Mechanisms of Resilience: Contextualizing the health of subtidal *Zostera marina* meadows around the San Juan Islands, Washington

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Abstract

Eelgrass (*Zostera marina*) is a vital ecosystem engineer in the Salish Sea and is established as an indicator for ecological health in Puget Sound. Declines within eelgrass meadows around the San Juan Islands from monitoring through 2000-2020 have gained attention, as losing eelgrass threatens the many ecosystem services they provide, potentially disrupting entire food webs. Seagrass wasting disease (SWD), caused by the protist *Labyrinthula zosterae*, and heat stress are primary concerns for these meadows; both of these stressors are moderated by cooler and deeper waters. In this study we provide new information on 11 subtidal eelgrass meadows around the San Juan Islands during the summer of 2023. Through specimens collected via snorkel survey, we report information on body size, leaf count, and disease, using one-way ANOVA tests to compare variables between locations. Sites significantly differed in canopy height, normalized sheath length, leaf count, and second youngest leaf area ($p < 0.05$). The prevalence of disease differed by survey site, from 0% at False Bay and Beach Haven to 74% at Fisherman's Bay ($p < 0.05$). The severity of disease between sites did not differ ($p > 0.05$). Sites were distinct in their environmental conditions. Variance in turbidity, depth, and water flow may have an influence on SWD susceptibility. In the face of rising global temperatures, this study reinforces the importance of holistic analyses of environmental conditions and future projections to identify resilience in subtidal eelgrass meadows.

Introduction

Seagrasses are aquatic angiosperms. Species of seagrass are widespread, found on every continent except for Antarctica (McKenzie et al., 2020). Healthy meadows of seagrass are integral to supporting the biodiversity of global ecosystems and cultures as they shelter juvenile organisms (Orth et al., 2006), support productive fisheries (Unsworth & Cullen, 2010), stabilize coastal sediments (de la Torre-Castro & Rönnbäck, 2004), cycle nutrients (McGlathery et al., 2007), clean seawater (Lamb et al., 2017), and sequester carbon (Fourqurean et al. 2012). Rapid environmental change, at global and regional scales, has led to widespread seagrass loss. (Orth et al., 2006). Rapidly changing coastlines, eutrophication, and warming temperatures harm seagrass, and have drastic impacts on surrounding ecosystems (Aoki et al., 2022; Kemp et al., 2005; Walker et al., 2006).

Eelgrass (*Zostera marina*) is a key species of seagrass found in coastal, temperate waters. Across the Northeast Pacific, eelgrass meadows provide habitat for a variety of marine life and are vital for the life history of many crustaceans, forage fishes, and salmon. Within the Salish Sea, eelgrass is an indicator of Puget Sound ecological health (Goehring et al., 2015). Eelgrass meadows act as essential nurseries in the life history of a variety of organisms (Kenworthy et al., 2007). Herring (*Clupea pallasii*), shiner perch (*Cymatogaster aggregata*), and surf smelt (*Hypomesus pretiosus*) use eelgrass meadows to successfully reproduce and survive (Rubin et al., 2018). Chinook salmon (*Oncorhynchus tshawytscha*) smolts preferentially reside in eelgrass habitats, and have greater survivorship in such environments (Semmens, 2009). Eelgrass also holds substantial value to indigenous communities, as the plants themselves and the organisms they protect are used for subsistence and cultural practices (Kenworthy et al., 2007).

Eelgrass and its services are threatened by Seagrass Wasting Disease (SWD). This infection is caused by the protist *Labyrinthula zosterae* (Muehlstein & Porter, 1991). SWD can limit plant growth and reduce the production and storage of sugars, potentially slowing eelgrass recovery during instances of disturbance (Graham et al., 2021). *L. zosterae* is more commonly observed in eelgrass located in shallower, more saline environments (Graham et al. 2023; Jakobsson-Thor et al., 2018). *In vitro*, *L. zosterae* grows faster in warmer environments (Dawkins et al., 2018). Increases in atmospheric and sea surface temperature (SST) will most directly affect shallow, easily influenced environments utilized by eelgrass. SWD prevalence (proportion of infected individuals) peaks during summer months in northern European meadows (Bockelmann et al., 2013). Other factors, such as light exposure and tissue damage, also influence the prevalence and severity (area of infected or necrotic tissue) of *L. zosterae* in eelgrass (Jakobsson-Thor et al., 2020). As meadows around the San Juan Islands, Washington experience different conditions, site-specific analysis is required for understanding the status of eelgrass populations and their futures amidst SWD outbreaks and environmental change. Current surveys indicate significant declines in the shallow edge of San Juan Island, Washington eelgrass meadows (Aoiki et al 2023; Christiaen et al., 2022; Groner et al 2021).

Visual analysis of eelgrass leaves has been used in the past to identify the prevalence and severity of *L. zosterae*. The Eelgrass Lesion Image Segmentation Application (EeLISA) artificial intelligence program analyzes scanned images of eelgrass leaves and calculates conservative disease metrics (prevalence, severity) measuring disease 5000x faster than human visual scanning (Rappazzo et al., 2021). EeLISA, which is ground truthed with qPCR diagnostics of *L. zosterae*, is an efficient program for measuring SWD prevalence and severity.

As part of the San Juan County Eelgrass Mapping, Change Analysis, and Conservation Project, this project evaluates the status of SWD in subtidal eelgrass meadows around the San Juan Islands through collaboration with University of Washington's Friday Harbor Labs, WA Department of Natural Resources (DNR), Friends of the San Juans, and volunteer citizen scientists. The data collected will aid in making informed conservation decisions on near-future site protections. By collecting eelgrass from subtidal meadows and using the EeLISA machine learning tool to record site-specific disease prevalence and severity, this study supplies novel data for historic monitoring sites, as well as data for newly included meadows of eelgrass.

The range of this study can help inform protections for eelgrass meadows around the San Juan Islands and further the understanding of the complex ecological systems that concern eelgrass conservation.

Methods

Field survey

Sites selected for the survey were located around the San Juan Islands. We selected 11 sites that were a combination of DNR monitoring sites, SWD survey sites, and herring spawning locations, that are spatially distributed throughout the archipelago. We surveyed subtidal eelgrass meadows on low tides on June 16, 19, 20, 21, 22 and July 17, 18, 2023.

