Epiphyte accrual patterns and effects on *Posidonia oceanica*
By Emily Hardison and Scott Borsum

**Abstract**
This study focuses on the relationship between *Posidonia oceanica* leaves and the epiphytes that live on those leaves at Stareso Research center in Calvi Bay, Corsica. We determine patterns of epiphyte accrual along *Posidonia oceanica* leaves for depths of 5m, 10m, 15m, and 20m and calculate the abundance of epiphytes across this depth range and the biomass of epiphytes in *Posidonia oceanica* bed at each depth, through in water measurements and characterizing epiphyte loads of samples brought onshore. We show that epiphytes aggregate in depth specific and leaf-side specific patterns along *Posidonia* leaves, cause degradation of live *Posidonia* tissue, and contribute a significant amount of biomass to *Posidonia oceanica* beds and detritus. We attempt to explain why certain patterns of epiphyte accrual are seen by determining that the convex side of the leaf most commonly faces up towards the sun.

**Introduction**
*Posidonia oceanica* is a protected endemic seagrass species in the Mediterranean Sea. This species is particularly interesting because of the large role it plays in maintaining a healthy and diverse Mediterranean ecosystem. It grows in vast meadows and provides habitats, food, and nursery areas for a wide array of species (Boudouresque C. et al., 2006). Despite its energy expensive lifestyle, *Posidonia* is able to survive in the nutrient poor waters of the Mediterranean Sea (Lepoint, 2002).

*Posidonia* is often inhabited by epiphytes. Previous research has shown that epiphyte load on *Posidonia* leaves has a negative relationship with depth (Lopez, 2012). Light attenuates with depth and can therefore become a limiting factor for primary producers, like *Posidonia*. The relationship between epiphytes and *Posidonia* has been studied in detail, but still many questions need to be answered in order to fully understand the depths of their interactions. Our current understanding is that epiphytes live on *Posidonia* leaves and therefore block those parts from photosynthesizing (Cebrian et.al, 1999). This study seeks to further our understanding of the relationship between epiphytes and *Posidonia* by analyzing the effects that epiphytes have on the lifecycle of *Posidonia* leaves. In order to accomplish this, we broke down the *Posidonia*-epiphyte interaction into three parts.

First, we surveyed the patterns of epiphyte accrual along *Posidonia* leaves. While doing this, we observed a difference in how epiphytes load themselves onto the leaf. *Posidonia* leaves have two distinct sides: a convex side and a concave side. Epiphytes were classified into two groups: plants and animals. We then calculated the percent coverage of epiphytes on both the convex and concave sides of the leaf at four depths: 5m, 10m, 15m, and 20m. This allowed us to determine the exact load of epiphytes across our depth range, and to calculate the biomass of epiphytes per meter squared.

We hypothesized that epiphyte loads would differ from 5m to 20m and between the concave and convex sides of the leaf. We also hypothesized that plant epiphytes prefer the
convex side of the leaf because it faces upward towards the sun more often than the concave side, and therefore has more access to light. If this is true, it will support the hypothesis that animal and plant epiphytes compete on Posidonia leaves for space. This competition should decrease as depth increases because of light limitation and a subsequent lower biomass of primary producers. This part of our study tests the importance of studying both sides of Posidonia leaves separately. Many studies either do not distinguish between the sides of the leaf, or only take measurements from one side of the leaf.

Our second goal was to evaluate the interaction between epiphytes living on Posidonia leaves and the live tissue of the Posidonia. We hypothesized that within the standing stock of Posidonia leaves there would be a significant amount of epiphyte biomass. We defined a “significant portion of the biomass” as 25% of the total. We also hypothesized that epiphytes cause Posidonia leaf tissue to degrade on the live plant. Once the plant begins accumulating epiphytes, the tissue breaks down. We could observe this degradation through a loss in plant biomass. We attempted to quantify this loss by comparing the weight of Posidonia covered in epiphytes to the weight of bare Posidonia and by demonstrating that the ends of Posidonia leaves lack curvature and that this correlates with increased epiphyte loads.

Our final goal was to determine how epiphytes play into the detrital cycle within Posidonia beds. Once a year, beginning in late fall, Posidonia sheds its older leaves, which are highly epiphytized and have lost their robustness. The ends of these leaves are discolored and lack curvature, structure and the ability to photosynthesize (Ott, 1980). This study took place at the beginning of this shedding event, which allowed for a unique opportunity to study the rate of shed and the biomass of shed over different depths.

