The effects of ocean acidification and increased nutrients on invasive amphipod herbivory of *Zostera marina* (eelgrass) in San Francisco Bay

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Introduction:

Seagrasses provide valuable ecosystem functions, yet they are declining globally due to environmental and anthropogenic stressors. To fully understand how stressors influence seagrass habitats, it is essential to test how global climate stressors like ocean acidification will interact with local environmental conditions (e.g. increased nutrient pollution) to affect ecosystem functions and key trophic relationships. In San Francisco Bay, it is unknown how stressor interactions will affect the feeding behavior of *Ampithoe valida*, an invasive amphipod who negatively impacts the local seagrass, *Zostera marina* (eelgrass), by directly feeding on its tissue. To further understand this relationship, we exposed *A. valida* and eelgrass to levels of ocean acidification expected with accelerating climate change, combined with levels of nutrient pollution that are observed along populated coastlines, to test if these combined stressors affect *A. valida* herbivory of eelgrass. We expected that exposure to stressors will modify *A. valida* herbivory of eelgrass, with low pH combined with high nutrient conditions potentially leading to increased *A. valida* consumption. These results will advance our understanding of *A.valida* impact on eelgrass meadows, informing potential shifts in feeding patterns amidst future environmental changes, and guiding effective eelgrass conservation and restoration efforts in San Francisco Bay.

Aim:

Examine the impact of ocean acidification and increased nutrients on *Ampithoe valida* consumption of eelgrass plant tissue.

Hypothesis:

a. *A. valida* will exhibit increased rates of consumption on eelgrass grown in higher nutrient conditions due to increased plant palatability of eelgrass and algae via reduced C:N ratios.
b. *A. valida* exposed to low pH conditions will exhibit higher rates of consumption due to unknown physiological changes that alter the amphipods.

Null Hypothesis:

A. valida will exhibit no change in rates of consumption on eelgrass grown in high nutrient and low pH bay conditions than eelgrass grown in ambient bay conditions.

Dataset Description & Approach

In Fall 2023, the SFSU Boyer Lab conducted a pilot project to collect preliminary data for a larger NSF project and my thesis research. This pilot project examined the impact of increased nutrients and reduced pH on *Ampithoe valida* herbivory of eelgrass tissue. To do this, we exposed eelgrass to four different treatment conditions, and *A.valida* to either reduced or ambient pH conditions, for thirty days. We consecutively conducted "choice" feeding assays to determine *A. valida* feeding preferences when *A.valida* from both treatments (ambient pH and reduced pH) are given eelgrass from each eelgrass treatment at the same time. Assays ran until there was a 50% reduction in eelgrass in the containers. There were 60 choice containers with 12 *A. valida* individuas in each container and four pieces of eelgrass, one per treatment. Control containers consisted of 60 choice containers with only eelgrass pieces and no *A.valida* to account for eelgrass changes in the container beyond the presence of *A. valida*.

Github repository link: https://github.com/ayoger/thesis_pilot

Analysis in R

```r
library(tidyverse)
```

```
## Warning: package 'ggplot2' was built under R version 4.3.1
```

```
## Warning: package 'tidyr' was built under R version 4.3.1
```

```
## Warning: package 'readr' was built under R version 4.3.1
```

```
## Warning: package 'dplyr' was built under R version 4.3.1
```

```
## Warning: package 'stringr' was built under R version 4.3.1
```

```
## Warning: package 'lubridate' was built under R version 4.3.1
```

```r
library(openintro)
library(nlme)
```

```
## Warning: package 'nlme' was built under R version 4.3.1
```

```r
library(lme4)
```

```
## Warning: package 'lme4' was built under R version 4.3.1
```

```
## Warning: package 'Matrix' was built under R version 4.3.1
```

```r
library(emmeans)
```

```
## Warning: package 'emmeans' was built under R version 4.3.1
```

```r
library(ggpubr)
library(lmerTest)
```

Data Preparation & Plots in R

```r
#import datasets

choicei <- read.csv("Data/2023.10_Feeding Assays_Choice_IDs.csv")

choicedat <- read.csv("Data/2023.10_Choice_EelgrassSegmentMass.csv")

choicedat2 <- read.csv("Data/pilot_assaydata.csv")

choicedat2man <- read.csv("Data/2023.10_AV_Choice_AssaySegmentScanProcessing_manualpost.csv")

avmassdat <- read.csv("Data/2023.10.27_AV_Choice_AVmasspercontainer.csv")

#data preparation

#change names in dataset
choiceid <- choicei %>%
  pivot_longer(A:D,
               names_to = "treatment",
               values_to = "section_ID") %>%
  relocate(c(treatment, section_ID), .after = AV_control)

#mutate AV mass data to calculate actual AV mass by subtracting the boat mass
avmassdata <- avmassdat %>%
  mutate(av_drymass_g = Boat_AV_dry_mass_g - Boat_mass_g) %>%
  dplyr::select(-Boat_AV_dry_mass_g, -Boat_mass_g) %>%
  rename(unit_ID = Container_ID)

#dataframes that join the data to IDs

choicedata <- left_join(choiceid, choicedat) %>%
  left_join(choicedat2man) %>%
  left_join(avmassdata, by = "unit_ID") %>%
  mutate(AVeel_treat = paste(tankpH_treat, "_", treatment),
         pH_treat = ifelse(treatment %in% c("A", "B"), "ambient",
                           ifelse(treatment %in% c("C", "D"), "reduced", NA)),
         nut_treat = ifelse(treatment %in% c("A", "C"), "ambient",
                            ifelse(treatment %in% c("B", "D"), "increased", NA)),
         plant_ID = str_extract(section_ID, '[0-9]+.[0-9]+'),
         section_age = str_remove(section_ID, "^([^.]+.){2}"),
         area_change_adj = area_change/av_drymass_g) %>%
  relocate(c(AVeel_treat, pH_treat, nut_treat), .after = treatment) %>%
  relocate(c(AV_no, mass_mg, area_change, area_change_adj, plant_ID, section_age), .before = Notes)

## Joining with `by = join_by(treatment, section_ID, Notes)`
## Joining with `by = join_by(section_ID)`
```