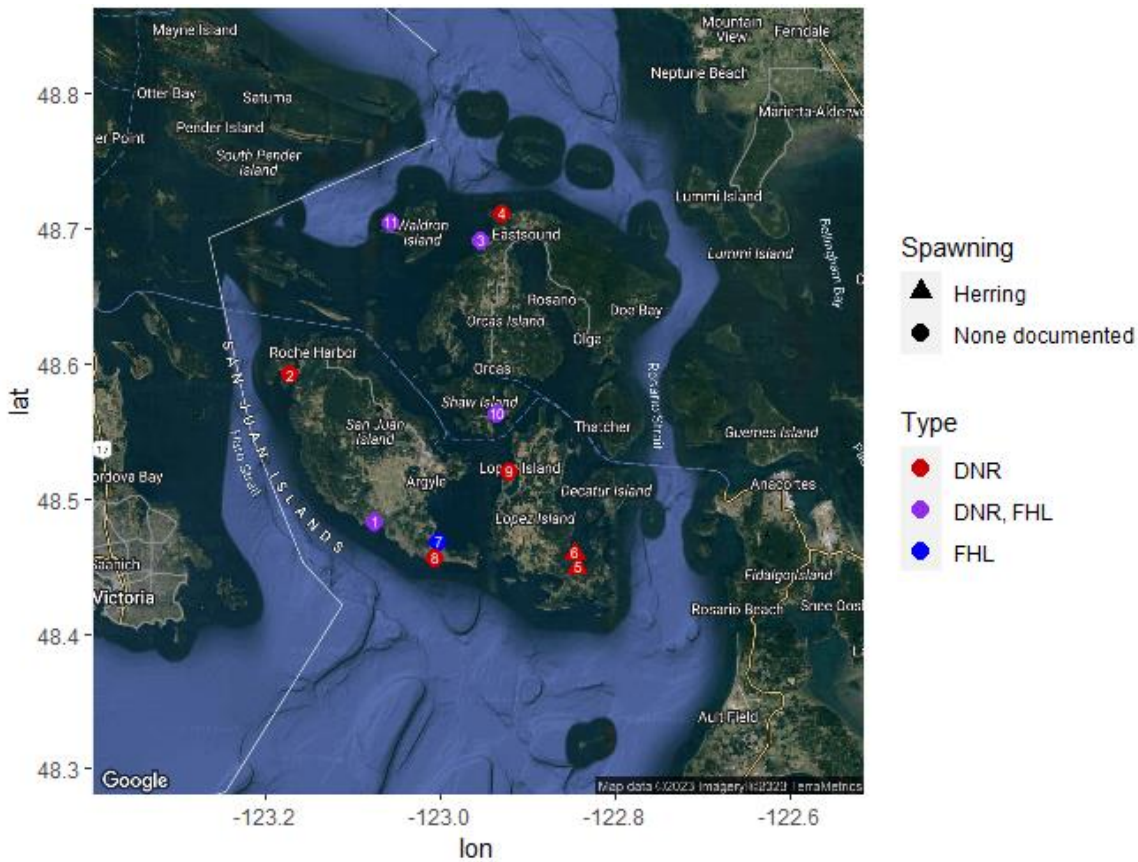

Figure 1. Map of subtidal survey sites around the San Juan Islands. Type specifies the group monitoring a particular site, as well as its status as a known herring spawning site.

Table 1. Sites listed with site type, date of survey, depth of survey, and coordinates. Meters Mean Lower Low Water (m MLLW) was calculated using tide data from nearby tide recording stations.

Site ID	Site	Site Type	Date of Survey (2023)	Depth Surveyed (m MLLW)	Latitude	Longitude
1	False Bay	DNR, FHL	6/16	-1.89	48.48262	-123.07436
2	Mosquito Pass	DNR	6/16	-2.74	48.59188	-123.17262
3	Beach Haven	DNR, FHL	6/19	-1.85	48.69165	-122.95287
4	North Shore	DNR	6/19	-2.33	48.70486	-123.05377
5	Mud Bay	DNR, Herring	6/20	-1.85	48.44957	-122.84359
6	Hunter Bay	DNR, Herring	6/20	-1.66	48.45970	-122.84650
7	Fourth of July	FHL	6/21 6/22	-2.77 (+0.40)	48.46862	-123.00211
8	Salmon Banks	DNR	6/21	-5.43	48.45633	-123.00688
9	Fisherman's Bay	DNR	7/17	-1.72	48.51982	-122.92082
10	Indian Cove	DNR, FHL	7/17	-1.81	48.56293	-122.93544
11	North Cove	DNR, FHL	7/18	-2.55	48.70432	-123.05718

We surveyed subtidal eelgrass meadows at each site through snorkeling, approximately occurring around the day's lowest tide. A small team (2-6 snorkelers) collected 20-25 eelgrass shoots from each subtidal meadow, retrieving a specimen shoot every 2 kicks of fins while following a consistent depth (Figure 2). The field team recorded depth and time of collection for each site, in addition to latitude/longitude coordinates. Teams collected shoots by snapping the plant at the base of the sheath, leaving rhizomes intact, and were stored in mesh bags before reaching shore. Teams then transferred the collected shoots to ziploc bags filled with seawater from the site, stored them in a cooler on ice for the return to Friday Harbor Laboratories, then processed the shoots for biometric data and disease prevalence.

Figure 2. The snorkel survey team at Fourth of July Beach retrieving eelgrass shoots from the sandy bottom.

Biometrics and disease prevalence

In the lab we measured eelgrass biometrics and SWD prevalence 1-2 hours following collection. We collected biometrics by measuring canopy height, sheath length, the length of the second youngest leaf, the width at the middle of the second youngest leaf, and the total count of leaves (Table 2). Length and width measurements were taken to the closest millimeter. We determined SWD prevalence visually by identifying signs of infection (dark lesions) from *L. zosterae* on the second youngest leaf. To prepare the second youngest leaf for analysis, we scraped the leaves with small plastic rulers to remove epiphytes and epifauna.

Table 2. Biometric data recorded when processing eelgrass shoots.

Metric	Collection Strategy
Canopy Height (mm)	Measure against a meter stick from the base of the shoot to the tip of the longest leaf.
Sheath Length (mm)	Measure against a meter stick from the bottom of the sheath to the last node (diverging leaves) of the sheath.
Leaf Count	Count the total number of intact leaves that extend past the last node of the sheath.
Second Youngest Leaf Length (mm)	Snap off the second youngest leaf at the base near the node. Measure against a meter stick from the base of the leaf to the tip.
Second Youngest Leaf Width (mm)	Measure against a ruler halfway up the leaf.
Prevalence	Scrape epiphytes and epifauna off the second youngest leaf using a plastic ruler. Visually scan for dark lesions.

