Unwitting Accomplices: Do Isopod Bite Wounds Provide a Head Start for the Progression of Eelgrass Wasting Disease?

Carston J. Haffner, Paul M. Foster, Sarah J. Anderson, and David L. Cowles

Walla Walla University

Rosario Beach Marine Laboratory

Abstract

Eelgrass beds (*Zostera marina*) are a vital part of intertidal and estuarine ecosystems. These eelgrass beds provide food and shelter to a variety of marine species including small invertebrates, fish, and seabirds. In the 1930s an infectious wasting disease caused by the protist *Labyrinthula zosterae* swept through Atlantic eelgrass beds, wiping out most of the population along the entire North Atlantic coast. To prevent future decimations of eelgrass populations, it is important to understand the mechanisms of disease spread. Our research focused on whether there was a spatial correlation between bite marks left on the eelgrass blades by the isopod *Pentidotea resecata* (Fig. 1) and the early lesions of wasting disease. The answer to this question may provide a better understanding of *P. resecata*'s potential role as a vector of disease spread among *Z. marina*.

Introduction

Zostera marina eelgrass is a wide-ranging aquatic plant ubiquitous to bays, lagoons, and estuaries in temperate regions across the Northern Hemisphere including the North Atlantic and North Pacific American coasts (Figure 1). Found intertidally and shallow subtidally (Ruesink et al., 2009), eelgrass serves not only as an important habitat for many marine and estuarine species but also shields coasts from erosion and traps sediment. Nevertheless, *Z. marina* is vulnerable to disease, including wasting disease which wiped out most of the Atlantic's population in a 1930's pandemic. *Labyrinthula zosterae,* a protozoan slime mold, is now known to be the pathogen which infects *Z. marina* blades, causing the wasting disease (Short et al., 1987). As a consequence of infection, dark brown or black lesions form and spread throughout the blades, indicating the movement of the protist after cell lysis. The lesions first appear as small black spots which gradually expand and eventually lead to complete deterioration and death of the blade (Burdick et al., 1993).

One common species found living and feeding in eelgrass beds in the Pacific Northwest is the isopod *Pentidotea resecata* (Figure 2), which can grow up to 5 cm long. This marine isopod is found in two color morphs: green and brown; the green color morph being most common in eelgrass beds. Easily blending in among the blades of eelgrass, these isopods cling to and readily swim from blade to blade while feeding on the eelgrass.

Figure 1. Global distribution of *Zostera marina* as shown in gold highlighted zones. (IUCN, 2010).

Figure 2. *Pentidotea resecata* on eelgrass blades infected with *Labyrinthula zosterae* (dark regions on blades).

Hypothesis & Predictions

Hypothesis: The isopods are facilitating the spread of wasting disease in the eelgrass by biting the eelgrass, thus breaking through the plant's defenses and allowing *L. zosterae* to become established in the bite wound.

Prediction 1: *L. zosterae* lesions will be more prevalent on eelgrass blades that have been bitten.

Prediction 2: On eelgrass blades with both bites and lesions, there will be a positive spatial correlation between bites and lesions.

Methods

Clean, disease-free eelgrass blades, diseased eelgrass blades, and isopods were collected from Padilla Bay, WA, and placed into running seawater tanks in the laboratory (Figure 3). One tank was used in the first round of experimentation and two were used in the second. The clean eelgrass blades were mounted in a frame to allow inspection of their surface (Figure 4). Diseased blades were placed just upstream of the clean eelgrass but not allowed to touch it (Figure 3). 20 isopods were placed into the tanks with the ability to roam freely between the clean and diseased blades. Each experiment proceeded for 12 days, with the clean blades photographed each day to monitor isopod bite wounds and the presence of wasting disease lesions.

Figure 3. (right) Blades of *Z. marina* mounted in a frame, with diseased blades placed upstream.

Figure 4. (above) Mounted eelgrass blades observed in experimentation, including some with wasting disease (dark spots).

We observed photographs from each day and chose to carry out analysis on photographs that contained both bitemarks and lesions, while not being completely ravished by the disease. Thus, we selected photographs from day eight for the first round of experimentation and from day ten for the second round. From these photographs the number and location of bite wounds and wasting disease lesions were quantified on each blade, and the distance of each lesion to the nearest bite mark was compared to the average distance that 20 points randomly chosen on the blade were from the nearest bite mark (Figure 5). These distances were normalized for each blade by dividing all measurements for the blade by the average distance between each random point and the nearest bite mark, to account for the varying amount of bite marks between the different blades. A chi-squared test was used to determine whether blades with bite marks were more likely to also have lesions, and a one-sample t-test was used to determine whether the lesions were significantly closer to the bite wounds than the randomly selected points were. Distances were cube-root transformed before analysis to normalize them.

