

# Diet specialization and grazing rate in eelgrass-associated mesograzers *Idotea resicata* and *Lottia parallela*.

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## Abstract

Grazers (herbivores) play a critical functional role in ecosystems by mediating bottom-up transfer as well as top-down control of primary producer biomass. In marine ecosystems where coexisting primary producers interact (ie. via epiphytic growth), grazers may have density-mediated indirect effects on one primary producer by removal of the other. This phenomenon applies to temperate seagrass (*Zostera marina*) systems which declining in abundance globally, partially as a result epiphytic microalgal overgrowth due to coastal eutrophication. Invertebrate mesograzers are known to mitigate decreases in *Zostera marina* abundance by relieving it of competitive stress with epiphytes, notably diatoms. Here, I performed two laboratory experiments on eelgrass-associated grazers *Idotea resicata* and *Lottia parallela* to (1) ground-truth the literature on diet preference in both species; (2) compare relative grazing contribution on the temperate seagrass *Zostera marina* (also known as eelgrass), and (3) determine whether coexistence of the two species affects relative grazing impact. I performed a choice experiment on *Idotea* and *Lottia* individuals using agar squares containing one epiphytic microalgae or *Zostera*. I also performed a feeding rate experiment on both grazers in monoculture and in coexistence using fresh *Zostera*, and quantified grazing rate per capita and per unit biomass of grazer. *Idotea* preferred eelgrass as a food source when given a choice, but was observed eating epiphytes in no-choice conditions. *Lottia* only fed on eelgrass. *Idotea* had a higher grazing rate on eelgrass per capita and per unit biomass. Total relative grazing rate per capita and per unit biomass differed when *Idotea* and *Lottia* were in coexistence; grazing rate was higher per capita, but lower per unit biomass, which adjusts for the fact that *Lottia* had a lower body mass. Overall, the results highlight the need to consider biomass contribution when using abundance to estimate grazing impact of whole populations.

## Introduction

Herbivores in all ecosystem types fill the critical role of transferring biomass generated by primary production to higher trophic levels. In doing so, they also impact the available

standing crop of primary produce and potentially overall ecosystem functioning; if grazing has a net negative impact on the ability of producers to sustain higher trophic levels, species diversity and abundance within these trophic levels may be compromised. To understand which grazers are beneficial and which are potentially harmful to the overall functioning of ecosystems, identifying species-specific grazing impact in vulnerable systems is necessary. Seagrass ecosystems are one case where understanding grazing impacts is particularly pertinent.

Seagrass meadows are recognized as a globally important ecosystem type for providing a suite of ecosystem services including carbon sequestration, sediment stabilization, and habitat provision for ecologically and economically important species<sup>1</sup>. They are experiencing a rapid global decline in abundance; in several cases this has occurred in association with overgrowth of epiphytic macro- and microalgae, which outcompete seagrass for nutrients and light availability<sup>2</sup>. Mesograzers (herbivorous invertebrates) are a group of particular interest in this system because numerous experiments have consistently demonstrated top-down control of seagrass biomass by grazers on various spatial scales, either by direct grazing on seagrass (negative control) or by grazing on epiphytic microalgae, thus relieving the seagrass of competition stress for light and nutrients<sup>3,4</sup>. Some studies have proposed a Biodiversity-Ecosystem Functioning relationship, wherein higher grazer diversity correlates with higher seagrass productivity as well as resistance to meadow disturbance<sup>5,6</sup>. However, it has been noted that this BEF relationship is variable across sites due to differences in the functional composition of grazer assemblages, and that trophic and functional complexity should be accounted for when considering the functioning of ecosystems<sup>7</sup>.