Disease severity

We prepared transparencies of any diseased second youngest leaf by placing whole or segments of diseased leaves flat between two transparencies, which were scanned using a Canon CanoScan LiDE220 scanner (Figure 3a). We utilized the EeLISA machine learning tool to analyze the eelgrass scans, calculating SWD prevalence and severity (Figure 3b).

Figure 3. a. Scans of the second youngest leaf of shoots 13-16 from Hunter's Bay, Lopez Island. Leaf 14 (second from the left) displays large bands of necrotic/infected tissue, identified as lesions from SWD. b. EeLISA output for shoots 13-16 from Hunter's Bay, Lopez Island. The prominent lesions on leaf 14 have been marked in red by EeLISA, while healthy sections are colored in green.

Analysis

Data analysis was conducted through R. We utilizes the packages tidyr, Rmisc, tidyverse, ggplot2, RColorBrewer, dplyr, plyr, and DescTools. Comparisons between sites were performed using a chi-square test for prevalence and one-way ANOVA tests with a confidence interval of 95%.

Results

<u>Site conditions</u>

The sites for this survey greatly differed in a variety of conditions. Depth, turbidity, and water flow were a few of the most observable conditions.

Depth

Sites were surveyed at different depths (Figure 4). The deepest survey, at Salmon Banks, was conducted at -5.43 m Mean Lower Low Water (MLLW). The shallowest survey, at Hunter Bay, was conducted at -1.66 m MLLW (Table 1).

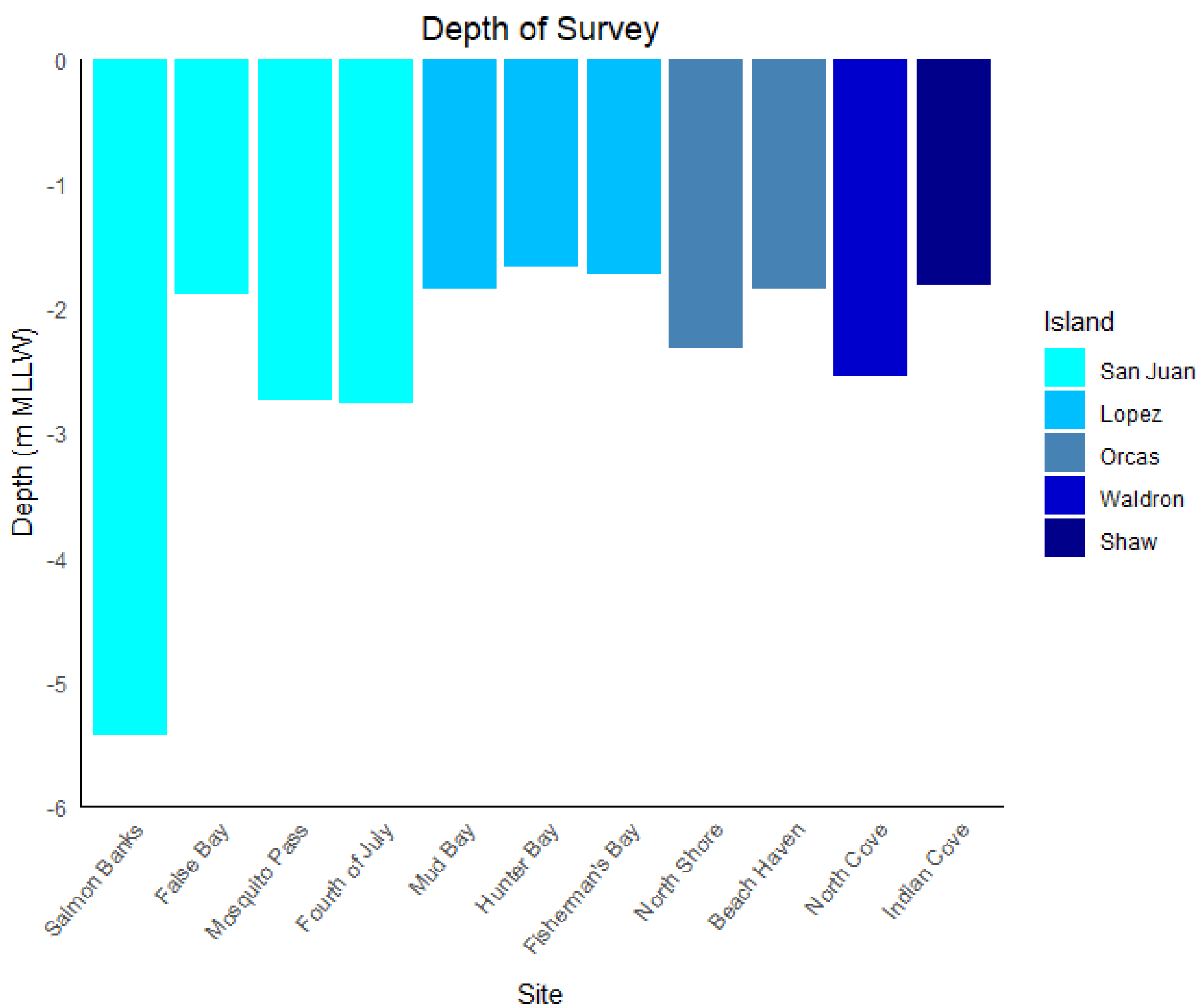

Figure 4. Depth (m MLLW) by site.

Turbidity

We observed varying levels of turbidity across sites. Mud Bay was shallow and harbored cloudy water full of suspended sediment and algae (Figure 5a). Salmon Banks and Fourth of July had few suspended particles, allowing for substantial clarity (Figure 5b).

