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Juvenile Dungeness Face High Predation Risk from Green Crabs Across Increased Temperatures at Different Salinities

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Juvenile Dungeness face high predation risk from green crabs across increased temperatures at different salinities.

by

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Abstract:

Marine heatwaves, altered precipitation regimes, and other changing climate conditions are altering the abiotic conditions of Oregon's estuaries. Furthermore, warmer water temperatures are correlated with the range expansion and population growth of the invasive and broadly tolerant *Carcinus maenas*, the green crab, in Oregon. *Metacarcinus magister,* Dungeness crab, one of Oregon's most valuable fishery species, is also facing these climate stressors, and their young that recruit to estuaries overlap with *C. maenas* adults. Therefore, we wanted to determine impacts of *C. maenas* on young-of-the-year (YOTY) *M. magister* and the roles of salinity, temperature, and predator size in mediating these impacts. We predicted that *C. maenas* would eat most M. magister under warm water scenarios and in the freshest conditions because *C. maenas* is expected to be less impacted than *M. magister* by these stressful conditions. We also predicted that larger *C. maenas* would consume more YOTY *M. magister.* A fully crossed, laboratory study examined predation upon Dungeness*,* with three temperature and two salinity combinations. One adult *C. maenas* was added into each replicate tank with four *Mytilus californianus,* as alternative prey, and three juvenile *M. magister.* We recorded predation across 48 hours. *C. maenas* ate the majority of Dungeness across all temperature and salinity combinations but ate the most Dungeness in 18.5°C and 32ppt. *C. maenas* at intermediate carapace width and mass consumed the most YOTY *M. magister*. Hence*,* Dungeness face high risk from *C. maenas* where they overlap in estuaries. We recommend increased trapping efforts of *C. maenas* during recruitment period by YOTY *M. magister* to mitigate impacts.

Introduction:

The global alteration of coastal ecosystems is a multifaced problem, with climate change and biological invasions often at the forefront (Teck et al., 2010). Increasing atmospheric CO_2 concentrations result in multiple positively reinforcing changes that drive the increase of temperatures in marine and terrestrial ecosystems, which will in turn affect rainfall and estuarine salinities (Mote et al., 2019). Increasing temperature affects the metabolic rates and demands of invertebrates, and therefore their food consumption, and both temperature and salinity affect the distribution and behavior of nearshore animals. Warmer temperatures can alter the biomass and trophic structure of marine ecosystems (O'Connor et al. 2009; Magel et al., 2022). Altered abiotic and biotic conditions can also be important variables in the establishment and abundance of select invasive species (Lewis et al., 2017). Past dispersal of invasive species can be used as a predictor of future invasive spread (Häussler et al. 2021). One such species that exhibits the ability to disperse and survive in a variety of environmental conditions is the green crab *(Carcinus maenas)*.

A successful invasive estuarine species, *Carcinus maenas* has a high tolerance to variable environmental conditions. This tolerance has enabled *C. maenas* to evade abiotic resistance in several recipient areas (Liegnel et al. 2014). *Carcinus maenas* is a euryhaline organism that performs efficient osmoregulation and osmoconformation and in laboratory conditions it can tolerate 10-50 ppt without mortality (Dal Pont et al., 2022). While *C. maenas* is able to tolerate a wide range of salinities, adjusting to changing salinity can be metabolically costly. Oxygen consumption by *C. maenas* can increase dramatically when exposed to a hyposaline environment (Penney et al., 2016). At lower salinities (50% s.w), *C. maenas* experiences increased heart rate and decreased cardiac volume output (McGaw and McMahon, 1996). Additionally, at lower salinities (<20ppt), the hemolymph of *C. maenas* has higher urea-N and ammonia levels and the concentration of hemolymph is dramatically altered (Dal Pont et al., 2022). In addition to being euryhaline, *C. maenas* can tolerate a wide range of temperatures.

The temperature tolerances of *C. maenas* and corresponding impacts are well documented. Depending on population and the season that organisms were collected, individuals can have critical thermal maximums (CTmax) ranging from 31.3-35.8°C (Cuculescu et al., 1998; Kelley et al., 2011). Individuals can also survive and continue consuming food in waters as low as 5°C (Kelley et al., 2011). Populations of *C. maenas* in the Eastern Pacific are distinguished by latitude which ultimately dictates their temperature tolerance and average carapace widths, with larger less temperature tolerant individuals found in higher latitude populations (Kelley et al., 2011). As water temperatures increase, carapace widths are expected to decrease, resulting in decreased egg-capacity for females, but the total egg laying period is expected to increase (Montiero et al., 2023). Water temperatures also impact the way *C. maenas* interacts with its environment in the presence of a predator crustacean, and foraging and feeding responses were found to be greatest at 12°C and 18°C (Connolly-Randazzo et al., 2022). Additionally, strong currents and warmer ocean temperatures associated with El Nino Southern Oscillation facilitate dispersal, recruitment, and survival of *C. maenas* larvae (Yamada and Kosro, 2010; Yamada et al., 2017; Yamada et al., 2021). Increased oceanic temperatures are expected to increase the biogeographic range of *C. maenas*, thus increasing the possibility of successful colonization in new areas (Compton et al., 2010; de Rivera et al., 2007; de Rivera et al., 2011b). Climate change is projected to alter the temperature and precipitation typical of nearshore environments in the Pacific Northwest, as elsewhere, and the eurytolerance of *C. maenas* suggests that they will not only survive in new environments, but they may be able to successfully invade them.

C. maenas, like other invaders, in a novel environment can have both direct and indirect impacts on resident species, consuming them and competing with them as well as impacting habitat and thus affecting ecosystem biodiversity. Directly, *C. maenas* has been found to decrease the abundance of native clams and shore crabs through predation, but native abundance recovered once *C. maenas* density decreased (Grosholz et al. 2000, de Rivera et al. 2011a). *C. maenas* is an opportunistic omnivore and preys upon members of the phylum Mollusca (Baeta et al., 2006; Cordone et al., 2022). *C. maenas* prefers thin shelled mussels over Olympia oysters (*Ostrea lurida*), but consumes both (Yamada et al., 2010). Multiple native organisms along the coast of the Pacific Northwest are impacted by the presence of *C. maenas* through direct predation (Fisher et al. 2023). Small adult *C. maenas* can outcompete juvenile *M. magister* for oyster shell habitat, reducing the amount of time juvenile *M. magister* seeks refuge in these areas (McDonald et al., 2001). In the presence of food, juvenile *M. magister* can spend less time feeding when near small adult

C. maenas (McDonald et al., 2001). *C. maenas* can also impact organisms and ecosystems indirectly.

Indirectly, higher densities of *C. maenas* increase its destruction of eelgrass beds, primarily though bioturbation (Howard et al., 2019), though they also eat it, clip blades, and dig it up (Garbary et al. 2015; Malyshev & Quijon 2011) Eelgrass is an ecosystem engineer, and its destruction can influence many marine and terrestrial species as they are utilized by a wide range of organisms (Thayer et al., 1984). The presence of *C. maenas* in a novel environment, can also facilitate the spread of other non-native species (Grosholz, 2005). Given *C. maenas'* eurytolerance, it may be able to outcompete native crustaceans as climate change continues to alter our coastal conditions (de Rivera and Heath, 2023). This competitive advantage against other marine species could possibly drive trophic level changes, and alteration of existing food webs.

