Feeding preference between two species of marine isopods (*Pentidotea*) on healthy and wasting eelgrass *(Zostera marina)*

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Abstract

Eelgrass meadows are critical habitat for biodiverse marine communities. San Juan Island eelgrass *Zostera marina* meadows are losing surface area. Eelgrass wasting disease is locally prevalent and correlated with the decline. Herbivores, including *Pentidotea* isopods, eat *Z. marina* and spread wasting disease. Recent studies on isopod feeding preference have found different preferences for wasting and healthy eelgrass between species.

This study addresses a data gap by testing surface area consumed by *Pentidotea wosnesenskii* and *Pentidotea montereyensis* isopods when offered both healthy and wasting *Z. marina*.

Both species of isopod consumed a larger surface area of healthy eelgrass. The difference between species was not statistically significant.

Feeding on healthy eelgrass may exacerbate eelgrass wasting disease. Another local species, *Pentidotea resecata*, prefers consuming wasting tissue. These differences have implications for herbivore-eelgrass-pathogen interactions and the different dietary needs of *Pentidotea* isopods.

Introduction

Eelgrass Ecosystems and Local Status

Eelgrass (*Zostera marina*) is an ecologically important species in the Pacific Northwest, forming coastal meadows in soft substrate, lower intertidal and shallow subtidal regions. These meadows anchor sediment and provide habitat and food for biodiverse communities (Fonseca et al. 1982). Commercially important and conservation priority species supported by eelgrass include Pacific herring (*Clupea pallasii*), salmonids (*Oncorhynchus spp.*), walleye pollock (*Gadus chalcogrammus*), tiger rockfish (*Sebastes nigrocinctus*), and Dungeness crab (Chalifour et al. 2019, Fernandez et al. 1993). Eelgrass also supports herbivores, which play important roles in meadows. Some herbivores include amphipods, isopods, gastropods, and waterfowl. (Valentine and Heck 2021).

Eelgrass meadows are rapidly declining globally. While the Salish Sea has seen fairly constant eelgrass coverage from 2009 to 2020, eelgrass area has declined in the San Juan Islands at four times more sites than it increased from 2000 to 2020 (Christiaen et al. 2022). Some sites in the Southern Gulf Islands of British Columbia have also documented declines, with 45% decline in coverage and 66% decline in meadow complexity (Nahirnick et al. 2020). A number of stressors are likely involved, including increased water temperature, decreased water clarity, human disturbance, and disease (Magel et al. 2022, Short and Wyllie-Echeverria 2009, Groner et al. 2016).

Eelgrass Wasting Disease

Eelgrass wasting disease is caused by the protist *Labyrinthula zosterae* and is increasingly prevalent in the Salish Sea. Increases in wasting disease are correlated with declines in shoot density (Groner et al. 2021). Eelgrass that is stressed by other factors is susceptible to wasting, which can cause high mortality and contribute to gradual decline in distribution and density (Groner et al. 2016).

Disease can change trophic interactions between plants and herbivores. Some herbivores prefer to feed on diseased tissue, which contains different nutrient concentrations than uninfected plants (Eberl et al. 2020). In wasting eelgrass, *Labyrinthula* produces important fatty acids in higher concentrations than most Pacific Northwest macrophytes (Yoshioka et al. 2019). Wasting disease could provide an important dietary resource for eelgrass herbivores.

Pentidotea Isopods

Some notable eelgrass herbivores in the northeast Pacific are isopods in the genus *Pentidotea* (formerly *Idotea*, Boyko et al. 2024). Eelgrass isopods (*P. resecata*) can be extremely abundant on *Z. marina*, especially during the summer (Lee and Gilchrist 1972). Rockweed isopods (*P. wosnesenskii*) can be found on a variety of substrates throughout the intertidal and subtidal, including *Fucus*, *Ulva*, and *Z. marina* (Buskey 1976, Carr et al. 2010). Monterey isopods (*P.* montereyensis) are commonly found on rocky intertidal surfgrass *Phyllospadix scouleri* and red algae spp. (Lee 1966). These three species of *Pentidotea* isopods mostly eat the substrate they are clinging to and are sometimes the dominant herbivores of that substrate (Lee 1966, Best and Stachowicz 2012).