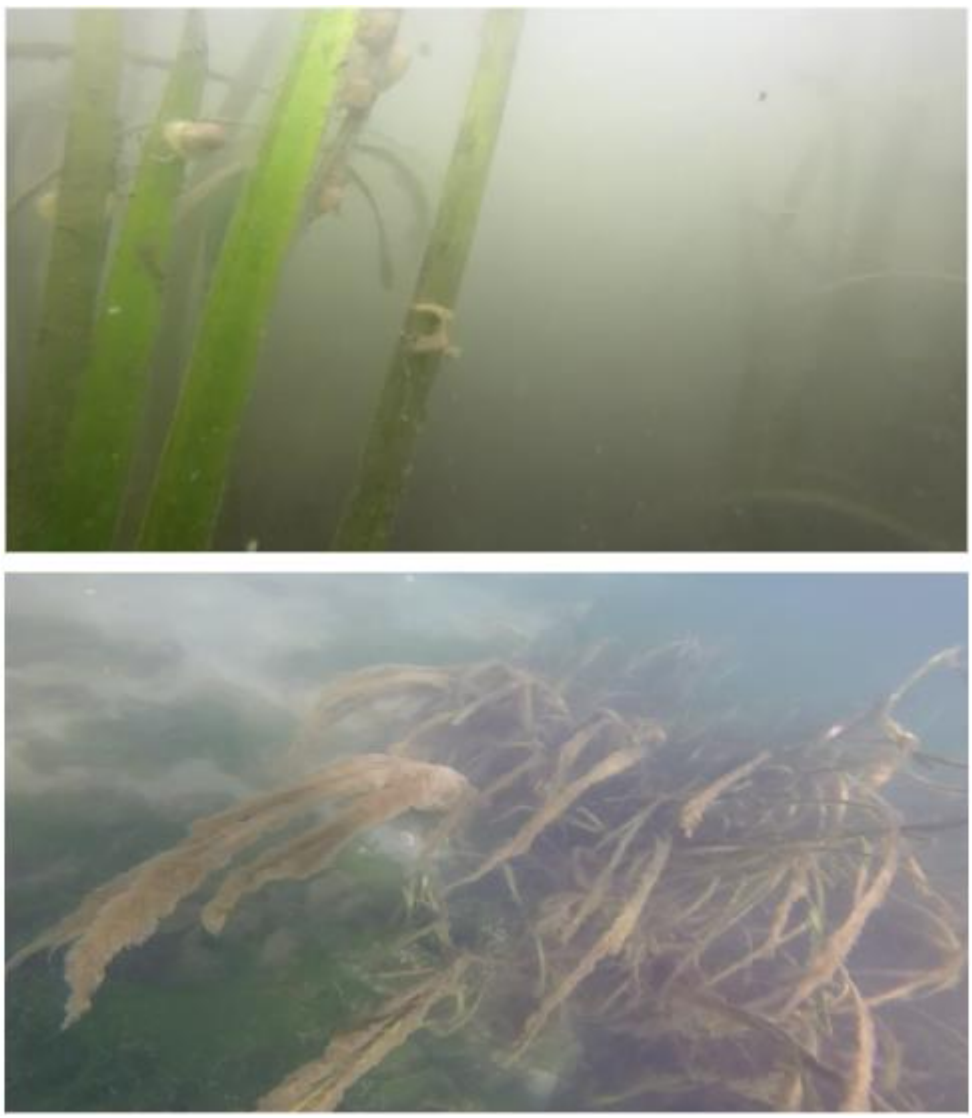

Figure 5. a. Mud Bay was a shallow site that had water clouded by suspended particles. b. Fourth of July Beach supported a deeper meadow with noticeably clearer water.

Current

The flow of water experienced at different sites was notably different. Salmon Banks experienced high flow rates, while embayments like Mud Bay and Indian Cove experienced slower, stable flows.

Body size

Biometrics were collected for all shoots (n = 251) on a site-specific basis for comparison.

Mean canopy height

The mean canopy height of shoots between sites differed significantly (Figure 6; $p < 0.001$). Post hoc analysis found the most significance between Salmon Banks and Indian Cove ($p < 0.001$). Minimum mean canopy height was found at Salmon Banks (891.88 mm). Maximum mean canopy height was found at Indian Cove (1812.63mm).

