Comparing *Metacarcinus magister* settlement preferences among the seagrasses *Zostera marina* and *Zostera japonica*

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**Introduction**

*Metacarcinus magister*, the Dungeness crab, is a commercially important species on the Pacific Northwest coast as the most profitable fishery in Oregon (Oregon Department of Fish and Wildlife 2012) and an important ecological player as both predator and prey (Pauley et al. 1986). Understanding the spatial distribution of *M. magister* throughout different life history stages is beneficial both ecologically and economically.

*M. magister* larvae are transported offshore after hatching, until they molt into the megalope (most advanced) stage of larval development, which is most abundant in Oregon in April and May (Pauley et al. 1986). Once the larvae settle and metamorphose into juveniles, they are found in shallow estuaries, largely among eelgrass (*Zostera* spp*.*) or other seagrasses, which provide food, protection, and substrate (Pauley et al. 1986). Juvenile crabs between molts are called instars, and are visible on size frequency plots as peaks; first instars generally have a carapace width of between 5 and 8.5 mm (Pauley et al. 1986). Settlement intensity of juveniles is most affected by larval survival to metamorphosis, resulting in many periods of fluctuation (Pauley et al. 1986). Settlement success is due to a combination of predation, temperatures, currents, and food availability (Eggleston and Armstrong 1995), but post-settlement juveniles are also affected by these factors.

Juveniles require protection and easy access to prey, both of which can be provided by the Pacific Northwest native *Zostera marina*, common eelgrass (Harrison and Bigley 1982). Greater numbers of species and densities of individuals in estuarine habitats occur near seagrass than bare areas (Lewis and Stoner 1983). *Zostera japonica*, dwarf eelgrass,is an established invasive species of seagrass that first occurred in the Pacific Northwest in 1957 (Harrison and Bigley 1982). In addition to direct competition with native seagrasses, *Z. japonica* may also have significant indirect effects through habitat modification. In the native range of *Z. japonica,* the physical canopy structure has positive effects on its associated fauna (Lee et al. 2001). Indirect effects, such as habitat modification foruse by macroinvertebrates like *M. magister*, are not well studied in the invasive range (Posey 1988). There is little knowledge on the potentially negative effects of habitat-forming invasive species on the life history of recruiting native species (Gribben et al. 2008).

Hedvall et al. (1998) found that when given a choice of mussel, eelgrass, algae, or sand habitats, 24-40% of megalopae and juvenile crabs preferred each one of three structurally complex habitats as compared to 0-2% preferring a sand habitat. This preference may be because predation is highest in open habitat (Ramsay 2012). Greater *M. magister* prey abundances are found in more complex habitats (Hedvall et al. 1998), suggesting that seagrasses can be a source of both protection and prey. Among different types of seagrass habitats, crabs are more abundant in areas with greater shoot density (Attrill et al. 2000). *Z. japonica* has a higher shoot density than *Z. marina* (Ruesink et al. 2009), and thus may be a better habitat for juvenile crabs. Higher shoot density causes a higher level of aboveground structural complexity available for macroinvertebrate use (Webster 1998). Given what is known about crab settlement in the Pacific Northwest, we hypothesized that juvenile *M. magister* prefer settlement in seagrass habitats to more open habitats because of the greater structural complexity. We also hypothesized that juvenile *M. magister* prefer to settle in *Z. japonica* over *Z. marina* because *Z. japonica* has the most aboveground structural complexity.

**Materials and Methods**

We collected the data for this study on the north band of the Yaquina Bay estuary in Newport, OR (44.6181° N, 124.0302° W). Yaquina Bay is a drowned river estuary. We chose a study site with a large mud flat that has open mud, *Z. japonica*, and *Z. marina* habitats. *Z. japonica* is closer to the shore and at a higher tidal height than *Z. marina,* and the open mud habitat is between the two seagrasses.