We hypothesized that epiphytes play a role in the detrital shed that stays within Posidonia meadows by providing biomass to the detritus. Degradation of Posidonia tissue is very slow. It has been estimated to take six months to degrade 64% of the plant at 5m and 44% of the plant at 20m (Boudouresque C. F., 2012). We hypothesized that Posidonia shed would have lower epiphyte abundance than on live leaves and that this would suggest that epiphytes have a much faster degradation rate than Posidonia leaves. We observed more Posidonia in the shallows, which led us to hypothesize that detritus increases as depth decreases within Posidonia beds. We believed that this increase of shed at shallower depths would correlate with a higher biomass of epiphytes living on leaves. Overall, we hoped to provide a more comprehensive understanding of the Posidonia-epiphyte interaction and how it plays into the Posidonia lifecycle.

Materials/Methods
Study Species

Posidonia oceanica is a long lived, slow growing seagrass. Its leaves can grow to be 100cm plus and exhibit both sexual and asexual reproduction. It is the bioengineer of the Mediterranean, creating vast areas of habitat that a wide array of species utilize. Posidonia meadows act like a large filters, and strain the water column of particulate matter, which allows them to support a diverse benthic environment (González-Ortiz et al., 2014). Posidonia meadows are one of the world’s most productive ecosystems, producing large amounts of oxygen and
acting as an important carbon sink (Mazzela L., Ott J.A., 1984). Epiphytes are commonly seen on *Posidonia* leaves, with some species living exclusively on the leaves. A change in epiphyte community can indicate an environmental stressor, as they are extremely sensitive to change (Panayotidis P. 1980).

Once detached, *Posidonia* leaves undergo one of three processes. Many leaves and epiphytes are consumed by microorganisms and decompose within the bed they came from, therefore contributing nutrients to their home environment. Other leaves are exported to nearby ecosystems and serve as a nutrient import there. Finally, many leaves are exported to beaches during winter and fall storms (Pergent, 1994). When lots of *Posidonia* builds up on the beach it is called a Banquette. Banquettes dampen wave action and therefore, help fight beach erosion. They also add nutrients into the sandy beach ecosystem. (Boudouresque C. F., 2012).

**Study Site**

Our study was performed on the North and South side of the harbor at Stareso Research Station in Calvi Bay, Corsica. All analysis was performed at four depths: 5m, 10m, 15m, and 20m.

![Figure 1. a) Places where transects were set around Stareso. b) Places where Detritus Traps were set around Stareso.](image)

**Posidonia Percent coverage Sampling**

20m transects were run out at depths of 5m, 10m, 15m, and 20m, in order to survey the epiphyte load on *Posidonia* across different depths. A leaf was picked and the length was recorded (cm) in the water, every other metermark along the transect. Leaves were bagged and labeled according to depth in order to avoid issues with breaking leaf tips during transport to the lab.

In the lab, we measured a square centimeter on a glass microscope slide and marked the slide with nine, evenly spaced points. We used the slide as a uniform point contact (UPC) and placed it on top of the leaf in order to classify what was under each point, every 5cm along the
leave. Points were either on top of *Posidonia* tissue, animal epiphyte tissue or plant epiphyte tissue. The sides of the leaf were surveyed separately to characterize potential differences in the epiphyte community. The goal of this survey was to map out the epiphyte load patterns and the relative abundance of plants and animals along the leaves. We also wanted to characterize the differences in epiphyte coverage between the convex and concave side of the leaf across our depth range. If abundance of plant epiphytes is greater on the convex than the concave side of the leaf, and animal epiphyte abundance is greater on the concave side of the leaf, it will support the hypothesis that competition exists between plant and animal animals on *Posidonia* leaves for space.

**Leaf Orientation**

One to two times a day, for seven days, we surveyed the orientation of the leaves relative to the surface. We used random fin kicks along each depth contour (5m, 10m, 15m, & 20m) to survey the leaves. We surveyed fifteen locations per depth on each survey. In each spot, three leaves were chosen by randomly holding a dive slate above a seagrass bed and looking at what the top right corner was pointing to. Selected leaves were described as being convex side up, straight up, or concave side up. For our analysis we disregarded straight up leaves and focused on convex up or concave up. If the convex side of the leaf faces up the majority of the time, we will conclude that plants out compete on the convex side of the leaf, because it faces up towards the sun more often than the concave side.