The Dungeness crab (*Metacarcinus magister)* is an Eastern Pacific native crustacean, one of Oregon's most valuable fisheries (ODFW, 2023), and its range currently overlaps with *C. maenas*. Compared to *C. maenas, M. magister* is less tolerant of wide-ranging abiotic conditions. *M. magister* is a weak osmoregulator and can tolerate salinities as low as 12 ppt, but not lower (Cleaver, 1957). While *M. magister* is a weak osmoregulator, in select situations this may not impact its consumption of food. The combination of time exposed to low salinities, time starved and salinity of water was found to not impact the rates at which it fed, but at lower salinities alone, *M. magister* both consumed less food and spent less time feeding than at higher salinities (Curtis et al., 2009). Adult *M. magister* occupy sublittoral habitats in estuaries, where resources may be limited which can result in interspecific competition with other crustaceans (Holsman et al., 2006). *M. magister* can experience death at temperatures exceeding 33°C (Prentice and Schneider, 1979). Given the abiotic tolerances of *M. magister*, it is restricted to narrower ranges within coastal landscapes than *C. maenas*, influencing the areas in which they overlap.

Both *M. magister* and *C. maenas* utilize estuaries and at times can overlap. Juvenile *M. magister* settle to the benthos of estuaries usually in early spring, but the exact time and abundance of settlement varies (Gunderson et al. 1990). Young-of-the-year *M. magister* are drawn to habitat areas with oyster shells or eelgrass (*Zostera marina)* and prefer these to bare mudflats (Fernandez et al., 1993). Being a weaker osmoregulator, salinity tolerance typically limits juvenile *M. magister* to the more marine and polyhaline areas of estuaries. *C. maenas* on the other hand, is euryhaline and can be found throughout estuaries, rocky intertidal zones and within mudflats along the Oregon and Washington coastlines, as both juvenile and adult lifestages (Macdonald et al., 2018; Yamada et al. 2005). Juvenile *M. magister* and adult *C. maenas* spatially overlap within estuaries, which potentially exposes the smaller *M. magister* to predation pressure by *C. maenas* (Yamada. 2001). Additionally, *C. maenas'* destruction of eelgrass beds (Malyshev & Quijon 2011, Garbary et al. 2015, Howard et al. 2019), can result in less vertical refuge for *M. magister* potentially exposing them to higher predation pressure. Each species possesses different salinity tolerances, and extremes may influence physiological or interspecies behaviors.

The outcome of the interaction between *C. maenas* and *M. magister* can be a function of species life history stage and location. Within sublittoral zones and at adult size classes, *M. magister* can outcompete *C. maenas*, in turn pushing *C. maenas* to intertidal zones where they endure higher metabolic costs (McDonald et al. 2006). But juvenile *M. magister* that develop in estuaries directly overlap with both juvenile and adult *C. maenas,* thus exposing them to predation and competition (Yamada. 2001; McDonald et al. 2001). Both *C. maenas* and *M. magister* are direct consumers of bivalves, and individually, *C. maenas* and *M. magister* prefer to prey on different bivalves but have been shown to each eat the other's preferential prey if hungry (Yamada et al., 2010). The habitat overlap of these intertidal crustaceans can lead to interspecific competition and altered community composition within estuaries. Climate change will alter the abiotic conditions of estuaries, namely the temperature and salinity (Lagerspetz and Vainio 2006). Knowing this, it is critical to understand how temperature and salinity shifts will may impact predator-prey relationships between *C. maenas* and juvenile *M. magister.* Previous research has explored impacts of temperature and salinity on *C. maenas* and *M. magister* individually. Research on predation and competition between similar size classes of these two species in ambient conditions found that *C. maenas* prey upon and outcompete *M. magister* (McDonald et al. 2001). Here we build on that study to address the remaining knowledge gap, how combined stressors, temperature and salinity shifts resulting from climate change, will impact the relationship between adult *C. maenas* and juvenile *M. magister*.

Given the projections of how climate change will impact estuaries, the overlap between juvenile *M. magister* and all sizes of *C. maenas* in estuaries, and the greater abiotic tolerance of *C. maenas* we asked: Do adult *C. maenas* prey upon juvenile *M. magister*? If so, does temperature and salinity influence the number of *M. magister* eaten, and does relative size play a part in mortality rates? We hypothesized that, (1) higher temperatures and lower salinities will increase predation of *M. magister* and that (2) larger differences in carapace width will result in increased predation of juvenile *M. magister* by *C. maenas*. We predict that due to the environmental tolerances of *C. maenas*, it will be able to forage and prey across thermal and saline conditions and the less tolerant *M. magister* will not be able to successfully evade attacks under these stressful conditions. We expect that temperature will affect mortality. Crab metabolism increases with temperature (Wallace 1972), likely increasing the need for greater foraging and thus predation pressure on *M. magister*. We also predict that larger crabs will have greater metabolisms and be more experienced hunters, thus resulting in larger *C. maenas* consuming more *M. magister*. This study aimed to determine the extent of *C. maenas* predation on *M. magister* under projected conditions resulting from climate change, and how size differences of *C. maenas* may impact predation. To implement this study, we tested predation of *M. magister*, at different treatment combinations, in a laboratory setting and recorded mortality.

Methods:

Field sampling and organism housing

All organisms were collected from Netarts Bay, Oregon between June 29th and July 15th, 2023. *M. magister* were hand collected from eelgrass beds (*Zostera marina*) and from mudflats near the Whiskey Creek Shellfish Hatchery (45.4226, -123.9376). *C. maenas* were collected using modified minnow traps and folding Fukui box traps baited with tilapia, placed in the lower intertidal by the shellfish hatchery and a site 0.8 km up the bay where there is an outfall pipe. Traps were deployed at low tide and left for one to two semi-diurnal cycles (~12-24 hours). Mussels (*Mytilus californianus)* and sea lettuce (*Ulva lactuca*) were collected and brought back to the Portland State marine aquarium laboratory. Animals were housed in species-specific tanks at Portland State University on recirculating water tables with 27ppt salinity and 13°C. All species were separated into different tanks by size class. All organisms were left to acclimate for 72 hours, before initial morphometric data was taken, and temperature acclimation was performed. During laboratory acclimating, crabs were fed tilapia filets every 24 hours and the excess was removed at the next feeding.