Herbivores and Disease Dynamics

There have been relatively few studies on how wasting disease affects herbivore feeding preference on eelgrass. *P. wosnesenskii* have been found to preferentially consume healthy eelgrass. Amphipods *Ampithoe lactertosa*, however, preferentially consume wasting eelgrass (Graham et al. 2023). Feeding preference was found to differ within isopods as *P. resecata* prefer wasting eelgrass (Murray et al. 2024). Depending on feeding preference, isopods could play a role in controlling disease by eating diseased tissue, or intensifying outbreaks by spreading the pathogen between blades and consuming healthy tissue (Murray et al. 2024). These findings imply that feeding preference cannot be generalized across related species and further research is necessary to understand the effects wasting disease will have on isopod-eelgrass interactions.

In this study, we examined isopod feeding preference by comparing surface area of healthy vs. diseased *Z. marina* consumed by *P. wosnesenskii*. We also compared the surface area consumed by *P. montereyensis*. We then compared the difference in surface area consumed between species. These experiments will identify feeding preferences at the species level and add context to the conversation on differences within *Pentidotea* and how the genus may affect eelgrass meadows.

Methods

Isopod and Eelgrass Specimen Collection

Rockweed isopods *Pentidotea wosnesenskii* and Monterey isopods *Pentidotea montereyensis* were collected from a number of locations around San Juan Island from late April to May 7th (Figure 1). Collection areas included intertidal substrates *Hedophyllum sessile* at Eagle Cove, *Phyllospadix scouleri* at Cattle Point, and Dr. Olivia Graham's tank of isopods at Friday Harbor Laboratories. A single eelgrass isopod *Pentidotea resecata* was collected from *Zostera marina* at Argyle Lagoon, but since only one specimen was located which was subsequently eaten by *P. wosnesenskii*, *P. resecata* was not able to be included in preference trials.

Eelgrass *Zostera marina* was collected from loose specimens at Argyle Lagoon and washed up against the dock at Friday Harbor Laboratories.