**Leaf Curvature**

Leaf curvature is the natural bend that occurs along the leaf and is common in many grass species. We ran 20m transects at each depth. Then, we took samples from every meter mark along the transect. Every 5cm along the leaf we measured the natural curve width of the leaf and the flattened width of the leaf. These measurements were used to determine the ratio of curve width to flat width at each location along the leaf length. This ratio indicates how curved the leaf is; smaller ratios indicate a larger curve. If the ends of the leaves, which are highly epiphytized, have lost curvature, it will support the hypothesis that epiphytes cause degradation and damage to *Posidonia* leaf tissue.

**Epiphyte to *Posidonia* leaf Biomass relationship**

We cut 1.0cm.$^2$ sections of leaf tissue at each depth from live leaves. Next, we determined epiphyte coverage for both sides of the leaf using the UPC method described in ‘*Posidonia* percent coverage sampling’. Then we dried the pieces at 70°C and weighed the samples to the nearest .0001 grams. We used these measurements to graph the relationship between dry weight and epiphyte coverage along the leaf. This relationship allowed us to elicit the total biomass of epiphytes and of *Posidonia* tissue within each sample and the degradation of *Posidonia* tissue on live leaves due to epiphyte coverage. We can compare the observed dry weight of 0% covered leaf pieces to the expected dry weight, and if the observed weight of 0%
covered pieces of leaf is greater than expected, we will conclude that epiphytes cause degradation of *Posidonia* tissue.

**Shoot Density and Number of Leaves per Shoot**

An estimate of the total number of shoots and leaves were needed in order to determine the standing stock of epiphyte and leaf biomass. Shoot density and number of leaves per shoot at 5m, 10m, 15m, 20m were measured. Three 20m transects per depth were performed on both the north and south side of the harbor. 45 x 45 cm^2 quadrats were placed every meter along the transect for the survey of shoot density. The leaves per shoot were surveyed with the same transect and quadrat methods as previous, but within the quadrat, five shoots were randomly selected to be record for leaf number. This data was used to calculate the standing stock of epiphyte biomass and *Posidonia* leaf biomass by multiplying the total leaf surface area/m^2 by the equations created from “Epiphyte to Posidonia Biomass relationship” and the percent coverage data determined in “*Posidonia* Percent coverage Sampling”.

**Leaf Shed Traps**

We set two (50cmX50cmX20cm) shed traps out at each depth of 5m, 10m, 15m and 20m inside *Posidonia* beds. We also set two traps outside of *Posidonia* beds at 5m, one on the edge of the bed and the other one meter away. We did not set traps outside of beds at the other depths because those depths were entirely covered by *Posidonia*. We collected detritus from these traps roughly every 24 hours for 7 days. From the trapped shed, 10 leaves were picked at random, from each depth, and epiphyte coverage was determined using the method discussed in the “epiphyte percent coverage sampling” section of this paper. Samples were dried at 70°C overnight and then weighed. If shallow depths (5m & 10m) had significantly higher rates of shed than deeper depths (15m & 20m), we will conclude that more shed accumulates in the shallows.

**Results**

For all of our results, we used a critical p-value of .05.

**Posidonia Percent coverage Sampling**

5m and 10m depths showed the greatest differences between the concave and convex side of the leaf. Analysis of covariance reveals that both depths show significant differences in the slopes of the convex and concave best fit lines in plant and animal epiphyte coverage. The only other significant difference in slopes was at 20m in the plant epiphytes. Figure 2 clearly shows that in at 5m and 10m plant abundance was greater on the convex side of the leaf, while animal abundance was greater on the concave side of the leaf. This figure supports the hypothesis that
plants and animals compete for space on *Posidonia oceanica* leaves.

Figure 3 shows the relationship between the sides of the leaves and the plants and animals living on them. There were higher epiphyte loads on the ends of the leaves across all depths. This figure supports the hypothesis that animals and plants compete for space on the leaf; because it shows an inverse relationship, where plants inhabit the convex side of the leaf, while animals inhabit the concave side.