Eelgrass (*Zostera marina*) meadows are distributed up and down the British Columbia



**Figure 1.** Aerial photograph of the *Zostera marina* meadow field site at Roberts Bank.

coast, from Haida Gwaii and Prince Rupert down to the Southern tip of Vancouver Island and the Lower Mainland. The subtidal meadow at Roberts Bank in Tsawwassen, BC (Figure1) is adjacent to the high-traffic BC Ferries terminal and a residential area, and therefore is relatively

disturbed compared to meadows in protected areas on Haida Gwaii and Vancouver Island. The two most abundant grazers at the site are eelgrass isopod *Idotea ressecata* and limpet *Lottia parallela*; they are therefore suitable study organisms for determining grazing impact at the site.

Both *Idotea ressecata* and *Lottia parallela* (henceforth referred to as *Idotea* and *Lottia*) potentially have both direct and indirect impacts on eelgrass abundance depending on their relative grazing rates on epiphytic microalgae and the eelgrass itself. The literature show mixed conclusions on diet preference and grazing rates. In an experimental mesocosm, *Idotea baltica* was shown positively increasing eelgrass biomass by removing epiphytes<sup>8</sup>. Other mesocosm experiments have shown *Idotea ressecata* grazing on the eelgrass itself<sup>9</sup>. Gastropod grazers like *Littorina littorea* and *Rissoa membranacea* have had strong positive impacts on eelgrass biomass via epiphytic grazing, however this is not the case for eelgrass-associated limpets<sup>8</sup>. The eelgrass limpet, *Lottia alveus* is a species on which little literature is available. The East coast subspecies, *Lottia alveus alveus* was declared extinct after not being found in the Western Atlantic for nearly a century<sup>10</sup>. In the paper documenting the extinction, a West coast subspecies, *Lottia alveus parallela* is briefly mentioned. At the time its distribution was poorly documented, with reports of specimens found in Haida Gwaii and Victoria, BC<sup>10</sup>. So far no one has written about *Lottia alveus parallela* in British Columbia, however a few informal articles and databases describe the species in Boundary Bay, BC, and refer to it as simply *Lottia parallela*<sup>11</sup>. The specimens collected for this experiment in Tsawwassen are most likely *Lottia parallela* based on this literature investigation as well as through the use of dichotomous keys and photo comparison, however no one has yet confirmed this. Assuming the limpets from Tsawwassen are *Lottia parallela*, I hypothesize that they will only eat the epithelial tissue of *Zostera marina* based on old literature on *Lottia alveus*<sup>10</sup>.

The overall aim of this study is to determine whether *Idotea* and *Lottia* have a net negative or positive grazing impact on *Zostera marina* standing stock. To meet this aim, I asked two questions: (1) Do *Idotea* and *Lottia* show preference between epiphytic microalgae and *Zostera marina*? (2) Does *Idotea* or *Lottia* have a higher grazing impact (3) Does species-specific relative grazing impact change when the two grazers are co-existing? I performed two separate laboratory experiments to answer these questions.

In response to question (1), I hypothesized that both species would graze on both food types based on previous experiments on *Idotea*, and the literature on local *Lottia* species. To

question (2), I hypothesized that *Idotea* would have a higher grazing impact due to its larger body size, as well as its significantly higher rate of travel; *Idotea* are able to crawl up and down eelgrass blades rapidly and are also very impressive swimmers (K. Stark, personal observation), which is conducive to easy transport between separate shoots. To hypothesis (3), I predicted a lower grazing impact on *Zostera* when grazers were in coexistence due to previous findings in laboratory mesocosms where higher species richness resulted in higher eelgrass standing crop. The findings from this study will hopefully ground-truth the literature, and contribute to our understanding of drivers of global seagrass habitat decline.

## Methods

### *Organism collection*

I collected *Idotea*, *Lottia*, and *Zostera* while snorkelling at a subtidal seagrass meadow at Fred Gingell Park, Tsawwassen, British Columbia. Collection occurred on March 20, 2017 in the evening during a 1.6 m low tide. The meadow contains both *Zostera marina*- the native species- and *Zostera japonica*, an introduced seagrass from Japan<sup>12</sup>. I cut bundles of *Zostera marina* shoots at the base above the substratum to avoid uprooting the plants, and placed them in large Ziploc bags and tupperwares with water. In doing so, I collected numerous *Lottia* and *Idotea* that were attached or crawling along blades. The experimental organisms were transported back to the laboratory at the University of British Columbia Vancouver Campus, and maintained in an aerated aquarium with eelgrass at approximately 13° C for five days until the experimental trial period.