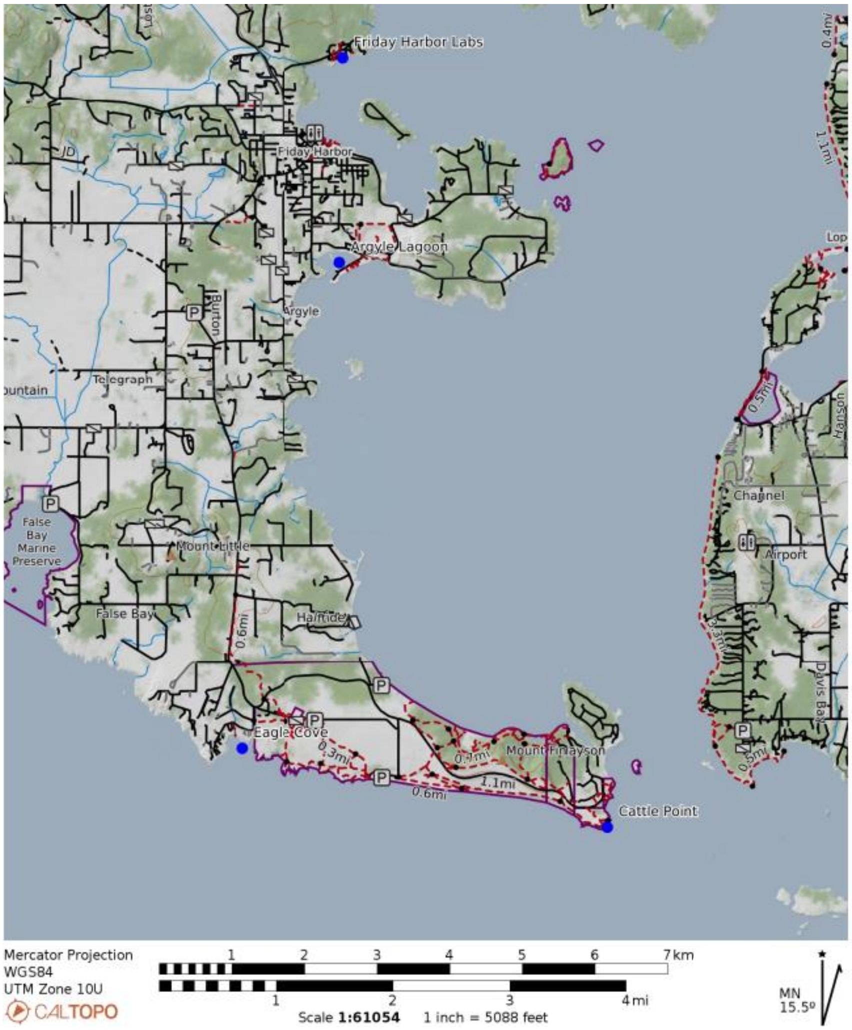

Figure 1 – Map of Sampling Locations including Friday Harbor Laboratories, Argyle Lagoon, Eagle Cove, and Cattle Point.

Between trials, eelgrass and isopods were stored together in a flow-through sea table in Lab 3 at Friday Harbor Laboratories. Sixty 3 cm strips each were cut from healthy, dark green *Z. marina* (Lighter, crunchier parts of the blade were too pale to show up on the scanner) and wasting *Z. marina*, evidenced by black lesions.

Feeding Preference Trials

Three feeding trials were performed May 14-16, 2024. One trial had 20 *P. wosnesenskii*, one 20 *P. montereyensis*, and one 10 of each, for a total of 30 replicates of each species. Since isopods were returned to the same holding setup after each trial, it is likely that some individuals were tested twice.

Isopods were placed in individual plastic containers with ~300 mL filtered seawater in the Fernald cold room at 10°C to match outside water temperature. They were given a starvation treatment from approximately 7:30 to 13:00 to clear their gut and encourage feeding during the experiment. Three-centimeter strips of wasting and healthy eelgrass were provided from approximately 13:00 to 19:00. The strips were stapled together to prevent differences in accessibility due to different buoyancies (See Figures 2 and 3 for experimental setup). The containers were covered with weighed-down paper towels to avoid isopods escaping.