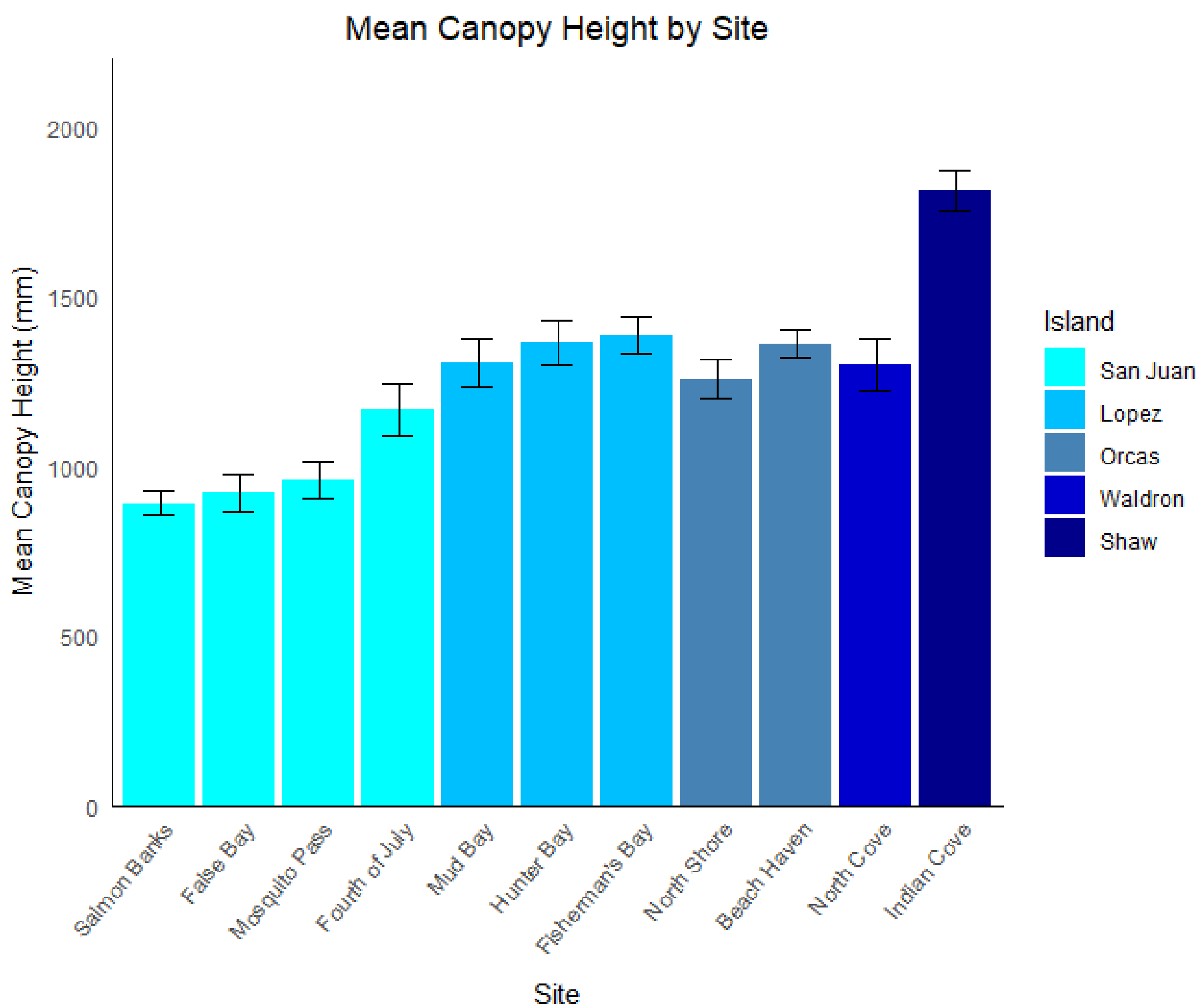

Figure 6. Mean canopy height (total length from the base of a shoot to the tip of the longest leaf) by site. Error bars represent standard error.

Mean normalized sheath length

Sheath length was normalized by dividing the individual sheath length of a shoot by that shoot's canopy height. There were significant differences between normalized sheath lengths across sites (Figure 7; $p < 0.001$). Post hoc analysis found significance between North Cove and Fourth of July ($p < 0.01$) and North Cove and Mud Bay ($p < 0.01$).

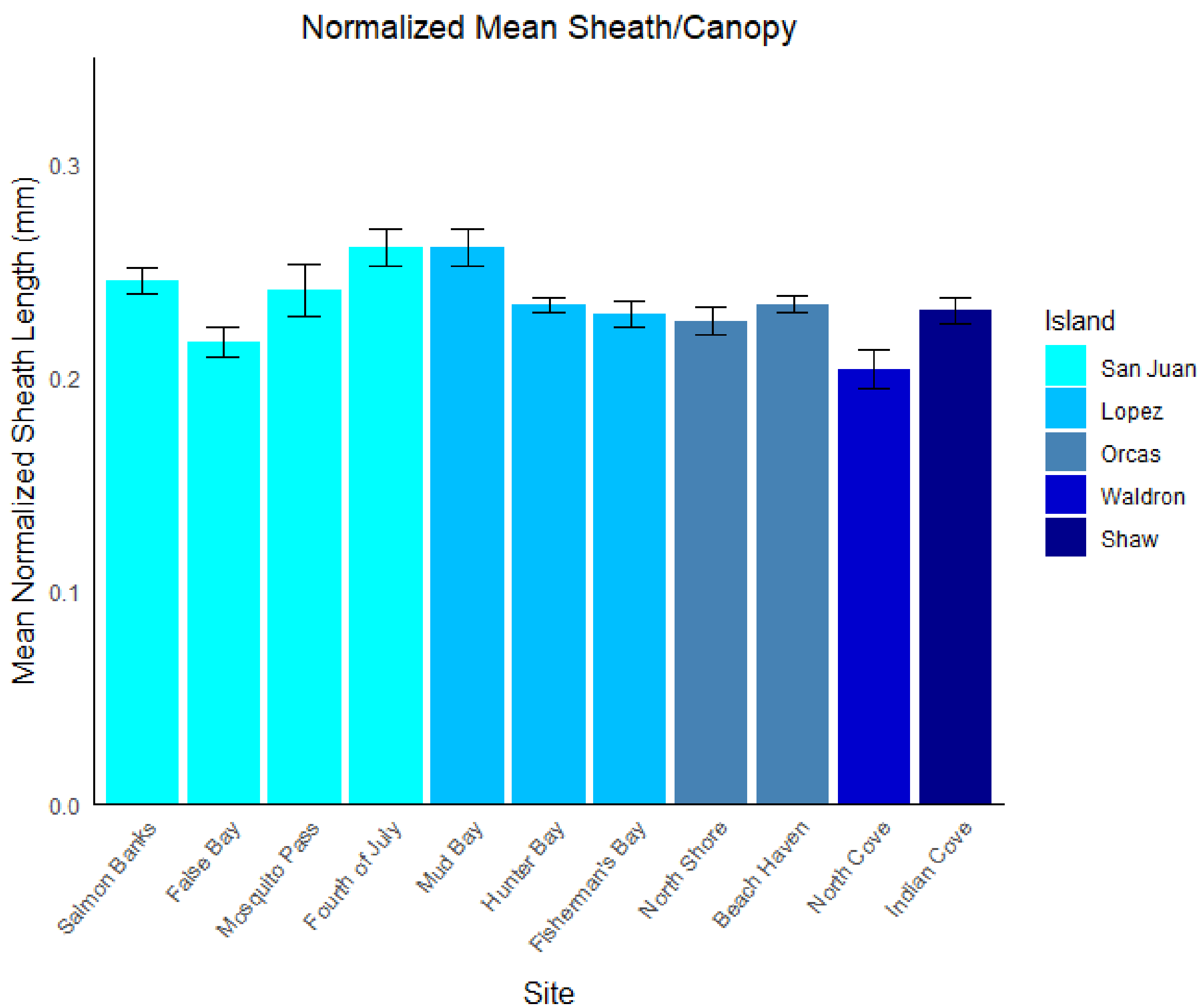

Figure 7. Mean values of normalized sheath length by site. Error bars represent standard error.

Mean leaf count

The mean count of leaves per shoot significantly differed between sites (Figure 8; $p < 0.001$). Post hoc analysis found the most significance between Indian Cove and False Bay ($p < 0.001$) and Indian Cove and Beach Haven ($p < 0.001$). We found the minimum mean leaf count at Indian Cove (4.08) and the maximum mean leaf count at False Bay (5.91).