### *Feeding Trial Preparation*

I made agar squares containing either epiphytic microalgae or *Zostera marina* for the choice experiment; this was the most effective method for measuring the amount of epiphytic microalgae consumed, as wet scrapings are difficult to weigh accurately and are liable to float around in the experimental vessel once removed from *Zostera* blades. Agar square preparation proceeded as follows: I scraped epiphytes from *Zostera* blades with a microscope slides, and filtered them with a 0.2µm glass fibre filter. The filtered epiphytes, along with fresh *Zostera* were placed in a desiccator oven for 48 hours. Upon removal, I scraped the epiphytes from the filter, and crushed the *Zostera* with a mortar and pestle until it became a fine powder. To make agar

cubes, I added 0.2 g of dried epiphyte/ *Zostera* to 0.25 g agar powder and 40 mL tap water (methods provided by Kathryn Anderson, personal communication). Once settled, I sliced the agar into approximately 1 cm<sup>2</sup> squares (thickness was approximately 0.25 cm). The dried food settled to the bottom of the cubes creating a 2-dimensional plane ideal for photo analysis of surface area before and after.

Because agar square consumption as a proxy for feeding behaviour has low ecological validity, I also ran grazing rate trials on both grazer species with fresh *Zostera marina* only. Unfortunately, this method was not feasible for epiphytic diatoms, so this experiment was solely for grazing rate comparison. To prepare fresh *Zostera* for these feeding trials, several blades were thoroughly pat-dried with a paper towel and weighed with a milligram scale. The same drying procedure was used when weighing the *Zostera* remaining after the experimental period.

### *Experimental design*

In the 24 hours leading up to the experimental period, all organisms were placed into the experimental vessels and starved to minimize confounding effects of variation in food grazed before the experiment. This was done for both the choice experiment and the grazing rate experiment.

The three treatments for the agar choice experiment were *Zostera* only (no choice), epiphytes only (no choice), and both *Zostera* and epiphytes (choice). I placed one agar square and one grazer individual in each beaker and filled to the top with salt water. For the choice replicates, I cut both squares in half to standardize the amount of food available. 500 mL beakers were used for *Idotea* and 250 mL beakers were used for *Lottia* replicates; this was to standardize the ease with which the individual could find the agar, since *Lottia* has a smaller body size and moves at a considerably slower rate. Salinity was not measured for the duration of the experiment, but assumed to have remained constant across trials, as all water came from the same source. Each beaker was aerated. I incorporated three replicates ( $n = 3$ ) for each of the three treatments for both species, to a total of  $N = 18$  for the choice experiment. The feeding trial ran for 48 hours before the agar squares were examined for proportion consumed. I took photos of each agar square before and after on a standard white background. To calculate proportion consumed, I used the imaging software ImageJ to divide the final surface area by the initial surface area of the agar square<sup>13</sup>.

For the fresh *Zostera* grazing rate experiment, I used 16x16x9 cm Tupperware with mesh windows as experimental vessels, since the beakers would have constrained the volume for grazers to move around with the extra floating *Zostera*. The Tupperware were housed in an aerated seawater table for the duration of the experiment at approximately 13° C. The treatments for this experiment were *Idotea* only (2 individuals per Tupperware), *Lottia* only (5 individuals per Tupperware), and both grazers in coexistence (2 *Idotea* and 5 *Lottia*). A pre-weighed amount of towel-dried *Zostera* was placed in each Tupperware along with the respective number of individuals. Three replicates of each treatment (n = 3) were performed to a total of N = 9. The feeding trial ran for 48 hours before the *Zostera* was towel-dried and weighed to calculate the mass consumed (1) per capita and (2) per g of mesograzer in the experimental vessel.