We used mesh bags filled with oyster shells as a settlement substrate for juvenile *M. magister*. We obtained oyster shells from Oregon Oyster Farms, Inc., in Newport, OR. We used oyster shells because megalopae settle generally 8-10 times more on oyster shell substrates than mud (Eggleston and Armstrong 1995), and bags of oyster shells have successfully been used as mitigation habitat to augment crab resource and reduce losses caused by dredging (Dumbauld et al. 1993). Each bag of shells was about a foot long and approximately ovoid. Using small metal stakes, we secured three bags in each of the three habitats, for a total of nine bags. The bags were each about 30 feet apart. The bags sat for two weeks to allow for *M. magister* settlement. At the end of two weeks we assessed the bags for *M. magister* abundance. We determined the tidal heights of the different habitats by recording the time that the water height reached the line of bags in each habitat, and used NOAA’s online tides and currents data to calculate tidal heights.

We then determined abundance of *M. magister* within each oyster bag. To do this, we placed a crab corral around the bag, and put its contents and the substrate (down to 2 cm) into a sieve. We counted the number of crabs and measure the carapace width of each one. To determine the normal density of *M. magister* in these habitats, we sampled seven representative areas in each of the three habitats, for a total of 21 samples in addition to the nine shell bag samples. The areas were in relatively close proximity to the bags, and represented the area the bags were placed in. The chosen areas were not too close to the bags that the settled crab would be frightened away. We enclosed each area with a crab corral, and put its contents and the substrate (down to 2 cm) into a sieve. We counted the number of crabs present and recorded the carapace width of each. We bagged the algae and seagrass rooted in each area, and later washed off the mud and measured the wet weight to find aboveground macrophyte biomass.

Lewis and Stoner (1983) recommend that any sampling for macrofaunal abundance must take plant distribution into account, so we also examined the shoot density of the seagrass habitats. We haphazardly placed 0.25m2 quadrats three times near each shell bag in each habitat, counting the number of shoots within a 0.09m2 sub-quadrat. Densities were extrapolated to m2.

We used Excel to create a size frequency distribution of all *M. magister* we found, and then used Minitab for a one-way ANOVA analyzing mean size among the three habitats, grouping similar habitats by the Tukey Method. We used Minitab for a one-way ANOVA comparing abundance among the three habitats, again grouping similar habitats using the Tukey Method. We also used Minitab for a multiple linear regression analyzing the abundance of *M. magister* as determined by shell bag presence and *Hemigrapsus oregonensis* abundance. We performed a linear regression using Minitab on the variance of abundance by shoot density, and again by seagrass biomass. Finally, we used Minitab to create a linear regression of tidal height and *M. magister* abundance.

**Results**

We created a size frequency distribution of all *M. magister* individuals we found (Fig. 1) to look at the levels of instars found. Habitat was not a significant predictor of length (one-way ANOVA, F2, 77= 2.22, p=0.115), and the Tukey Method grouped all habitats together for similarity in average size and variation.

A one-way ANOVA showed that habitat was a significant predictor of abundance (data were natural log transformed for normality; F2,27=9.43, p=0.001; Fig. 2). A grouping using the Tukey Method showed mud to have significantly lower abundances of *M. magister* than the other two habitats.

*Hemigrapsus oregonensis,* the yellow shore crab, was present in some sampled areas. However, neither *H. oregonensis* abundance nor shell bag was a significant predictor of magister abundance (multiple linear regression, F3.26=1.30, p=0.296) and there was no interaction between bag presence and *H. oregonensis* abundance (p=0.232).

Shoot density was not a significant predictor of *M. magister* abundance (data were natural log transformed for normality; linear regression, p=0.942, R2=0.001; Fig. 3). However, the wet biomass of collected seagrass was a significant predictor of *M. magister* abundance, (data were natural log transformed for normality; linear regression, p=0.001, R2=0.446; Fig. 4), with *Z. marina* having the most biomass in each area.

Tidal height was a significant predictor of *M. magister* abundance (linear regression, p=0.015, R2=0.597), with higher tidal heights having lower abundances of crabs.

**Discussion**

The carapace width of first instars is generally between 5-8.5 mm (Pauley et al. 1986). Comparing this to the peaks in our size-frequency graph suggests the crabs we collected were mainly first and second instars. A simple one-way ANOVA showed habitat was not a significant predictor of size, but it appeared that more second instars were found in habitats closer to shore, suggesting juveniles might switch habitats as they grow. An interesting avenue for further research would be a proper analysis of size frequency distribution to compare the location and size of instar peaks among different habitats.