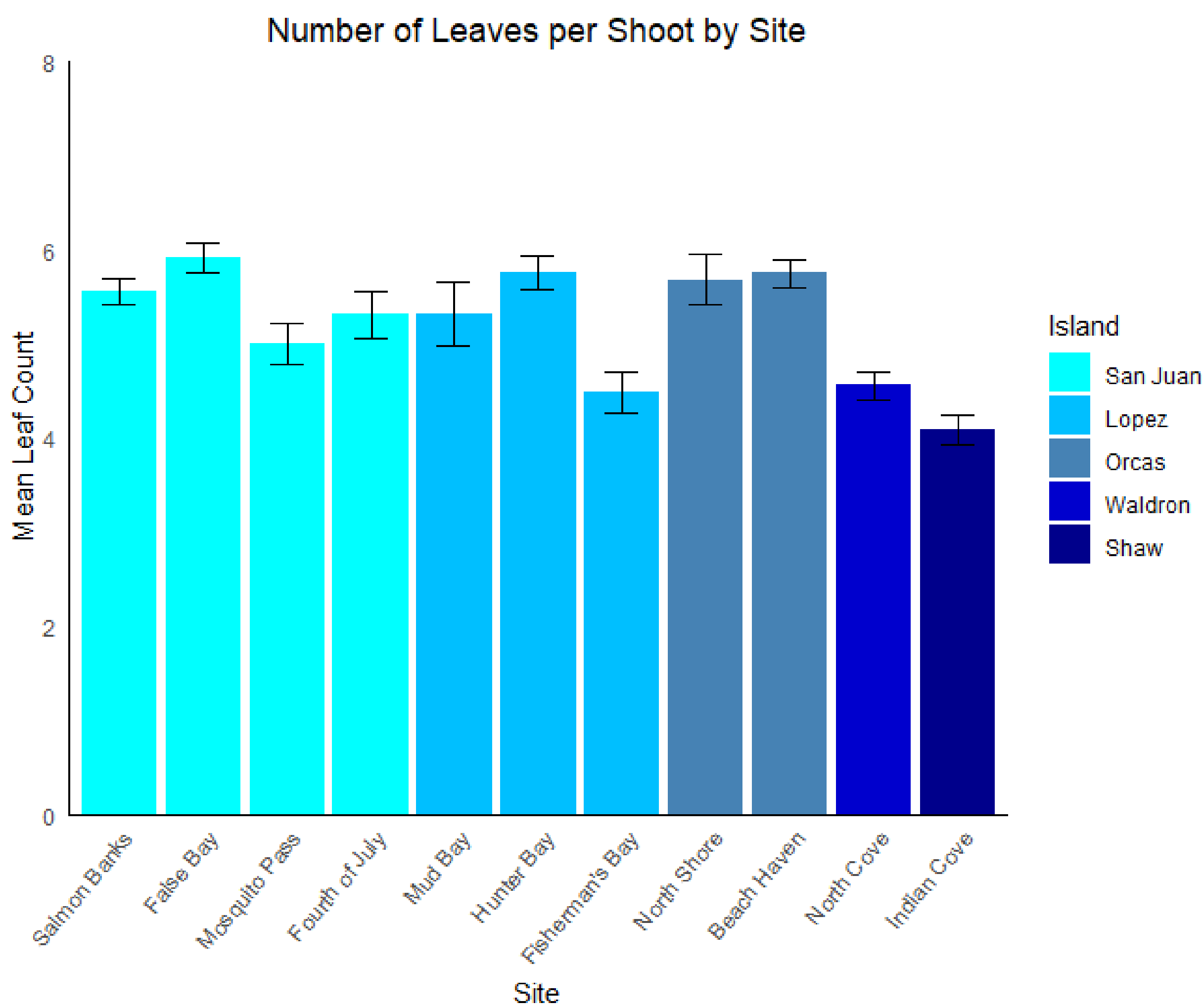

Figure 8. Mean count of leaves per shoot by site. Error bars represent standard error.

Second youngest leaf size

From the length and width of the second youngest leaf, leaf area was calculated. The area of the second youngest leaf significantly differed between sites (Figure 9; $p < 0.001$). Post hoc analysis found the most significance between Salmon Banks and Hunter Bay ($p < 0.001$) and Salmon Banks and False Bay ($p < 0.001$). We found the minimum mean area of the second youngest leaf at Salmon Banks (4812.24 mm^2). We found the maximum mean area of the second youngest leaf at Hunter Bay (10345.24 mm^2).