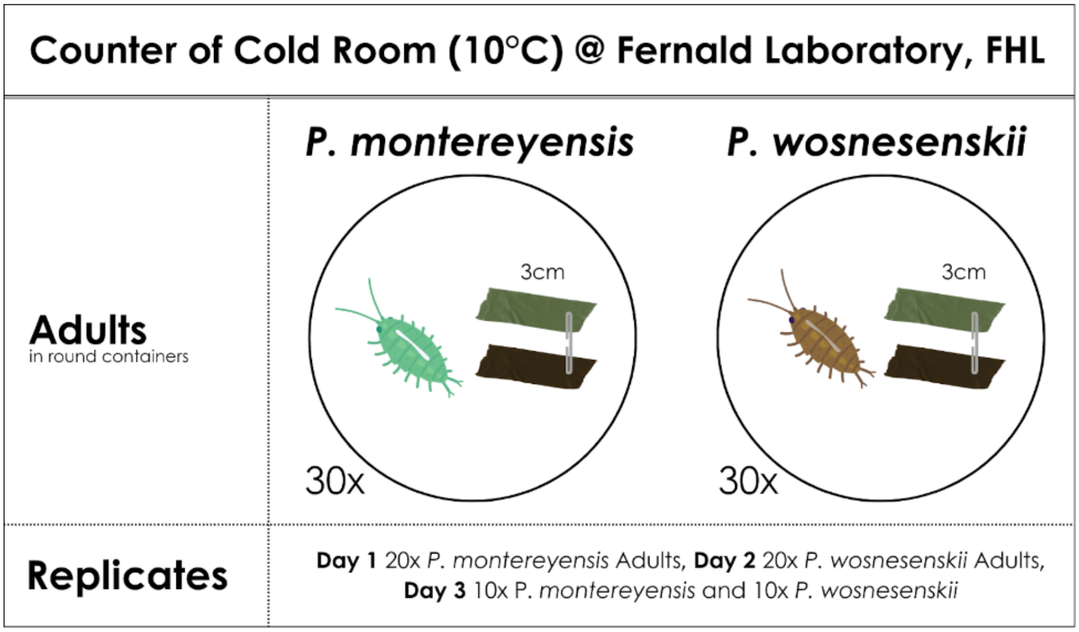

Figure 2 – Feeding preference trials experimental setup concept. Image credit: Samantha-Lynn Martinez.

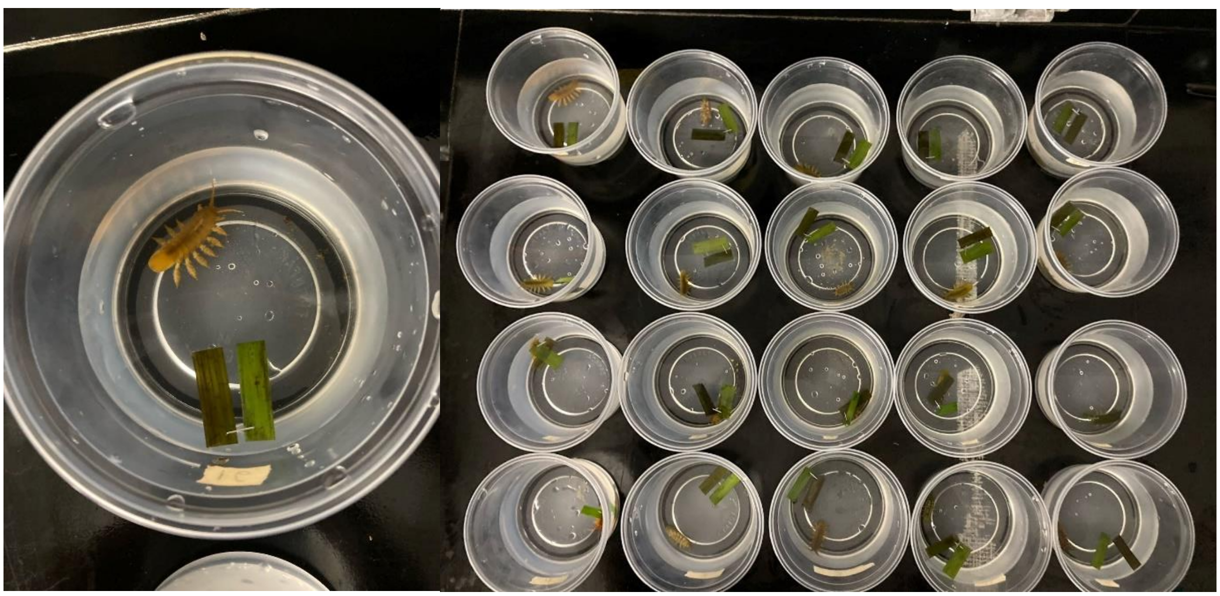

Figure 3 – *P. wosnesenskii* experimental setup immediately after addition of *Z. marina*.

The eelgrass strips were arranged between transparency sheets and scanned with a Canon LiDE scanner before and after feeding trials. Surface areas were measured using ImageJ. The scale in ImageJ was set to 236.2 pixels/10mm. Images were converted to binary and surface areas of each strip were measured with the wand tool. Surface areas were recorded in an Excel spreadsheet for calculations described below.

Data Analysis

Area of eelgrass after feeding trials was subtracted from initial area to calculate amount eaten. Amount eaten was compared between healthy and wasted eelgrass with a two-tail t-Test. We set a p-value of 0.05 as the threshold for significance. Change in surface area of wasted eelgrass was subtracted from change in surface area of healthy eelgrass to calculate preference. Preference was compared between *P. wosnesenskii* and *P. montereyensis* with a two-tail t-Test.