### *Statistical analysis*

Statistical analyses were performed in RStudio (Version 0.99.903). To determine preference between epiphytic microalgae and eelgrass in both species, I performed a two-way Analysis of Variance with “choice vs no choice” and “food type” as the two explanatory variables. An interactive effect between choice and food type would indicate that the respective amount of epiphyte or eelgrass grazed differs based on whether or not grazers are given a choice. Grazing rate comparison for the agar choice experiment are not presented because (1) *Lottia* did not eat epiphytes and (2) *Lottia* appeared to be at a significant disadvantage in accessing the agar, thus introducing confounding effects on grazing rate.

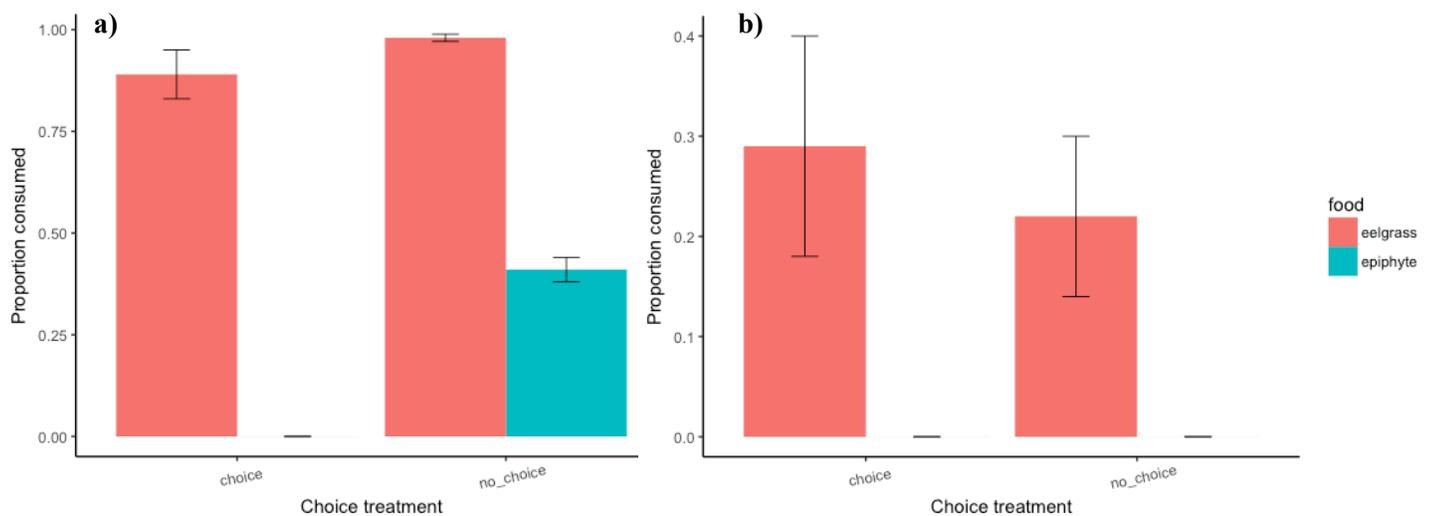
To compare fresh *Zostera* grazing rate between *Idotea* and *Lottia*, I performed 2 two-sample t-tests; one using mass grazed per capita, and one using mass grazed per unit mass (g) of grazer. I also performed a one-way ANOVA and post-hoc Tukey’s HSD test to compare grazing rates between *Idotea*, *Lottia*, and both together to see if coexistence between the two species affected grazing rate per capita and per g of grazer.