<table>
<thead>
<tr>
<th>Depth</th>
<th>P-value from Analysis of Covariance between convex and concave side of blade for Animal epiphytes</th>
<th>P-value from Analysis of Covariance between convex and concave side of blade for Plant epiphytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>.0021</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>10</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>15</td>
<td>.9345</td>
<td>.6904</td>
</tr>
<tr>
<td>20</td>
<td>.4588</td>
<td>.0453</td>
</tr>
</tbody>
</table>

Table 1. Shows the analysis of covariance results on Figure 3. graphs. Comparison is between the slope of the convex and the concave side of the blade best fit lines for each depth. Critical p-value is .05. 5m and 10m contain observed p-values <.05 for both animal and plant epiphyte coverage. 20m has p-value<.05 for plant epiphytes.
Figure 4 shows average epiphyte coverage as a function of depth on each side of the leaf. The convex side of the leaf showed significantly more epiphytes at all depths measured. A T-test revealed that average epiphyte coverage on the convex side of the leaf was significantly different at 10m (p-value <.0001) and on the concave side of the leaf the percent coverage was significantly different between all depths except 5m & 10m and 15m & 20m. This supports the hypothesis that epiphyte coverage differs between the concave and convex side of the leaf.

Leaf Orientation

Across all of the depths studied, the convex side of the leaf faced upwards towards the sun an average of 86.4% of the time. When compared to the expected 50% if leaf orientation were random, chi squared analysis revealed a p-value of <.0001. Figure 5 illustrates the leaf orientation averaged across all depths, but this result was consistent across all depths. These results strongly support the hypothesis that across all depths leaf orientation is predominantly convex up.
Leaf Curvature

Leaf curvature along the leaf did not seem to vary significantly with depth. T-tests of average curvature all had P-values > 0.05. The curvature across all depths started with no curve and ends with almost no curve. The curvature was most pronounced around the middle of the leaf. This data supports the hypothesis that high epiphyte coverage causes degradation of *Posidonia* tissue.

Dry Weight of Epiphytes on *Posidonia*
In Figures 7-9 each plotted point represents the weight of a $1\text{cm}^2$ area with a measured percent coverage. 15m was not included in the results here, because the dry weight sample data was flawed. Best fit lines are significant for 5m, 10m, and 20m. All best fit lines have y-intercepts that are lower than the actual average dry weight of Posidonia with 0% epiphyte coverage, which is depicted as a flat blue or green line. This 0% line was created by averaging all the dry weights of the larger than predicted 0% covered $1\text{cm}^2$ leaf piece and assuming it is constant. This supports the hypothesis that epiphyte coverage causes degradation of Posidonia tissue because the observed 0% value is larger than the predicted value.

**Figure 7.** Graph showing how dry weight varies with epiphyte coverage at 5m. Red line indicates best fit line and has p-value of <.0001. Blue line shows average weight of Posidonia with 0% epiphyte coverage.

**Figure 8.** Graph showing how dry weight varies with epiphyte coverage at 10m. Red line indicates best fit line and has p-value of <.0001. Green line shows average weight of Posidonia with 0% epiphyte coverage.
Table 2. Table showing p-values for best fit lines and the equations for best fit lines on Dry Weight vs. Epiphyte Coverage graphs.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Best fit line</th>
<th>P-value of Best Fit Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Dry Weight = .0039889 + 0.0000695*Percent coverage epiphytes</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>10</td>
<td>Dry Weight = 0.0038816 + 4.1649e-5*Percent coverage epiphytes</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>20</td>
<td>Dry Weight = 0.0016314 + 6.6434e-5*Percent coverage epiphytes</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

**Epiphyte to Posidonia Biomass relationship**

Figure 10 shows the total biomass per m^2 of leaves and what is living on them in live *Posidonia* beds, and then shows the epiphyte biomass/m^2 and the *Posidonia* leaf biomass/m^2. T-tests reveal that 5m is significantly different across all measures of biomass than 10m and 20m (with p-values <.05). Epiphytes make up ~50% of the leaf biomass across all three depths. This supports the hypothesis that epiphytes make up a significant portion of the biomass in *Posidonia* beds.
Detritus Traps

Figure 11 shows that detrital accumulation rates were greatest in the shallows, but T-tests indicate that because of large standard deviations, none of the depths show significantly different average dry weight/day. All p-values were >.05 when comparing all possible combinations of means. This supports the null hypothesis that there will not be any significant differences in shedding rates between the depths studied.