Specimen Management

Zostera marina was returned to the water off the Friday Harbor Laboratories dock after the completion of the experiment. Isopods, including those collected in the field, were returned to Dr. Olivia Graham.

Results

Overall, both *P. wosnesenskii* and *P. montereyensis* showed a preference for healthy *Zostera marina*, consuming a greater amount of healthy than wasting tissue. Not every individual was recorded consuming *Z. marina* (20 and 15 individuals ate less than 10mm^2, respectively), but isopods that ate almost exclusively consumed healthy eelgrass (Figure 4).

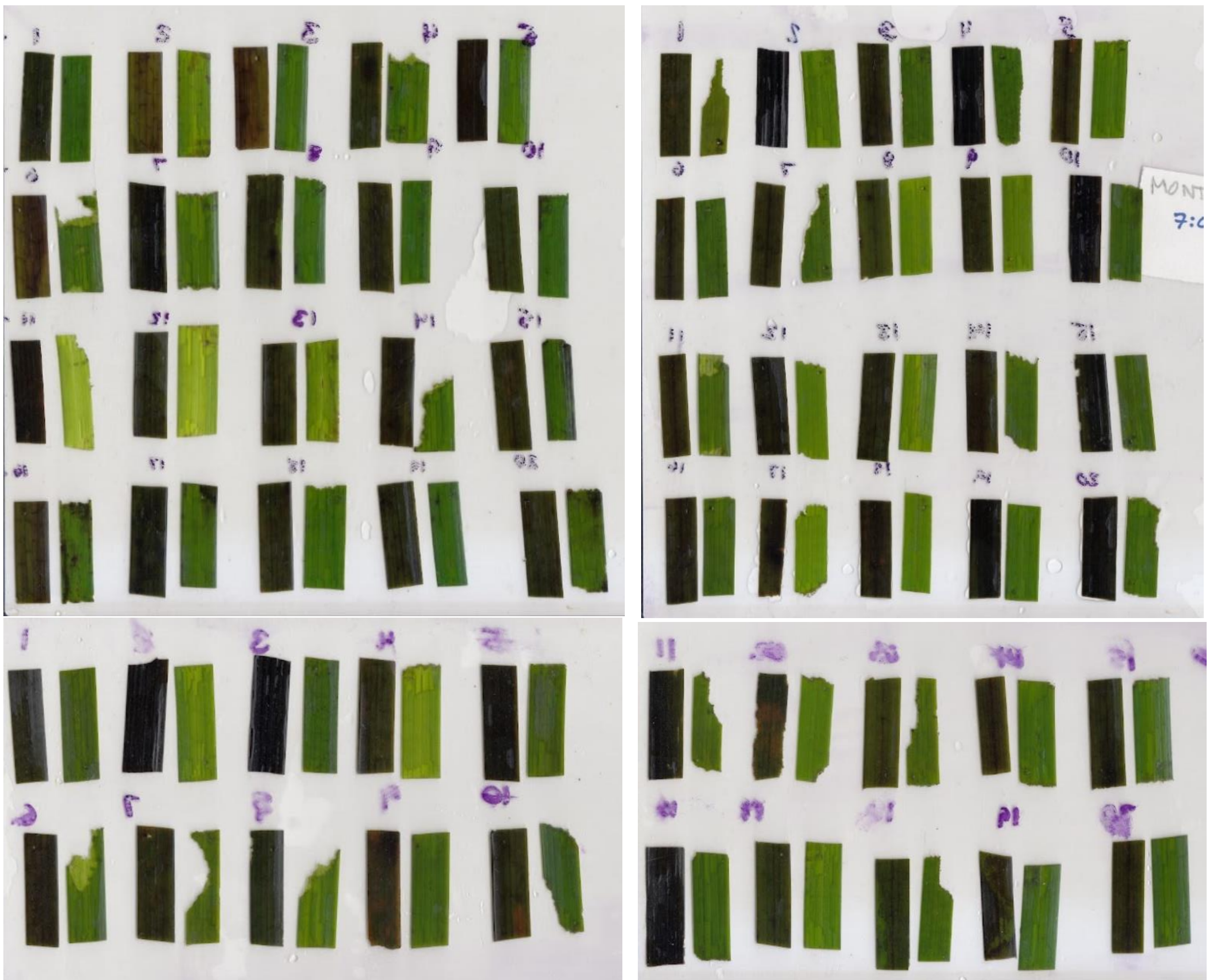

Figure 4 – Post-trial scans of wasted and healthy eelgrass showing bite marks on healthy eelgrass. Left - 30 pairs from *P. wosnesenskii* trials. Right - 30 pairs from *P. montereyensis* trials. Wasted eelgrass blades are on the left and healthy blades are on the right in each pair.

P. wosnesenskii ate 20 $\pm$ 40 mm^2 more healthy blade area relative to wasted blade area (Figure 5). There was a significant difference when analyzing this comparison using the

Student's t-test (p=0.01). *P. montereyensis* ate 19 ± 20 mm^2 more healthy blade area than wasting area (Figure 5). There was also a significant difference when analyzed by a t-test (p=0.0001). The difference in area eaten between heathy and wasting eelgrass (hereafter referred to as "preference index value") was not significantly different across species (p=0.9, Figure 6).