Experimental Tank composition

Experimental trials were conducted in independent, temperature-controlled self-recirculating 80 L tanks (*Little Giant, U21B*). Bioballs were placed into a mesh bag and added to each tank to remove nitrogenous waste. To inoculate the Bioballs with denitrifying bacteria each tank was dosed one time, prior to organism introduction, with one capful (~10ml) Seachem's Stability. Five-pieces of 3-4cm round river rock (250g) and half of a *Tresus capax* shell were placed in the tank for refuge. One 8cm x 12cm piece of *U. lactuca* and three whole mussels of three different size classes and one smashed mussel were placed in the river rock. Each tank was covered with black plastic film to prevent external stimuli (Robles 1987). Tanks were filled with artificial seawater (Instant Ocean) filtered with two spin down filters (200µm, 50µm) to remove microplastics and other particles suspended in Instant Ocean. Each tank was constructed 24 hours before introduction of organisms.

Temperature acclimation, treatment assignment, morphometric data collection

Each organism was randomly assigned an experimental treatment, which included three replicates of each experimental temperature-salinity combination, then transferred into independent 80L, recirculating holding tanks (*Little Giant, U21B, 10gmp*) with an external chiller (*Aqualogic DS-3WHC*) for acclimation. Temperature was incrementally adjusted, 1 degree Celsius per 4 -7 hours, until all species were at their designated treatment temperature. No acclimation to experimental salinities were performed on any organisms due to lack of tanks. 26 hours before introduction into experimental tanks, crabs were fed two pieces of tilapia (2cm^3 for *C. maenas*, and 0.5cm^3 for *M. magister*) per individual to ensure each crab was adequately fed. Crabs were allowed to feed freely for 2 hours then food was removed. Then we recorded mass, carapace width (widest point to widest point) and injuries (missing legs or claws), marked them with nail polish for identification, and distributed them into the experimental tanks. Each tank received three *M. magister* (13.1 mm - 22.1 mm carapace width) and one *C. maenas* (43.4 mm - 83.1mm carapace width).

Experiment 1: Effect of increased temperature and variable salinity on predation

To determine the impact of temperature and salinity on the predation of *M. magister* we tested six fully crossed treatment combinations. Three temperatures were selected: 16.5°C, which reflected current water temperatures in Netarts Bay, Oregon in July, and 18.5°C and 20.5°C, which represented increased water temperatures resulting from climate change (Magel et al., 2022; Khangaonkar et al., 2021). Two salinities were selected, 22ppt, which represented salinity changes caused by heavy rains (Kreag 1979) or mid estuarine areas, and 32ppt, which reflected the historic average for Netarts Bay, Oregon (Kreag 1979).

The experiment was repeated over the course of three different time blocks. We intended for two tanks for each treatment combination in each time block but due to two external chiller failures in block 2, block 3 had three replicates of 32ppt x 16.5°C and 22ppt x 20.5°C. Each experimental tank contained one *C. maenas,* three *M. magister* and four *M. californianus*. Four *M. californianus*, were added to make the experiment more realistic as multiple prey options are available in natural settings. To prevent the premature predation of organisms, *M. californianus* were introduced to the tank before *M. magister,* and *M. magister* were introduced to the tank one hour prior to the introduction of *C. maenas*.

Organisms were left in the tank for a 48-hour period where predation was monitored. Predation of *M. magister* and *M. californianus* was counted every 8-hours, and a final predation count was taken at the end of the 48-hour experiment. At the end of the 48-hour experiment, morphometric data were recorded again. Tanks were drained, flushed with deionized water, and allowed to dry for 24 hours before refilling for the next trial period. All *C. maenas* were humanely euthanized by freezing, and *M. magister* were transferred back to a holding tank on the water table until the end of the experiment. Each animal was experimentally naïve to the experiment.

Experiment two: Effect of size difference on predation

Organisms were subject to the same field sampling, acclimation, final feedings and random treatment assignment processes as described earlier. Tank setup was the same as outlined in the tank composition section except there was no *Ulva*. Tanks were not wrapped with black plastic, as the purpose of this experiment was strictly predation and there was little activity in the lab throughout. No individuals had been used in experiments prior to this.

To determine the impact of size difference on predation, three *M. magister* and one *C. maenas* of varying size differences were added to 11 tanks. Tanks also included four *M. californianus* individuals of varying sizes, one of which had a broken shell for easy consumption even by small *M. magister*. The experimental tanks were filled with 32ppt artificial seawater (*Instant Ocean*) and their temperature was maintained at 18.5°C. Predation of *M. magister* and *M. californianus* was counted every 8-hours, and total predation was tallied at the end of the 48-hour experiment. Post experimental procedures were the same as above.

Tethering juvenile M. magister

To explore rates of predation of juvenile *M. magister* we tethered 20 of the surviving juveniles from previous experiments and placed them either in eelgrass or in mudflats. One juvenile *M. magister* was leashed with ~30cm of 6lb, clear monofilament, and secured with LocTite super glue over the knots and where the monofilament overlapped with the carapace. Two tethered individuals were tied to one green 4' gardening stake, with one individual fixed at a point where it could freely move along the benthos and the other tied at a point where it was completely suspended in the water column, to allow access to seagrass blades and an escape from purely benthic predators or the equivalent height. Five stakes were then placed into open mudflats, and five were placed in eelgrass (*Z.* marina) beds in Netarts Bay, Oregon, USA (45.4226, -123.9376), at low tide on July 27th, 2023, and left in place for 12-hours. Stakes were removed and surviving individuals were tallied.

Organism morphometric data

C. maenas used in experiment 1, had carapace widths between 43.4 mm, and 83.1 mm with an average carapace width of 66.7 mm. Mass of *C. maenas* used in experiment 1 ranged from 17.7 grams to 158.4 grams, with an average weight of 70.7 grams. *M. magister* in experiment 1, had carapace widths between 13.1 mm and 22.1 mm, with an average value of 15.4 mm. The mass of *M. magister* ranged from 0.45 grams to 1.98 grams, with an average of 0.73 grams. Intact *Mytilus californianus* used in experiment 1 had weights that ranged from 0.20 grams to 2.10 grams, and the average weight was 0.71 grams. Smashed *M. californianus* weights ranged from 0.18 grams to 0.79 grams, with an average weight of 0.42 grams.

C. maenas used in experiment 2, had carapace widths between 41.9 mm, and 94.4 mm with an average carapace width of 66.0 mm. Mass of *C. maenas* used in experiment 2 ranged from 17.5 grams to 208.0 grams, with an average weight of 92.5 grams. *M. magister* in experiment 2, had carapace widths between 13.4 mm and 25.5 mm, with an average of 18.8 mm. The mass of *M. magister* ranged from 0.52 grams to 2.34 grams, with an average weight of 1.18 grams. *M. californianus* weights were not recorded for experiment 2 but sizes were visually similar to experiment 1.