Figure 12 illustrates the differences in epiphyte coverage between live and dead Posidonia. T-tests reveal that only 10m. on the Live Posidonia has a significantly different average epiphyte coverage (p-value <.0001) and that none of the mean Posidonia epiphyte coverages for the dead samples are different across depth (p-value >.05). With the exception of 10m, all depths show a much higher average coverage in dead Posidonia, when compared to live Posidonia. This does not support the initial hypothesis that epiphytes will degrade quickly from shed and will therefore have lower biomass than on live Posidonia.
Discussion

When attempting to understand any processes within the lifecycle of *Posidonia oceanica* leaves, it is important to understand the role and effects of the epiphytes living on those leaves. The relationship between epiphytes and *Posidonia* leaves is more complicated than just a simple negative one where epiphytes block *Posidonia* tissue from photosynthesis. To further understand the depth of epiphyte-*Posidonia* interactions, we broke down their relationship into three parts: epiphyte accrual patterns, effect on live *Posidonia* tissue, and role in detritus. We studied each of these interactions across a depth range of 5m to 20m in order to see if the relationship changed with depth. Light is a large limiting factor for benthic aquatic primary producers and has large influences on the epiphyte-*Posidonia* relationship.

Comparisons between the epiphyte coverage on the different sides of the leaf revealed a strong preference for plant epiphytes on the convex side of the leaf and animal epiphytes on the concave side of the leaf (Figure 2,3,4). This preference was stronger at the shallower depths studied (Figure 2). This leads us to believe that light availability may be responsible for driving this pattern. We determined, through surveys, that the convex side of the leaf more commonly faces up towards the sun. This means that the convex side of the leaf has more access to sunlight. These results suggest that plant epiphytes outcompete animal epiphytes on the convex side of the leaf, because it affords them greater access to light. The animals instead grow on the concave side of the leaf, because they are not limited by light, but by space. Figure 3 supports this hypothesis by demonstrating the inverse relationship between plant and animal epiphytes. It shows how the percent coverage changes across the leaf. The line representing plant epiphyte coverage on the convex side of the leaf is almost identical to the animal line on the concave side of the leaf. Both sides of the leaf show there was an increase in epiphyte coverage on the ends of the leaf. This is probably because these are the oldest parts of the leaf and have therefore had the most time to accumulate epiphytes on them.
The results of our leaf curvature research suggest that the ends of *Posidonia* leaves have lost their curvature. This supports the hypothesis that the ends of the leaves have become highly epiphytized and that this epiphytization has a damaging effect on *Posidonia* tissue. Figures 8-10 also support this hypothesis. They show the expected weight of 0% covered *Posidonia* and how the expected weighed less than the observed weight of 0% covered *Posidonia* at 5m, 10m and 20m. This suggests that once epiphytes begin accumulating on *Posidonia* leaves they cause degradation of tissue, which can be observed through the underestimation of bare *Posidonia* weight compared to the observed seen in our results.

We found that across all depths the epiphyte biomass was almost half of the total leaf biomass in *Posidonia oceanica* beds. This means that the epiphytes on *Posidonia oceanica* leaves weigh about the same as the leaf itself. Figure 13 illustrates the difference between dead *Posidonia oceanica*, or leaf detritus, and live *Posidonia*. The leaf shed had significantly more epiphyte coverage across all depths studied than live *Posidonia* leaves. This pattern could be because shed *Posidonia* contains the oldest, most highly epiphytized leaves. From this result, we can infer that epiphytes on leaf shed make up even more than half of the biomass in the detritus.

Once a *Posidonia* leaf is detached it provides valuable resources to its environment, or it is exported to nearby ecosystems and serves as a nutrient input there (Pergent, 1994). Epiphytes make up at least half of the biomass on this shed. Therefore, in order to understand the nutrient recycling within *Posidonia* beds, or the export of nutrients out from *Posidonia* beds, it is imperative that the contribution of epiphytes be included in the calculations.

Unfortunately, in our study we did not have access to the tools or the time needed to determine and trace the nutrient input of epiphytes in *Posidonia* beds. Instead, we hoped to provide a platform for further nutrient and detritus studies by emphasizing the contribution of epiphytes to *Posidonia* beds and by characterizing the relationship between epiphytes and their host *Posidonia* leaves.
Bibliography

Boudouresque C. F., Bernard G., Bonhomme P., Charbonnel E., Diviacco G., Meinesz A., Pergent G.,