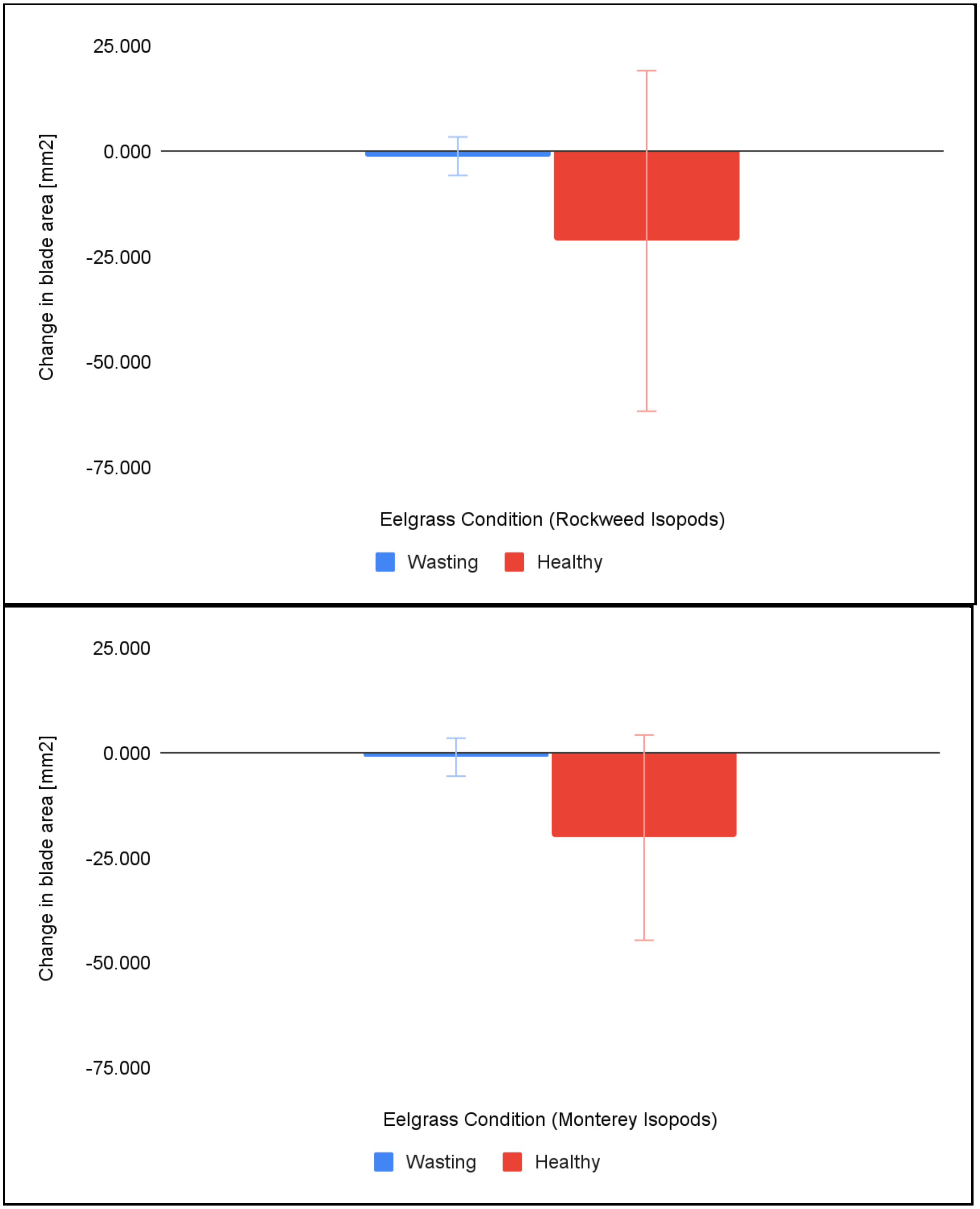

Figure 5 – Area of wasted and healthy eelgrass consumed, by *P. wosnesenskii* (top) and *P. montereyensis* (bottom). Areas of healthy and wasted eelgrass consumed were significantly

different between *P. wosnesenskii* (p=0.01) and *P. montereyensis* (p=0.0001) when analyzed using the Student's t-test. Error bars indicate standard deviation.