Statistical Analysis

All statistical analyses were performed using R studio (*2023.12.1 Build 402*). In experiment 1, generalized linear mixed effects models (*lme4* package) (GLMM) were used to determine if temperature, salinity, or their interaction and heterospecific mass difference had a significant impact on *M. magister* predation. The time block was utilized as a random variable in case conditions changed across experimental time blocks. In all the GLMMs performed, temperature and salinity were scaled as their units are not comparable. In experiment 2, a GLMM was also used to determine if heterospecific carapace width or mass differences, between *C. maenas* and the three *M. magister*, had a significant impact on predation. Replicates from Experiment 1 with the same tank conditions as Experiment 2 (32ppt and 18.5°C) were added to the analysis to increase power. The temporal differences between these replicates were utilized as a random variable. Preferred prey choice by *C. maenas* at eight hours of exposure was calculated using a Mann-Whitney U test. Successful predation events of specific prey species were calculated using General Linear Models (GLM) for both *M. magister* and *M. californianus* to determine if the temperature, salinity, or the interaction had a significant impact on prey choice. To determine if carapace width or mass of *C. maenas* influenced the predation of *M. californianus* and *M. magister* at 8-hours exposure, GLMs were used. We tested: salinity, temperature, *C. maenas* carapace width and mass as variables in prey choice models. A forward selection process was used to determine the factors that were retained for final GLMM and GLM models (Table 1; Appendix 3). We only retained factors that either were explicitly tested, temperature, salinity, and their interaction or if they helped reduced the Akaike Information Criterion (AOC), thus strengthening the model.

*Table 1. Comparison of GLMM model strength indices, AIC, BIC and logLik and the corresponding deviance and residual degrees of freedom for the different GLMMs performed. Each GLMM had temperature (T), salinity (S) and their interaction (T*S) as a fixed effect, but the other two incorporated either mass difference or carapace width difference. All GLMMs use experimental block as a random effect. The corresponding model strengths from this table were the basis for selecting which fixed effects were to be selected for the final GLMM.*

Fixed effects	**AIC**	**BIC**	**logLik**	**deviance**	**df.resid**
T, S, T*S, No size difference	101.1	109.0	-45.5	91.1	31
T, S, T*S, Mass difference	99.6	109.1	-43.8	87.6	30
T, S, T*S, Carapace width difference	100.9	110.4	-44.5	88.9	30

Results

Experiment 1.

Mean predation of juvenile *M. magister* was highest at 32ppt x 18.5°C, the intermediate temperature at marine salinity, and lowest at the warmer two polyhaline treatments: 22ppt x 18.5°C and 22 ppt x 20.5°C (Fig. 1). The interaction of temperature and salinity had a significant impact on predation of *M. magister* (figure *X*, GLMM, $z = 2.65$, $p = 0.008$, df = 31). Salinity significantly affected predation, with *C. maenas* eating more *M. magister* in marine than polyhaline conditions (figure 1, GLMM, $z = 2.39$, $p = 0.02$, df = 31), but temperature alone did not affect predation (figure 1, GLMM, $z = -0.735$, $p = 0.462$, df = 31). Heterospecific mass difference did not have a statistically significant impact on predation, though there was a non-significant trend for more predation when a *C. maenas* was relatively closer to its prey size (GLMM, $z = 1.716$, $p = 0.08$).

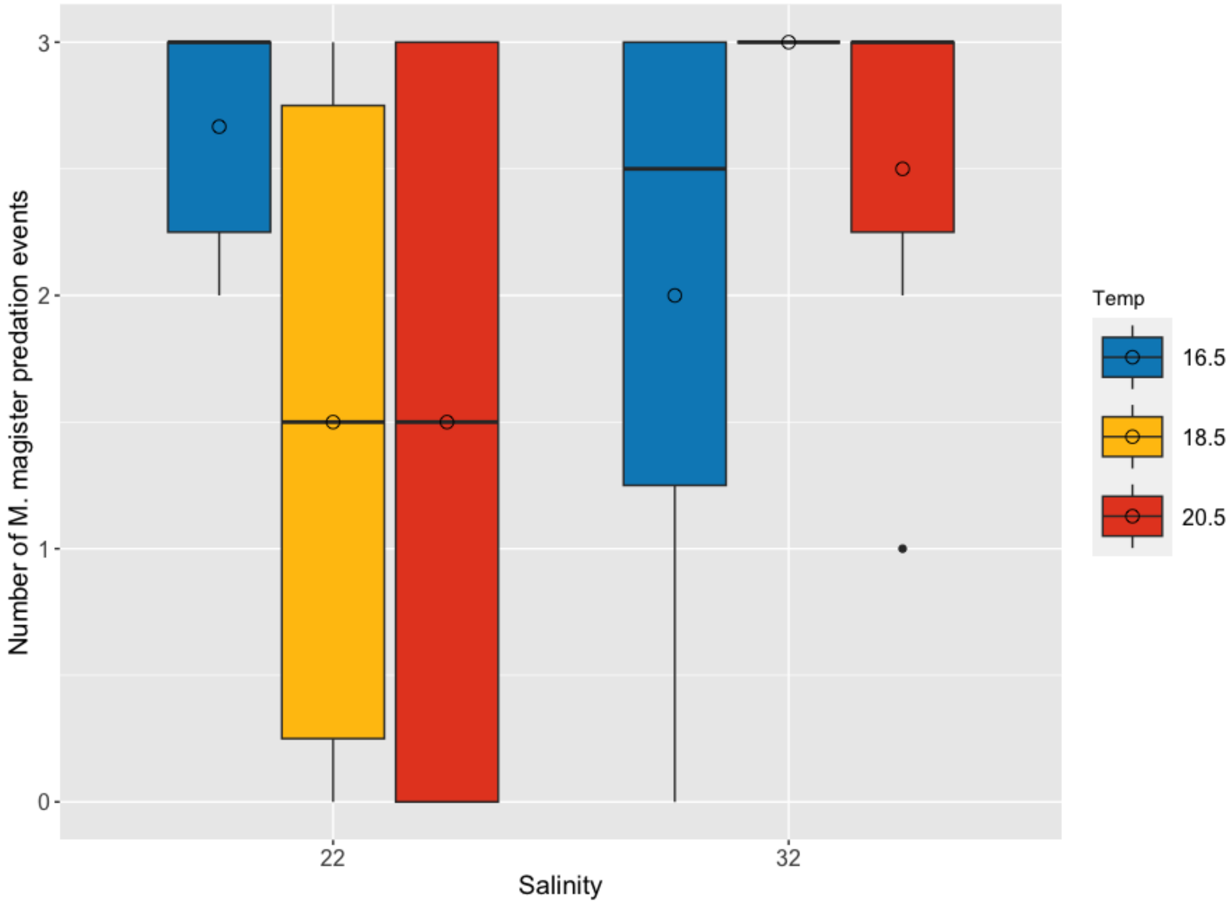

Figure 1. Clustered boxplot of M. magister predation across different temperatures and salinities. The x-axis represents salinity, and the y-axis represents the number of predation events per tank with a maximum value of three. The colors represent the temperature of the treatment. The open black circles are the mean values. At 22ppt salinity, mean predation was highest at 16.5°C, but decreased at 18.5°C and 20.5°C. At 32ppt salinity mean predation peaked at 18.5°C, with 16.5°C and 20.5°C having lower mean predation. (N = 6)

Table 2. Statistics generated by the chosen GLMM. Temperature and Salinity were scaled as their units are not comparable, which facilitated the successful implementation of this GLMM. The final row indicates the interaction between scaled temperature and scaled salinity, scale(Temp):scale(PPT).