## **Results**

### *Epiphyte vs eelgrass diet choice experiment*

*Idotea* consumed almost 100% of the eelgrass-containing agar squares in all replicates for both the choice and no-choice treatments. They only consumed the epiphyte-containing agar squares in the no-choice treatments, and only consumed 41% of the square on average. The

results demonstrate that *Idotea* has a strong preference for eelgrass regardless of whether it is given a choice (as demonstrated by the “food type” term: ANOVA:  $F_{1,8} = 829.9$ ,  $p = 2.28e-9$ ). However when not given a choice, *Idotea* will eat epiphytes (as demonstrated by the interaction term:  $F_{1,8} = 97.4$ ,  $p = 9.37e-6$ ). The “choice” term was also significant ( $F_{1,8} = 44.7$ ,  $p = 0.0002$ ), however this only applied to the epiphyte variable; eelgrass grazing did not differ significantly between choice treatments (Tukey’s HSD,  $p > 0.05$ ).

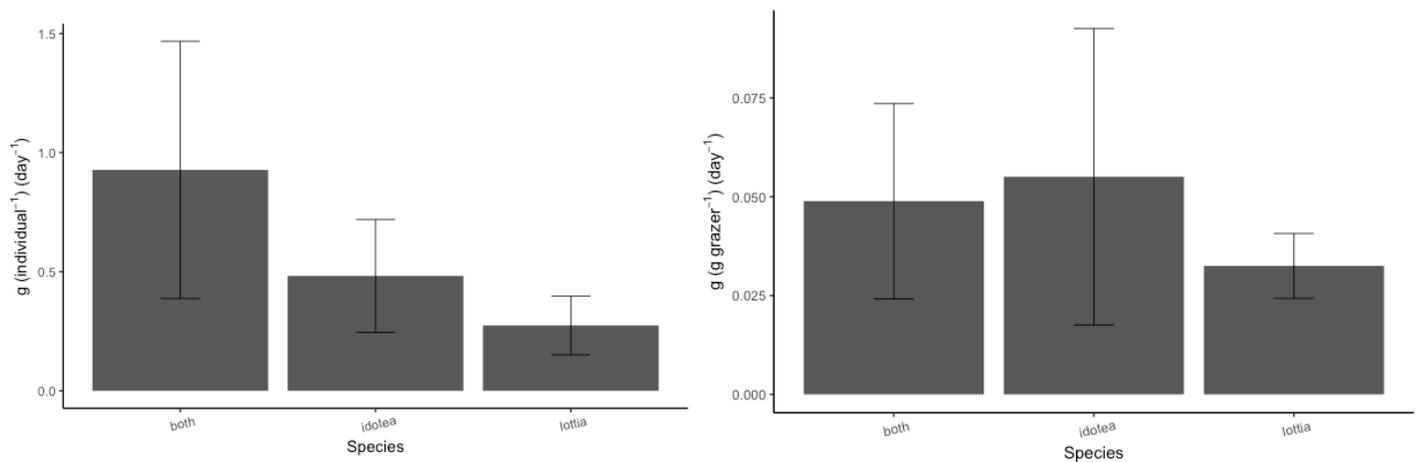


**Figure 2.** Bar plot depicting mean proportion of agar square consumed of each food type for each choice treatment for a) *Idotea resecata* (N = 9) and b) *Lottia* sp. (N = 9). Error bars depict standard error of the mean (n = 3). Note y-axes differ between the two bar plots.

*Lottia* did not consume epiphyte-containing agar squares at all (Figure 1b). They therefore showed strong preference for eelgrass over epiphytes, ( $F_{1,8} = 243.8$ ,  $p = 2.82e-7$ ). Whether or not they had a choice between the two food types was insignificant ( $p > 0.05$ ).

#### *Fresh eelgrass grazing rate experiment*

*Idotea* had a higher grazing rate than *Lottia* both per capita and per unit mass (Figure 2), however not significantly so ( $p > 0.05$ ). Interestingly, when calculated in terms of grams per unit biomass of grazer ( $\text{g g}^{-1} \text{day}^{-1}$ ), grazing rate was lower in the “both” treatment than the maximum individual grazing rate (*Idotea*).