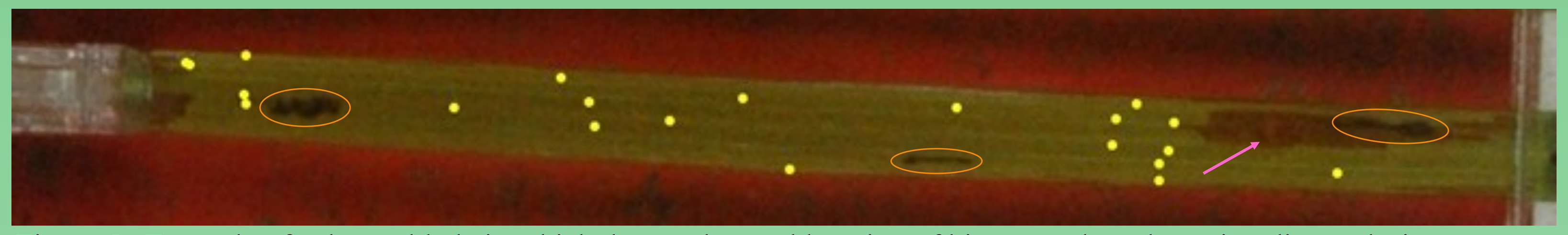

Figure 5. Example of eelgrass blade in which the number and location of bite wounds and wasting disease lesions were quantified. The yellow dots represent randomly selected points superimposed on the photo via ImageJ, the pink arrow indicates a bitemark, and the lesions are circled in orange.

Results

Our statistical analysis demonstrated that there was a significant difference ($p < 0.05$, Table 1) between average lesion-bitemark and random point-bitemark distance. Non-transformed data indicates that the median and mean distances between lesions and the nearest bitemark were, respectively, 67% to 47% of the distance between lesions and random points. Of these two values, the median more closely resembles the value you would get from cubing the transformed data which would be 40% of the distance. Considering all theses values, the lesions were about twice as close to bitemarks as would be expected if they were randomly distributed on the blade. Additionally, division of the bitemark-lesion distances into separate distance bins revealed that many more lesions were within a half centimeter of a bitemark than could be expected if the points were randomly distributed (Figure 6). However, chi-square analysis (Table 2) found lesions to be slightly but not significantly more common on blades with bitemarks than on those without ($\chi^2 > .05$). 72% of blades with bites had lesions, while only 62% of blades without bites had lesions.

Table 1: Data for the distance from lesion to bitemark, normalized then transformed with a cube root transformation. sd is standard deviation, n is the number of distances measured, t is the calculated t-statistic value, p is the probability.

(Normalized Lesion to Bite)$^{1/3}$	
mean	0.746
sd	0.352
n	177
t	-9.601
p	0.00001