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.07632	0.88249	-0.086	0.93108
Temperature	-0.19975	0.27163	-0.735	0.46213
Salinity	0.61516	0.26421	2.328	0.01990
Mass Difference	0.01871	0.01091	1.716	0.08622
Temperature*Salinity	0.79249	0.28413	2.789	0.00528

Experiment 2

The mass difference between *C. maenas* and averaged *M. magister* within a tank, was found to significantly impact predation (GLMM, z = -2.47, p = 0.01, df = 14). As the mass difference between *M. magister* and *C. maenas* decreased, the number of *M.*

magister preyed upon, also decreased (figure 2A). Similarly, the difference between carapace width of *C. maenas* and averaged *M. magister* within a tank, was found to impact predation (GLMM, z = -2.32, p = 0.02, df = 14). As the average carapace width difference increased, the number of *M. magister* predation events decreased (figure 2B).

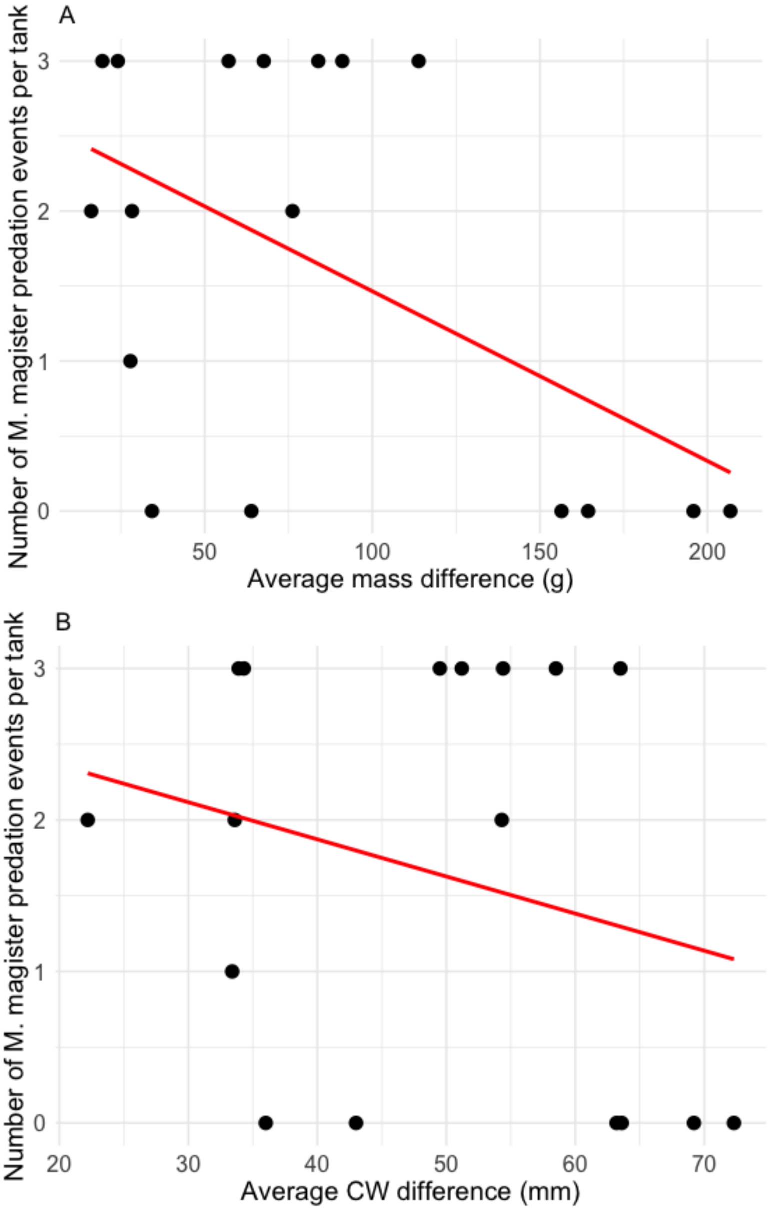

Figure 2. Scatterplots representing the relationship between average size difference and predation. The y-axis represents number of predation events per tank on both scatterplots. On figure 2A, the x-axis represents the mass difference (grams) between the C. maenas and the average of the three M. magister in one tank (n=17). The most predation events occurred when average mass differences were intermediate (~55-110g). On figure 2B, the x-axis represents the carapace width difference (mm) between the C. maenas and the average of the three M. magister carapace widths in one tank (n=17). The most predation events occurred when average carapace widths were intermediate (~45-60mm).

Tethering M. magister

Sample sizes were too low to run statistical analysis between predation occurring in and out of *Z. marina* beds at both tethering heights. The highest predation observed was for the benthic tethered *M. magister* outside of eelgrass, followed by the suspended (20 cm up) tethers inside eelgrass beds, and both were higher than benthic in eelgrass or suspended outside of eelgrass (Table X).

Table 3. Predation rates of tethered M. magister at two different heights, both inside and outside of Z. marina beds. Each combination contained five tethered M. magister.

	Top predation	Bottom predation
Inside eelgrass	2	1
Outside eelgrass	1	3

Prey Choice

At eight hours into each time block, at all treatment combinations, mussels were found to have been eaten before *M. magister* (figure 3, Mann-Whitney, w = 1243, $p<0.001$). At 8 hours exposure to treatment combinations, 29 of the 36 tanks had all of the *M. californianus* eaten and seven of the 36 tanks had all of the *M. magister* eaten. Of the six tanks with both mussels and *M. magister* remaining, only three had fewer *M. magister* than mussels. *M. californianus* and *M. magister* were consumed indiscriminate of the predator *C. maenas'* carapace width or mass. The carapace width of *C. maenas* was not found to be influential in the consumption of mussels (GLM, z = -0.439, p =0.66), or *M. magister* (GLM, z = 0.183, p= 0.85). The mass of *C. maenas* was not found to be influential in the consumption of *M. californianus* (GLM, z= -0.200, p=0.84), or *M. magister* (GLM, z= 0.438, p=0.66

Carcinus maenas preference of *M. magister* alone as prey, was not found to be influenced by temperature (figure 4a, GLM, z = -0.787, p = 0.43, df = 32) or by treatment combination (figure 4a, GLM, z = 1.60, p = 0.118, df = 32). However, a non-significant trend of greater predation on *M. magister* and of *M. californianus* when salinity was higher suggests that differences in salinity impacted *C. maenas* prey choice or consumption amount (figure 4a, GLM, z = 1.793, p = 0.07, df = 32). Prey preference of *Mytilus californianus* alone was not influenced by temperature, and there were no discernable trends observed (figure 4b, GLM, z = 1.567, p = 0.12, df = 32), salinity, (figure 4b, GLM, z = 0.726, p = 0.47, df = 32) or the combination (figure 4b, GLMM, z = 0.338, p = 0.74, df = 32).