**Figure 3.** Bar plots depicting mass of fresh *Zostera marina* consumed a) per individual and b) per unit biomass of grazer. Error bars depict standard errors of the mean (n = 3).

## Discussion

I performed two laboratory experiments on eelgrass-associated grazers *Idotea ressecata* and *Lottia parallela* to (1) determine diet preference, (2) compare relative grazing rate on *Zostera marina*, and (3) determine whether coexistence affects grazing rate. The overall aim was to determine whether each species has a net negative or positive impact on *Zostera* standing biomass.

In the agar square choice experiment, *Idotea* ate both eelgrass and epiphytes, however showed strong preference for eelgrass. Given this result, I am confident that *Idotea* does eat both in nature, however due to the ecological validity constraints of these experiments, I am not confident that *Idotea* always prefers eelgrass to epiphytes. The isopods had to consume the same amount of agar to reach the food source in both treatments, which standardized feeding effort. However, the agar-making process (ie. desiccating the food sources, boiling in water with agar powder) might have altered the nutritional properties of one or both food types, thus altering the palatability. Ecological validity also came into question with respect to the manipulability of the agar squares compared with natural food sources. The squares tended to sink to the bottom of the experimental beakers. However, when agitated by swimming *Idotea*, the squares drifted about, making them more challenging to grasp.

*Lottia* did not consume epiphyte-containing agar squares at all, which indicates that it likely does not eat epiphytic microalgae. This is synonymous with descriptions of *Lottia alveus*, as well as other eelgrass-associated limpets on the West Coast of North America such as *Tectura depicta* in Bodega Bay, which significantly reduces *Zostera marina* biomass by direct consumption of epithelial tissue<sup>14</sup>. This finding is a strong case for my organisms indeed being *Lottia alveus parallella*. Assuming my finding is true, a question that arises is whether *Lottia* actively avoids eelgrass shoots that are overgrown with epiphytes. If they do not, it is worth determining whether they simply slough off the epiphytes while crawling up the shoot, or instead they do in fact ingest epiphytes. During the field collection, I found most *Lottia* attached to clean eelgrass blades with no epiphytic growth, however this does not allow us to discern which of these three possibilities occurs.

*Idotea* had a higher grazing rate on fresh *Zostera marina* than did *Lottia* both per capita and per unit biomass. However, additional trials with more replicates are required to confirm the statistical significance of this result, given  $n = 3$  had relatively large standard errors. Using the same data to perform two different calculations of grazing rate, I found that relative grazing impact was lower than the maximum grazing rate (ie. in *Idotea*) when expressed as eelgrass consumed per unit weight (g) of grazer. This finding suggests that relative grazing impact depends both on the relative abundance and mass of each species. The explanation is fairly intuitive: *Lottia* individuals are significantly lighter than *Idotea* individuals. When relative grazing contribution is quantified in terms of the number of individuals, *Lottia*'s contribution is inflated. An interesting albeit challenging follow-up experiment might be to replicate the entire procedure, and measure weight gain in each *Idotea* and *Lottia* individual. Individual weight gain in grazers in the coexistence treatment may be compared to weight gain in individuals in the monocultures to determine whether one species' grazing rate is compromised more in the presence of the other. There are even further complexities to the suite of species-specific characteristics contributing to total ecosystem functioning; Best (2012) found that varied susceptibility to predation in eelgrass-associated grazers played a key role in determining which species had the largest net impact on eelgrass standing crop<sup>9</sup>.

This finding has implications for field studies. Given that relative biomass per individual affects our measurement of grazing impact, it may be worthwhile to incorporate biomass contributions (as opposed to abundance) to estimates of grazing impact in the field. Incorporating

these considerations may be particularly beneficial in Biodiversity-Ecosystem Functioning studies, which in some previous cases have treated species as billiard balls that simply contribute to richness.

### Acknowledgements

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