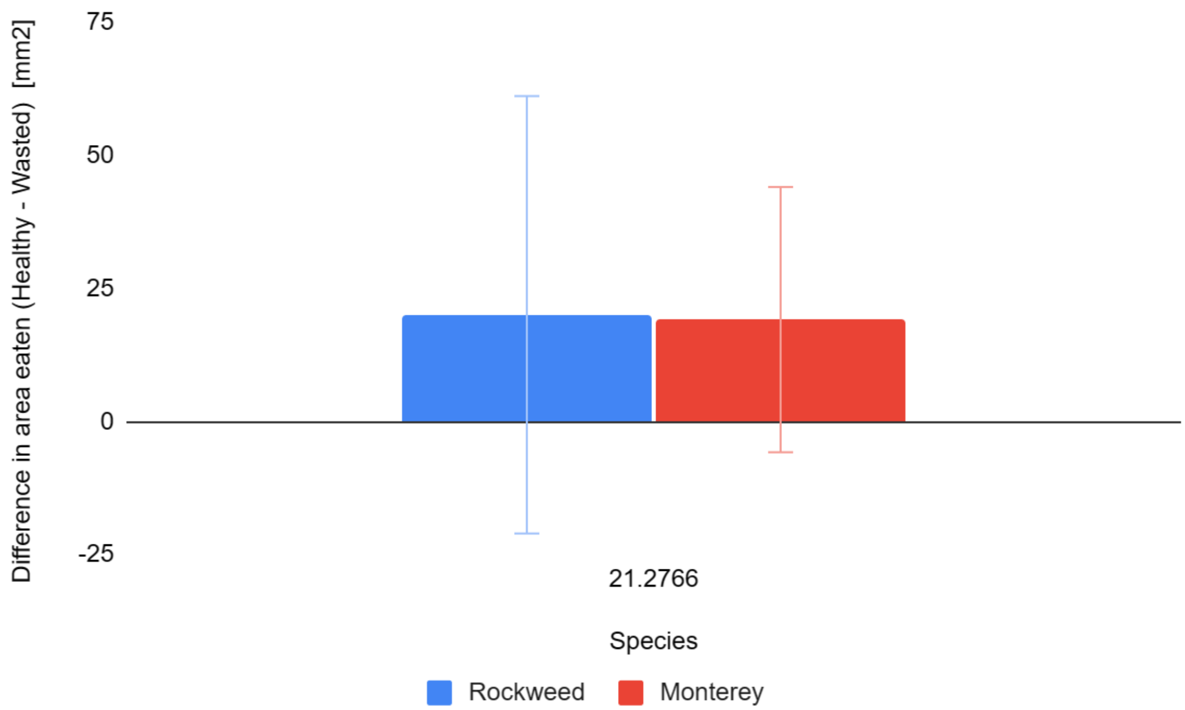

Figure 6 – Difference in preference index values between *P. wosnesenskii* and *P. montereyensis*. Both species preferred healthy eelgrass to wasting eelgrass. The preference index values are not significantly different with a two-tailed, equal variance t-Test (p=0.9). Error bars indicate standard deviation.

Discussion

Isopod Feeding Preference

Our results agree with previous work that *P. wosnesenskii* prefer to consume healthy eelgrass (Graham et al. 2023). *P. wosnesenskii* have been found to disregard color when picking substrate (Nelson 2021), and individuals in our study preferred green, healthy eelgrass despite being brown in color themselves. If the color of the eelgrass is not involved, preferences could be influenced by chemicals made due to *Labyrinthula* infection. Wasting softens eelgrass and produces fatty acids, which would normally encourage herbivory (Murray et al. 2024, Yoshioka et al. 2019. Indeed, softer tissue has been proposed as a reason *P. resecata* prefer wasting eelgrass (Murray et al. 2024). Another chemical change *Labyrinthula* causes is increased production of phenols (Vergeer et al. 1995). Decreased levels of polyphenols in eelgrass has been associated with stronger feeding preference in *P. resecata* (Murray et al. 2024). Future research could examine if phenol levels are responsible for differences in preference among isopod species.

Preference Influencing Wasting Disease

Depending on feeding preference, isopods could have different effects on eelgrass-disease dynamics. *P. resecata* preferentially consume wasting eelgrass. While herbivore bites have been found to spread wasting disease, *P. resecata* might have a net positive impact on eelgrass by targeting diseased tissue (Murray et al. 2024). This could maintain widespread, low severity wasting (Murray et al. 2024). Our findings demonstrate that this relationship cannot be generalized across *Pentidotea* species. Isopods that avoid consuming wasted tissue likely cause additional stress to eelgrass meadows by targeting healthy tissue and spreading *Labyrinthula* through their bites.