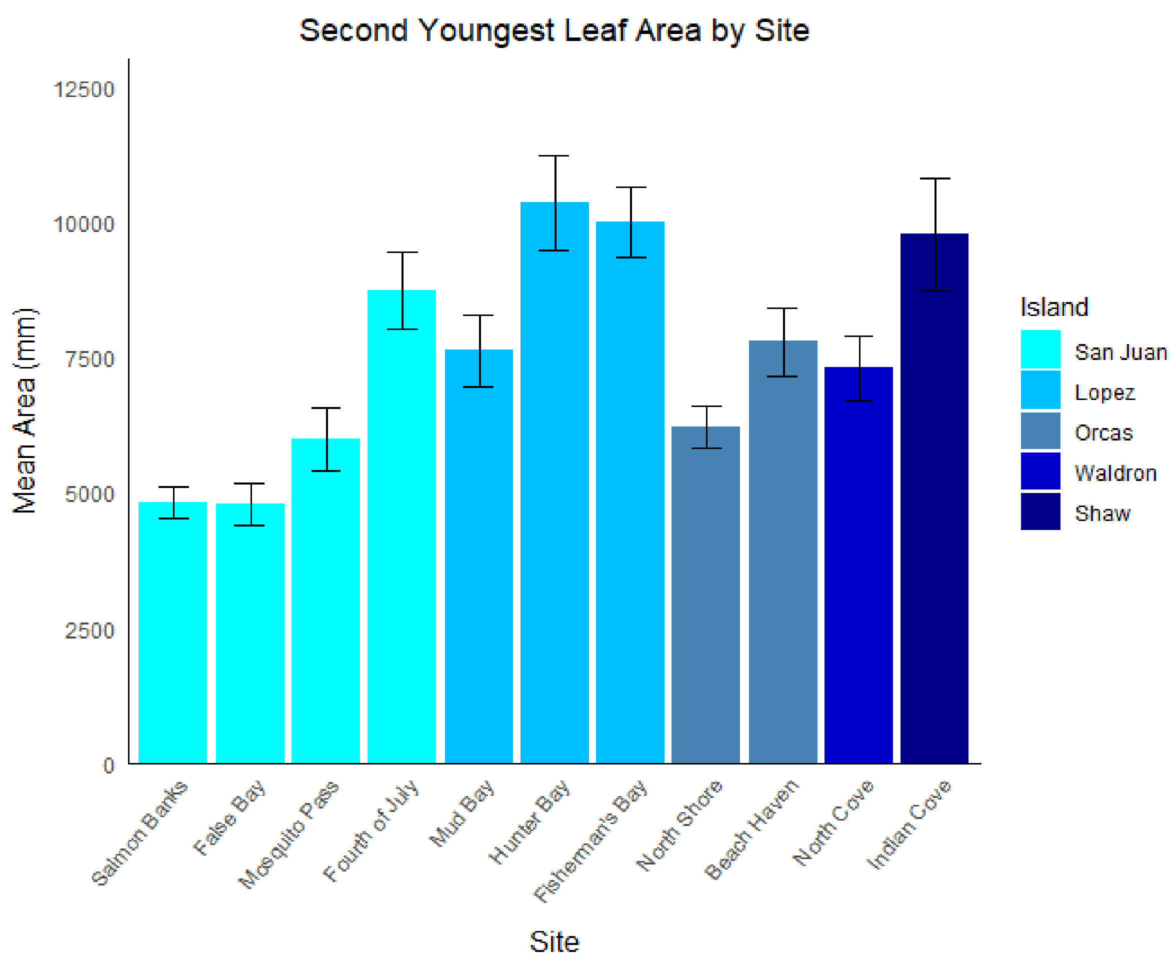

Figure 9. Mean of the total second youngest leaf area by site. Error bars represent standard error.

Disease metrics

Prevalence

Using a chi-square test, the prevalence of infected shoots showed significant differences between sites ($p < 0.001$). We report a prevalence of 0% for Beach Haven and False Bay, and a maximum prevalence of 74% for Fisherman's Bay (Figure 10).

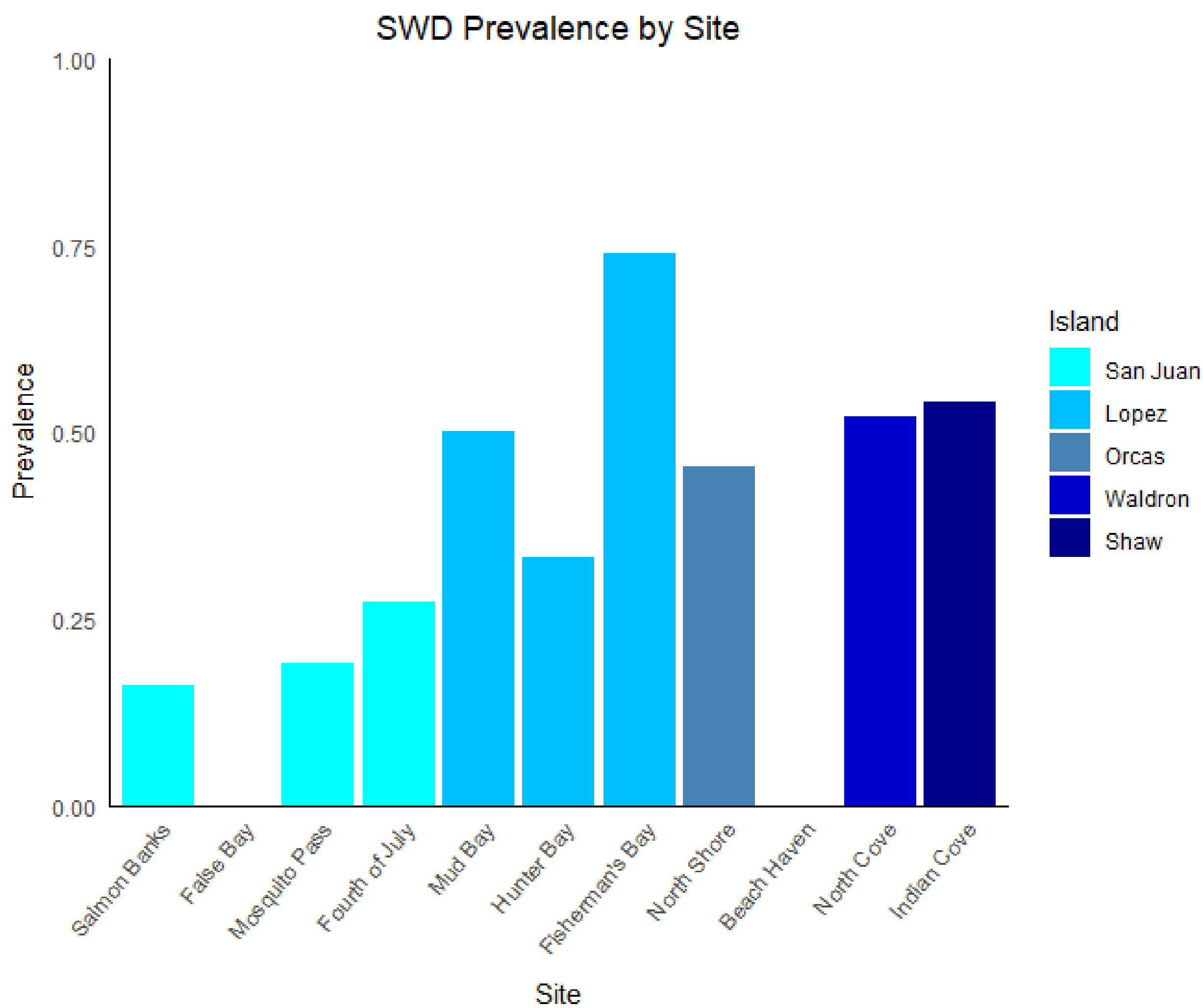

Figure 10. Prevalence (% of shoots with lesions on the second youngest leaf) by site.

Severity

Severity did not significantly differ between sites (Figure 11; $p > 0.05$).

