatoms
<i>Smithora</i>	
<i>Blidingia minima</i>	
<i>Punctaria</i>	
<i>Leptonematella</i>	
<i>Ulva</i>	

Discussion

Depth

Our results suggest that the depth of eelgrass habitat does not strongly influence epiphyte abundance or species richness. The comparable distribution of epiphyte species and abundance between the two depths sampled in this study suggests that the stress induced by epiphytes remains an important factor for eelgrass in deeper meadows. While deeper meadows may still be recognized as refugia, the impacts of epiphytes must be considered. As light attenuation is a primary predictor of eelgrass distribution across depth (Krause-Jensen et al., 2011), it is possible that epiphytes induce higher stress at deeper depths by limiting already decreased levels of light. However, we observed substantial turbidity in the shallow portion of the meadow during both surveys. While these shoots may have access to higher levels of light when turbidity is low, there appear to be periods in the day (often during low tide) where light would be limited by suspended sediment. This may induce more stress than depth-related light attenuation. It must be considered that these shoots are also more susceptible to warming and disease (Graham et al., 2023). Further research will benefit from investigating these stressors across depth and provide insight on site-specific stressors in the San Juan Islands.

Leaf age

Our results suggest that the age of eelgrass leaves strongly influences both epiphyte abundance and species richness. Older leaves had significantly higher species richness and epiphyte abundance. This is expected, as leaf epiphyte loads accrue over time. Older leaves have more time to collect epiphyte species, and these species have more time to grow and become more abundant across the leaf. The difference in epiphyte species richness between young and old leaves may imply further variation in light availability impacts based on the age of the leaf, decreasing productivity potential in older leaves. Additionally, invertebrate communities appeared more abundant and diverse on older leaves. This is likely related to the significantly higher epiphyte abundance (Saunders et al., 2003), as many eelgrass grazing amphipods were identified between tube-dwelling diatom tubes. Assuming that limited light and increased grazing primarily impacts older leaves, younger leaves may face less difficulty in growth and maintenance relative to older leaves. Diatoms were the only taxa observed on young leaves. This consistently low species richness across young leaves suggests that the recruitment of epiphytes on young leaves, while minimal, may be predictable. Succession in microbial communities has been described (Fierer et al., 2010). Studies observing leaves overtime may be able to describe the temporal component influencing epiphyte growth, which may find use in restoration efforts.

The influence of epiphytes grazers on eelgrass health should be considered. One important grazer, the *Phyllaplysia taylori* sea hare, is sensitive to temperature changes (Tanner et al., 2020). This suggests that these sea hares may be vulnerable in the face of continued climate change. If a key epiphyte grazer is removed from the ecosystem, increased epiphyte settlement may occur on eelgrass leaves, further limiting light availability and providing more microhabitat

to eelgrass grazers. Assuming epiphyte communities will grow under these conditions, it is necessary to understand the distribution of epiphytes to confidently describe the current and future eelgrass stressors across the San Juan Islands.

Limitations

The lateral distance between our deep and shallow categories was substantial, measuring approximately 35 m. However, there was only a minor difference in depth between these locations (0.4 - 0.6 m MLLW). Our low range of depth limits the application of this study to meadows extending beyond -2 m MLLW. A study of these deeper shoots would aid in testing the claims of the present study. Our method for measuring depth was also inconsistent. The tape applied to the dive float rope was not static. The tape slid up and down across the rope, without maintaining the 20cm increments. This limits our ability to confidently describe our sample depths. This could have influenced our similarity between samples and should be recognized by future researchers utilizing this study system.

The consistent turbidity of the water around the shallow eelgrass made use of the quadrat for collections ineffective and made sampling more difficult. The sample size for young, shallow leaves decreased from 8 to 5, simply due to losing already collected eelgrass. By using an approximation method for collections instead of the quadrat method and decreasing the sample size for young, shallow shoots, the strength of our results is limited.

There was limited time in lab to analyze leaves, as the present study prioritized analyzing leaves while epiphyte communities were fresh. The author suggests the inclusion of more scientists in the lab to process the leaves more thoroughly, granting more time to each analysis. The present study provides only a broad understanding of eelgrass epiphyte community structure.

The present study sought to implement a new method of sample collection for eelgrass: to retrieve only specific leaves from an eelgrass shoot so as to not disturb the rest of the plant. This was made in the interest of leaving minimal impact on the study system. While successful, this was difficult to perform in the field. Some collected leaves were snapped too far away from the sheath to be considered complete, limiting their value as a representative sample. Further studies may benefit from improving upon these collection methods.

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