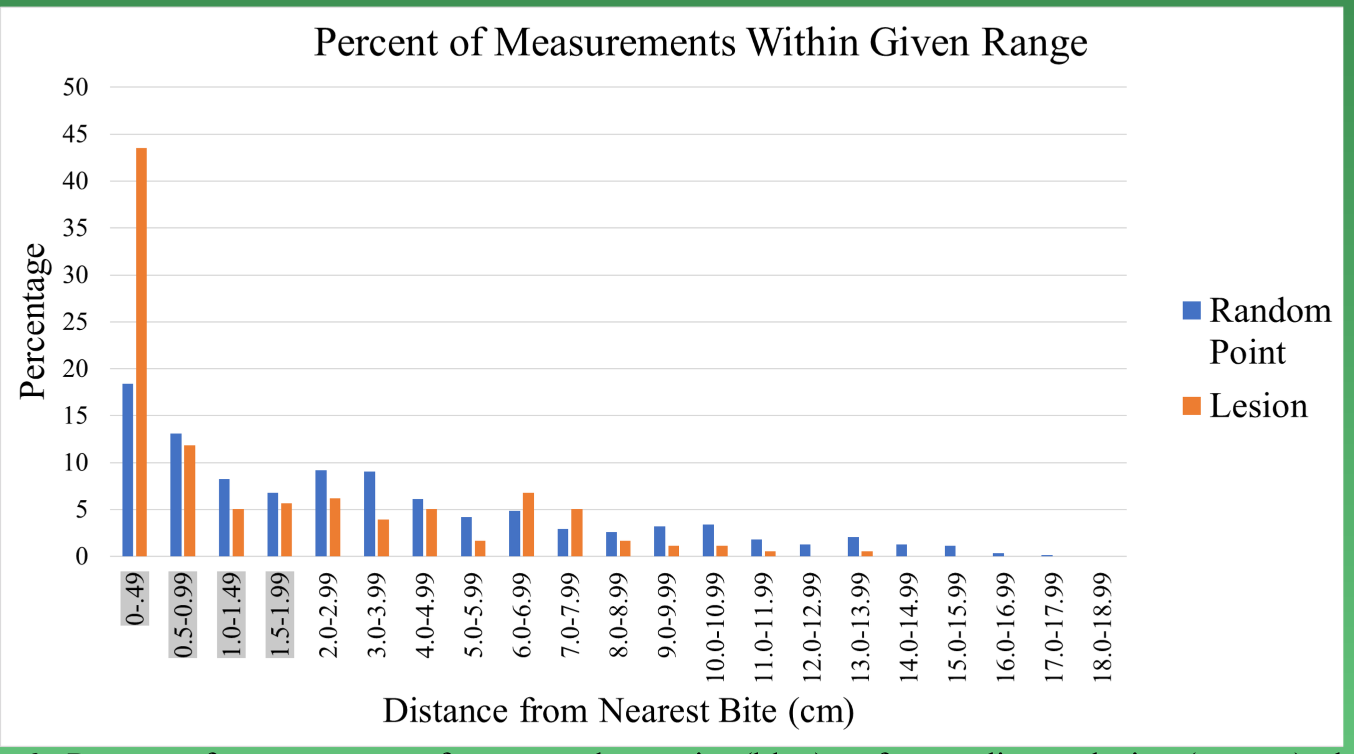

Figure 6: Percent of measurements from a random point (blue) or from a disease lesion (orange), that fall within a certain distance away from the nearest bitemark. The highlighted bins each cover a half centimeter, while the other bins all cover a whole centimeter.

	Bites	No Bites	Total
Lesions	33	16	49
No Lesions	13	10	23
Total	46	26	72

Table 2: Association of bites with lesions on eelgrass blades. Blades with bites were slightly more likely (72%) to also have lesions than were blades without bites (62%), but a chi-squared test showed that this difference was not significant [$\chi_2 = 0.791$, df = 1, $\chi_2(0.05, 1) = 3.85$].

Conclusions

- Statistical analysis using a t-test indicated that wasting disease lesions are significantly more likely to be nearer to a *P. resecata* bite mark than would be expected by chance. The results from this test support the idea that the oral cavity or bites of *P. resecata* may play a role in transmitting *L. zosterae* between diseased and healthy blades of eelgrass.
- Chi-square analysis demonstrated that lesions were not significantly more likely to occur on blades with bitemarks than those without. While it is still plausible that herbivory of *Zostera marina* by *P. resecata* provides a weakened entry point for the pathogen, this analysis suggests it is also likely that *L. zosterae* is able to infect a blade of eelgrass through other means.
- Our results confirmed our second prediction, that there is a spatial correlation between lesions and bitemarks, supporting the idea of the facilitative nature of the isopods in regards to the disease spread in *Z. marina.* However, the ambiguous results found in testing our first prediction leave some of our questions unanswered. Therefore, we aim to continue this research, possibly exploring other methods of data analysis.

References

Burdick, David M., Frederick T. Short, and Jaimie Wolf. 1993. An index to assess and monitor the progression of wasting disease in eelgrass *Zostera marina*. Marine Ecology 94: 83–90.

International Union for Conservation of Nature (IUCN) 2010. *Zostera marina*. The IUCN Red List of Threatened Species. Version 2018-1

Ruesink, J.L., J.-S. Hong, L. Wisehart, S.D. Hacker, B.R. Dumbauld, M. Hessing-Lewis, and A.C. Trimble. 2009. Congener comparison of native (*Zostera marina*) and introduced (*Z. japonica*) eelgrass at multiple scales within a Pacific Northwest estuary. Biological Invasions 12: 1773-1789.

Short, F.T., L.K. Muehlstein, and D. Porter. 1987. Eelgrass wasting disease: cause and recurrence of a marine epidemic. The Biological Bulletin 173: 557–562.

Acknowledgments

We would like to thank and acknowledge the following people and institutions for their invaluable help. Without their expertise and resources this research would not have been possible.

- Rosario Marine Beach Laboratory: For providing the space and materials used in research.
- Technical Support Services: For constructing the racks and manifold used for the project.
- WWU Biology Department: For support and sponsorship.
- Dave Habenicht: For maintaining and ensuring that the water pumping system used in experimentation was functional.