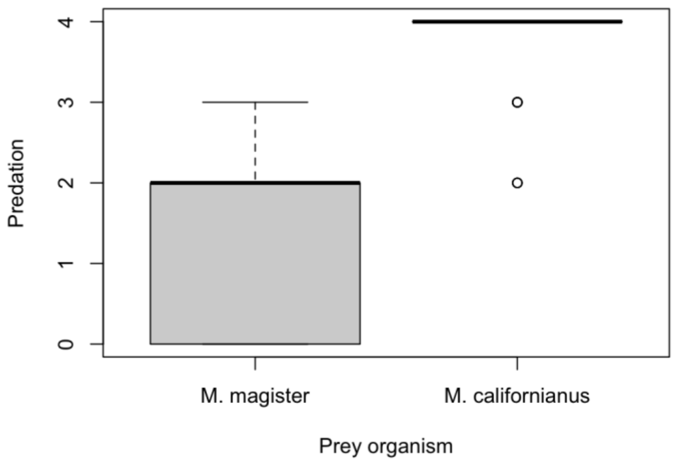

Figure 3. Prey choice at 8 hours of exposure to treatment combination in all time blocks. The x-axis represents the prey organism, and the y-axis represents the number of individuals eaten. N = 17

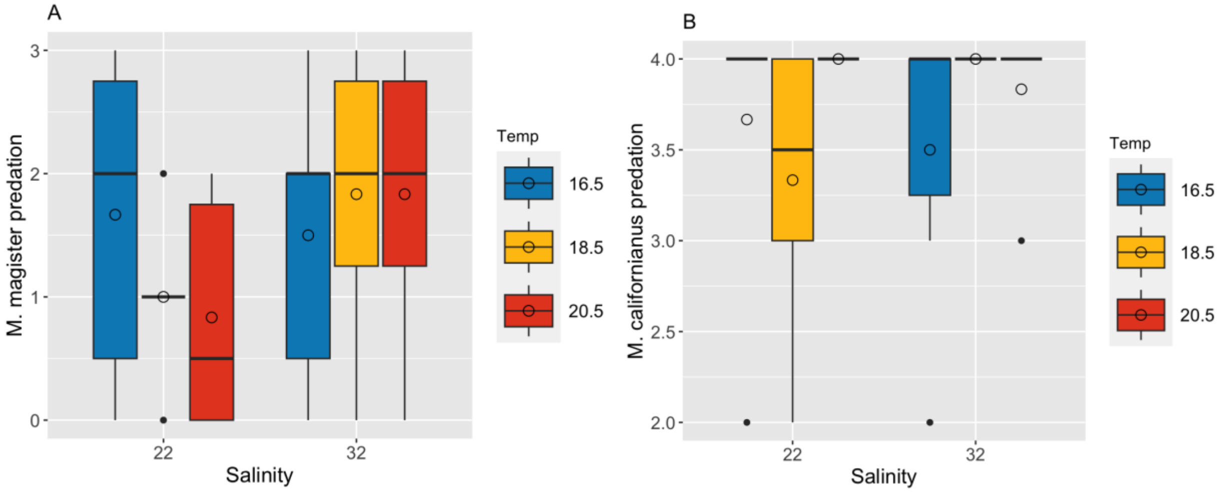

Figure 4. Clustered boxplots of the amount of predation events that occurred at 8 hours. figure A (N= 17) displays the M. magister predation across all treatment combinations. figure B. (N=17) displays the M. californianus predation events across all treatment combinations. For both graphs the x-axis is salinity, y-axis is predation, and the colors reflect the temperature.

Discussion:

Multiple stressors are likely to affect crab habitats and trophic relationships, potentially impacting *M. magister* populations. Over the past few decades, the Eastern Pacific has experienced novel marine heatwave events that have varied in both length and their influence on temperature anomalies. During the 2015-2016 heatwave event, estuaries along the Oregon and Washington coasts experienced periods of increased water temperatures and after these heat events the biomass of *Z. marina* and other macrophytes decreased (Magel et al., 2022). These *Z. marina* beds play an important role in the lifecycle of juvenile *M. magister* (Fernandez et al.,1993), and many other organisms (Thayer et al., 1984). *C. maenas* is also supported by these eelgrass beds and can negatively impact them in many ways (Garbary et al. 2015; Howard et al., 2019; Malyshev & Quijon 2011). The use of these eelgrass beds by both *C. maenas* and *M. magister* can increase the probability of overlap.

C. maenas and *M. magister* occupy different parts of the estuary at different life stages and there are conflicting thoughts on the level of overlap between the two (McDonald et al., 2001; Yamada, 2001). During the field collection of test organisms from June 26th, 2023, to July 15th, 2023, we witnessed both adult *C. maenas* and juvenile *M. magister* in the same *Z. marina* beds in Netarts Bay, Oregon. Juvenile *M. magister* individuals were inverted and clasped to the top of the *Z. marina* blades, presumably to evade predation by benthic predators and aerial predators. The *Z. marina* beds that we collected organisms from were in close proximity to both mudflats and oyster shelled habitat, but contrary to findings from Fernandez et al., (1993), we found the majority of *M. magister* in *Z. marina* beds, few in mudflats and none in the oyster shell rich location. This observation of juvenile *M. magister* in *Z. marina* in during our July sampling is consistent with findings by Fernandez et al., (1993) and aligns with timelines suggested by Gunderson et al., (1990). Adult *C. maenas* were also found in a gradient of *Z. marina* densities but were not found in mudflats, which may serve to camouflage themselves from a variety of seabird predators. Although *M. magister* may prefer oyster shell habitat to *Z. marina* beds (Fernandez et al., 1993), we did not detect them in oyster shell habitat, perhaps due to competition with, avoidance of, or direct consumption by *C. maenas*. The direct observation of overlap, the body of literature on the impacts by *C. maenas*, and the threat of altered ecosystems by climate change, prompted us to examine the intersection with *M. magister.*

Experiment 1: Temperature and Salinity on Predation

The pattern of *M. magister* predation that we observed did not support our predictions. We predicted a trend of increased predation as abiotic conditions increased stress on *M. magister* more than *C. maenas* given its broad tolerances, with the lowest salinity and highest temperature resulting in the most *M. magister* predation. However, results from our first experiment found that the combination of 32ppt x 18.5°C, resulted in the highest mean predation of juvenile *M. magister.* Mean predation was generally higher across all 32ppt treatments, with the exception of 22ppt x 16.5°C (Fig X). We found that higher temperatures resulted in more *M. magister* predation only at the higher salinity treatment. Our GLMM indicated that when isolated, salinity alone played a larger role than temperature in influencing the rates at which *C. maenas* preyed upon *M. magister*, and predation pressure was higher in fully marine salinities, the opposite response of our predictions (figure 1).