*M. magister* abundance was significantly higher in seagrass habitats over mud habitat, which supported our hypothesis that juveniles prefer structurally complex habitats. However, there was no difference in abundance between the two species of seagrass we studied. We expected a higher abundance of *M. magister* in *Z. japonica* due to a higher shoot density, but shoot density was not a significant predictor of abundance. On the other hand, biomass did significantly predict abundance, showing that the overall aboveground complexity of the habitat might be more important for juvenile *M. magister* than the number of shoots alone. The importance of overall complexity is supported by previous studies showing that differences in epifaunal abundance are related to macrophyte biomass (Heck and Wetstone 1977; Lewis and Stoner 1983). High aboveground biomass causes increased habitat heterogeneity, food availability, refuge from predators, and more living space due to greater plant surface area (Heck and Wetstone 1977), all of which are suitable for vulnerable juveniles. As biomass directly affects juvenile survival to adulthood, it should be considered as part of overall seagrass habitat conservation.

Our results showed that crab abundance decreased with increasing tidal height, which is consistent with a study performed by J. Ramsay (2012). This may be due to a higher immersion time at a lower tidal height, decreasing risk of desiccation and bringing more food to the crabs.

Some sample areas contained *H.* *oregonensis* individuals, which we did not expect when planning the study. Although some predation of *M. magister* megalopae by *H. oregonensis* does occur, the relationship between the two species is mostly a result of density-dependent competitive interactions, as *H. oregonensis* is capable of evicting the smaller *M. magister* from desirable shell habitat, and high densities of the former cause emigration and avoidance by the latter (Visser et al. 2004).

Overall, *M. magister* abundance varies widely over the habitats we studied, and was highest in seagrass habitats, especially seagrass with a large biomass, as well as at a low tidal height. Further studies would be useful to narrow down the relative importance of each of these factors.

As an introduced species in the Pacific Northwest, *Z. japonica* needs to be studied further in order to determine its ecological role, whether predominantly harmful or beneficial. *Z. marina* and *Z. japonica* coexist because of differences in morphological and life history traits causing *Z. japonica* to generally occur at a higher tidal height than *Z. marina* (Ruesink et al. 2009). Our study shows that *Z. japonica* may be beneficial to *M. magister*. Both absence of competition with native seagrass and benefits for native invertebrates should be considered when considering the eradication of the introduced *Z. japonica*.

Juvenile *M. magister* settlement preferences can have many implications for coastal and bay development. Dredging in Grays Harbor in Washington led to an estimated killing of 161,561 subadult *M. magister* in 1990 (Wainwright et al. 1992). Assessing the relative importance of different habitats can allow for informed decisions on the significance of the ecological effects of habitat removal. Juvenile *M. magister* habitat, such as estuaries in general and seagrasses specifically, must be protected to maintain a sustainable adult population and supply a lasting profitable fishery. A fishery cannot be comprehensive without understanding the life cycle and habitat preferences of the target species. Sustainable yields may be achievable if certain estuarine habitats are conserved and protected.

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Fig. 1. Size-frequency distribution of *Metacarcinus magister* we found in two seagrass habitats (*Zostera marina* and *Zostera japonica*) and mud habitat.

Fig. 2. Average abundance of *Metacarcinus magister* we found in samples from one mud and two seagrass habitats (*Zostera marina* and *Zostera japonica*). Error bars represent one standard deviation.



Fig. 3. Shoot density (number of shoots/m2) shown with the corresponding abundance of *Metacarcinus magister* found in various areas of the seagrasses *Zostera marina* and *Zostera japonica*. Linear regression line is shown. Data were natural log transformed for normality before analysis; p=0.942, R2=0.001.



Fig. 4. Abundance of *Metacarcinus magister* found within areas of the seagrasses *Zostera marina* and *Zostera japonica* with varying biomasses of seagrass measured wet. Linear regression line is shown. Data were natural log transformed for normality before analysis; p=0.001, R2=0.446.