We did not find any species of isopods aside from *P. resecata* on the eelgrass at Argyle Lagoon, so it is not clear which herbivores may be having the strongest effect on San Juan eelgrass. Eelgrass decline is more severe in the San Juan Islands than other areas in the Puget Sound. It is unknown whether different herbivore communities and preferential feeding could be influencing disease severity or local decline.

Isopod Distribution

An original goal of this study was to compare feeding preferences of *P. wosnesenskii* to *P. resecata* under the same experimental conditions. Due to collection limitations, we were not able to collect enough *P. resecata* to run preference trials. This could be because *P. resecata* are less common at Argyle Lagoon specifically, or in the San Juans more broadly. *P. resecata* is described as being very abundant in the summer, so there may also be a seasonal aspect at play (Lee and Gilchrist 1972).

This study examined the feeding preference of *P. montereyensis*, although it is not clear that this species eats *Z. marina* in the wild. *P. montereyensis* are often found on *Phyllospadix*, so their preference may be more important for surfgrass communities than for eelgrass.

Life Stage and Feeding Preference

Organisms often have different dietary requirements at different developmental stages. Juveniles of *Idotea balthica*, another isopod found on seagrass, have been found to have different diets than adults (Sturaro et al. 2010). We originally wanted to compare the feeding preferences of juvenile isopods, but they didn't eat enough eelgrass to be measured in a pilot trial. After running trials with adults where many individuals ate neither strip, it is likely that an experiment with more replicates would be able to measure preference.

In summary, our findings that both species preferentially consume healthy eelgrass agree with previous research on *Pentidotea wosnesenskii*, add new information on the feeding preferences of *Pentidotea montereyensis*, and open up new questions on how isopod distribution and species affects eelgrass abundance in the Pacific Northwest.

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