We believe that our findings of *M. magister* predation at different temperature and salinity combinations can potentially be explained by greater physiological effects of combined stressors to *C. maenas* and to other confounding factors. *C. maenas* is tolerant of both temperature (Cuculescu et al., 1998; Kelley et al., 2011) and salinity variation (Dal Pont et al., 2022; McGaw et al.,1999). Often laboratory experiments have exposed *C. maenas* to one stressful condition at a time (Nancollas and McGaw, 2021). However, the interaction between the two stressful conditions, lower salinity, and higher temperature, may have caused a decreased efficiency in its ability to effectively hunt *M. magister*, thus explaining our findings of the lowest predation occurring at the middle and warmest temperatures at polyhaline salinities (Fig 1). Although the temperatures and salinities we used were well within the tolerance ranges for both *C. maenas* and *M. magister* (Cuculescu et al. 1998; Kelley et al. 2011; Prentice and Schneider, 1979*)*, the combination of these stressors at the more stressful values (higher temperature and lower salinity) may in part explain patterns of predation we observed. Low salinities (<20ppt), are metabolically demanding for *C. maenas* (Dal Pont et al., 2022) and even though we tested organisms at 22ppt, there may have been instances of increased metabolic demand. Increased thermal stress, regardless of its magnitude may have interacted with stress induced by altered salinity. Cumulative impacts of increased temperature and salinity alterations, on estuarine organisms can result in reduced fecundity as maintaining tolerance can be energetically demanding (Kelly et al., 2016).

Altered behaviors due to increased stress may not be isolated to *C. maenas* alone. *M. magister* are weak osmoregulators (Cleaver, 1957) and when exposed to lower salinities they may spend less time feeding and consuming food (Curtis et al., 2009). Less time spent foraging by juvenile *M. magister* could have reduced the amount of overlap it experienced with *C. maenas*, ultimately decreasing the potential for predation. We noticed that individual *M. magister* preferred higher spots within the tank, such as on floating algae or the side of the tank, where it would remain stationary and out of reach of *C. maenas*. Although *C. maenas* can swim, they were not observed swimming during this experiment, whereas the *M. magister* juveniles swam frequently. Less feeding time, and more time stationary at higher points could explain trends of predation we found at lower temperatures.

Alternatively, several factors other than multiple abiotic stressors could have influenced the results including, genetic variability among individual *C. maenas*. Different populations of *C. maenas* vary in their thermotolerance, and Oregon, where we collected the crabs, has a mixture of genetic backgrounds represented (Kelley et al., 2011; Tepolt et al., 2021). Differences in CTmax values were found to be a function of recruitment population and the temperature they were acclimated in during laboratory trials (Kelley et al., 2011). Additionally, the impacts of tank acclimation could have influenced the trends of predation we observed. All individuals had been acclimated to 27ppt and then were rapidly transferred to either 32ppt or 22ppt, it is possible that organisms that were then introduced to 22ppt experienced a greater osmoregulatory shock, resulting in greater stress. This has been observed in both test organisms and when exposed to quick decreases in salinity, *M. magister* (McGaw and McMahon, 1996) and *C. maenas* (Hume and Berlind, 1976) respond with altered cardiac response. It's possible that the lack of salinity acclimation altered the rates of predation we found.

The consumption of alternative prey may have influenced the predation trends displayed by *C. maenas*. In each tank there were four mussels, which served as alternative prey. *C. maenas* prefers thin-shelled mussels (Yamada et al., 2010), and at 8-hours exposure to treatments it consumed all of them in 29 of the 36 tanks. We found that prey preference was not significantly influenced by temperature but there was a weak trend with salinity, which may have influenced later predation on *M. magister*. The initial consumption of these mussels by *C. maenas* at hyposaline conditions (22ppt) could have increased its oxygen consumption for up to 40-hours after ingestion (Penney et al., 2016). When exposed to hyposaline conditions on an empty stomach *C. maenas* faces a short window of dramatically increased oxygen consumption occurring shortly after exposure, lasting up to 24 hours (Penney et al., 2016). Therefore, this increased oxygen consumption at lower salinities, in combination with stress resulting from higher temperatures may have decreased the ability of *C. maenas* to continue to forage, explaining our observed patterns of mortality. The salinity-influenced behavior of *M. magister* may also have influenced the pattern of predation we observed. For example, when food was introduced to tanks at 24 hours of exposure to conditions, decreased foraging demand caused by lower salinity (Curtis et al., 2009) may have allowed *M. magister* to remain hidden and out of reach of *C. maenas*.

Tank composition and design may have influenced the predation rates we found compared to another laboratory study performed on predation (McDonald et al., 2001). The McDonald et al. (2001) design allowed for juvenile *M. magister* to freely emigrate and immigrate from the internal cage, where *C. maenas* was restricted. The interior of their two-cage set up was oyster shell habitat and lacked the vertical refuge juvenile *M. magister* is known to inhabit (Fernandez et al., 1993). Knowing that *M. magister* also seeks refuge in *Z. marina* beds we believe that the predation they observed more accurately reflects areas where oyster shell habitat is dominant. While our design had some shells, and *Ulva* as vertical refuge, it lacked eelgrass due to restrictions with harvesting. Our experimental tanks also did not contain sediment, as tank recirculation prohibited sediment from settling. *M. magister* can seek refuge by burrowing into mudflats (Fernandez et al., 1993) , while not preferred, the lack of this substrata could have reduced total time spent on the bottom of the tank, reducing overlap with *C. maenas*.

Experiment 2: Size Class

We found that both mass difference and carapace width difference between juvenile *M. magister* and *C. maenas* significantly impacted predation. We expected that larger differences in mass and carapace width between *C. maenas* and *M. magister* would result in more mortality due to increased metabolic costs and superior claw power of larger *C. maenas*; however, our data did not support this hypothesis. We found that larger differences in size resulted in less predation of *M. magister*, and that more predation occurred when differences were intermediate (~50-100g;~50-60mm), whereas larger and smaller differences resulted in less or variable predation. An earlier study that examined predation by small (~35 mm) *C. maenas* on juvenile (~15mm) *M. magister* found much less predation than we did (McDonald et al., 2001). We attribute this to their smaller difference in carapace width and mass compared to our intermediate size difference (~50mm); this intermediate size difference was found to produce the highest rates of predation.

We found that variation in carapace width and mass, did not dictate prey preference of *C. maenas*, and individuals preyed on *M. californianus* over *M. magister* regardless of their size. Our findings of preferential consumption of thin-shelled mussels align with and expanded on findings by Yamada et al. (2010). They found that larger carapace width *C. maenas* (~80-90mm) preferred thin shelled mussels. We found that thin shelled mussels were preferred over *M. magister*, when carapace widths of *C. maenas* ranged from 41.9 mm, and 94.4mm.