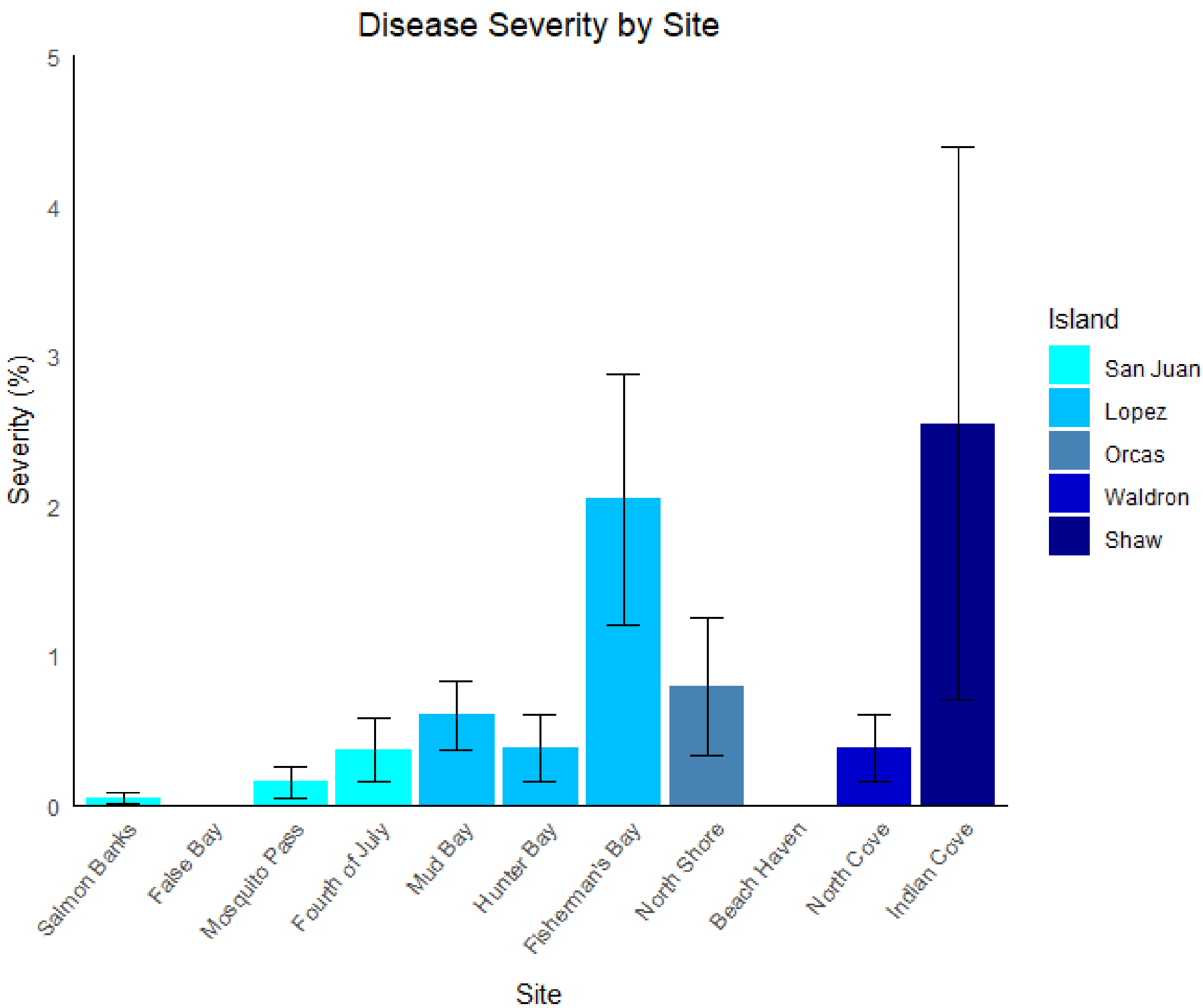

Figure 11. Severity (% of a leaf covered in infected tissue) by site. Error bars represent standard error.

Discussion

While the impacts of SWD on eelgrass is well researched, little is known about the complex environmental factors that may potentially play a role in modifying plant morphology and disease susceptibility. Increased *L. zosterae* severity has been correlated with reductions in leaf growth rates (Graham et al. 2021). Our initial attempt to connect overall plant body size to severity is not supported by our findings. Plants of different canopy height, normalized sheath length, leaf count, and second youngest leaf area did not significantly differ in severity on a site-wide basis. This is inconsistent with Groner et al. (2014) which found that larger leaf size was correlated with increased prevalence and severity.

The most surprising finding was how significantly the prevalence of disease differed across sites. We reported no prevalence of disease at two sites, Beach Haven and False Bay, which is inconsistent for *L. zosterae* in these systems. This also appears to be equally unaffected by differing plant sizes. The environmental conditions at these sites may contribute more to dictating morphology than disease. For example, plants in deeper sites that experience more current may experience an environmental pressure on leaf length. With longer, broader leaves, drag potential increases, which threatens the placement of the plants in sediment. This may be why we see our shortest plants at our deepest site. There is a potential genetic component to this question of plant morphology as well. Wyllie-Echeverria et al. (2010) found differing levels of genetic diversity within and between sites around the San Juan Islands. Future work on the microbiome found around eelgrass may also provide answers to why sites differed in prevalence.

Looking further at the differing environmental conditions experienced at these sites, there are many factors that may influence resilience to *L. zosterae* in eelgrass meadows. Resilience will be defined here as a measure of biotic and abiotic factors that support or hinder the health of eelgrass meadows. Energetic stress may lead to disease susceptibility, which would decrease resilience. Moore et al. (2012) found that increased summer temperatures and increased turbidity have resulted in lower light availability for *Z. marina* meadows, leading to energetic stress. Mud Bay and Indian Cove exhibited high levels of turbidity, while Salmon Banks and Fourth of July were mostly clear of sediments. Both of these factors are compounded, however, by depth and the rate of water flow in these regions. Future trends of sea surface temperatures and the mechanisms that influence turbidity should be taken into account for establishing meadow resilience. The intertidal meadows that most directly experience heat stress have already greatly reduced in range (Aoki et al. 2023).

This large range study on subtidal *Z. marina* meadows around the San Juan Islands presents new data for locations currently changing. The status of eelgrass meadows today may not accurately reflect their futures. It is necessary to understand the complex mechanisms underlying these ecosystems to make effective conservation decisions that will allow for long term success. It is better to make informed decisions now than learn from mistakes later; previous restoration efforts of seagrass communities around the world have proved expensive and unreliable for returning eelgrass meadows to their prior healthy states (Kenworthy et al., 2007). This stresses the contemporary importance of producing informed site-based protections for eelgrass populations that will be more resilient to impending environmental and developmental change.

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