Ecological Impact

When size and mass differences between *C. maenas* and *M. magister* are intermediate, we recorded the highest predation, and since *C. maenas* utilizes many parts of estuaries at multiple life stages, we anticipate that after postlarval *M. magister* recruit to estuaries, there is a higher possibility of overlap with intermediately sized predators. Our results suggest that higher heat, and marine salinities pose the greatest threat to juvenile *M. magister*. Salinity provided the biggest buffer against predation at higher temperatures, and if *M. magister* can recruit or seek refuge in areas with lower salinity, they may be able to lower their risk of direct impacts by *C. maenas*. We found that climate change may alter how *C. maenas* can directly impact *M. magister* through predation, but we also are concerned with how climate change will indirectly influence how *C. maenas* impacts *M. magister*. Under current conditions we know that *C. maenas* destroys *Z. marina* beds (Howard et al., 2019), and after high heat events, biomass of *Z. marina* beds decreases (Magel et al., 2022) which both indirectly impact *M. magister.* As abiotic conditions continue to change in estuaries, we stress the importance of preserving and restoring *Z. marina* beds which are a refuge for juvenile *M. magister* (Fernandez et al., 1993) and which we observed facilitating their escape from *C. maenas*. We suggest that to buffer against *C. maenas* predation during critical juvenile *M. magister* estuarine recruitment, managers implement increased trapping efforts along eelgrass beds, and areas with high oyster shelled habitat to reduce predation pressure. During high temperature events, these intensified trapping efforts become more important.

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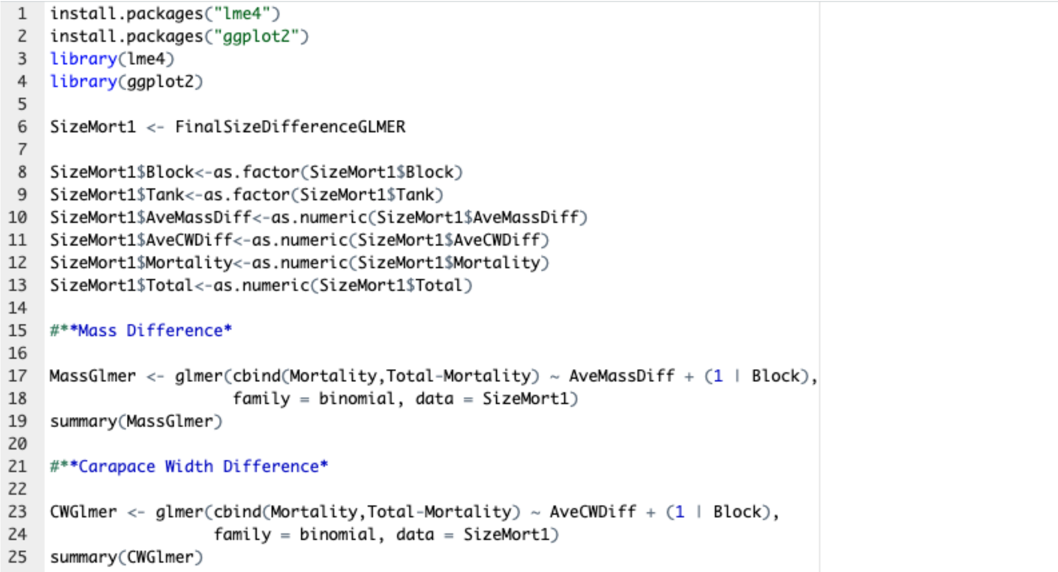
Appendix:

```r
install.packages("lme4")
install.packages("ggplot2")
library(lme4)
library(ggplot2)



countpredation <- PredationDataExpOne
names(countpredation)
list(countpredation)

countpredation$Block<-as.numeric(countpredation$Block)
countpredation$Replicate<-as.factor(countpredation$Replicate)
countpredation$Tank<-as.factor(countpredation$Tank)
countpredation$PPT<-as.numeric(countpredation$PPT)
countpredation$Temp<-as.numeric(countpredation$Temp)
countpredation$Mortality<-as.numeric(countpredation$Mortality)
countpredation$Total<-as.numeric(countpredation$Total)
countpredation$MassDiff<-as.numeric(countpredation$MassDiff)
countpredation$CwDiff<-as.numeric(countpredation$CwDiff)

#**With Mass Difference*
FinalGlmer <- glmer(cbind(Mortality,Total-Mortality) ~ scale(Temp) * scale(PPT) + MassDiff + (1 | Block),
                family = binomial, data = countpredation)

```

Appendix 1. GLMM R-script used to determine the impact of temperature, salinity and heterospecific mass difference on the predation of juvenile M. magister



```r
install.packages("lme4")
install.packages("ggplot2")
library(lme4)
library(ggplot2)

SizeMort1 <- FinalSizeDifferenceGLMER

SizeMort1$Block<-as.factor(SizeMort1$Block)
SizeMort1$Tank<-as.factor(SizeMort1$Tank)
SizeMort1$AveMassDiff<-as.numeric(SizeMort1$AveMassDiff)
SizeMort1$AveCWDiff<-as.numeric(SizeMort1$AveCWDiff)
SizeMort1$Mortality<-as.numeric(SizeMort1$Mortality)
SizeMort1$Total<-as.numeric(SizeMort1$Total)

#**Mass Difference*

MassGlmer <- glmer(cbind(Mortality,Total-Mortality) ~ AveMassDiff + (1 | Block),
                   family = binomial, data = SizeMort1)
summary(MassGlmer)

#**Carapace Width Difference*

CWGlmer <- glmer(cbind(Mortality,Total-Mortality) ~ AveCWDiff + (1 | Block),
                 family = binomial, data = SizeMort1)
summary(CWGlmer)
```

Appendix 2. GLMM R-script used to model the impact of average carapace width difference and mass difference on the predation of juvenile M. magister by C. maenas.

GLMM 1				
Fixed Effects	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
(Intercept)	1.1905	0.4461	2.668	0.00762
Temp	-0.1205	0.2638	-0.457	0.64780
Salinity	0.5942	0.2609	2.278	0.02275

Temp*Salinity	0.7414	0.2795	2.653	0.00798
AIC	*BIC*	*logLik*	*deviance*	*df.resid*
101.1	109.0	-45.5	91.1	31
GLMM 2				
Fixed Effects	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
(Intercept)	-1.16483	1.70579	-0.683	0.49469
Temp	-0.17274	0.27061	-0.638	0.52325
Salinity	0.61271	0.26409	2.320	0.02034
Cw Difference	0.04651	0.03231	1.439	0.15007
Temp*Salinity	0.78908	0.28458	2.773	0.00556
AIC	*BIC*	*logLik*	*deviance*	*df.resid*
100.9	110.4	-44.5	88.9	30
GLMM 3				
(Intercept)	-0.07632	0.88249	-0.086	0.93108
Temp	-0.19975	0.27163	-0.735	0.46213
Salinity	0.61516	0.26421	2.328	0.01990
Mass Difference	0.01871	0.01091	1.716	0.08622
Temp*Salinity	0.79249	0.28413	2.789	0.00528
AIC	*BIC*	*logLik*	*deviance*	*df.resid*
99.6	109.1	-43.8	87.6	30

Appendix 3. Summary statistics of three different GLMMs used during the first experiment. This table was used during backward selection to determine which was the strongest model.