Plots

```
# **Plots for Eelgrass Area**

#bulk area loss for AV *not control, not adjusted*
boxplota1 <- ggplot(data = choicedata[choicedata$AV_control == "AV",],
                   aes(x = treatment,
                       y = area_change,
                       color = tankpH_treat)) +
  #introdataviz::geom_split_violin(alpha = .5, trim = FALSE) +
  geom_hline(yintercept = 0,
            color = "black",
            size = 0.8) +
  geom_boxplot(position = position_dodge(0.8),
              size = 0.5) +
  geom_point(position = position_dodge(0.8),
            size = 1)  +
  # geom_text(aes(label = area_change),
  #          position = position_dodge(0.8),
  #          vjust = -0.5,
  #          size = 3) + # Adjust position and size of text
  scale_color_manual(name = expression(paste(italic("A. valida") ~ "pH")),
                    breaks = c("ambient", "reduced"),
                    values = c("cyan4", "red4")) +
  scale_x_discrete(breaks = c("A", "B", "C", "D"),
                 labels = c("ambient pH\nambient nut.", "ambient pH\nincreased nut.",
                            "reduced pH\nambient nut.", "reduced pH\nincreased nut.")) +
  labs(x = "Eelgrass treatment",
      y = expression(paste("Change in Segment Area ("*cm^2*")")),
      title = "A. Valida Choice Assays: Eelgrass Bulk Area Loss by Treatments") +
  theme_bw(base_size = 14) +
  theme(axis.text = element_text(color = "black", size = 12))+
  theme(plot.title = element_text(size = 13))
```

```
## Warning: Using 'size' aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use 'linewidth' instead.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.
```
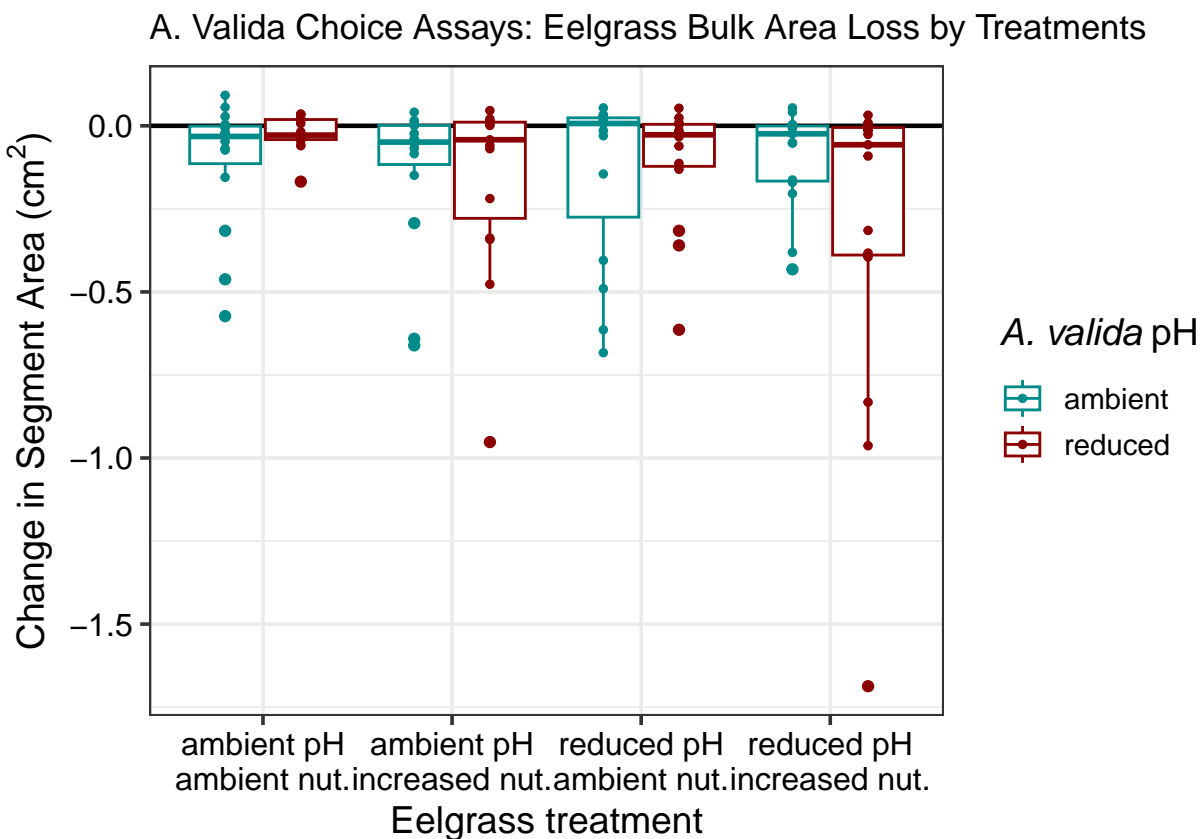
```
boxplota1
```

A. Valida Choice Assays: Eelgrass Bulk Area Loss by Treatments

Figure 1: This figure shows the bulk change in segment area (cm^2) of eelgrass segement area (x) by eelgrass nutrient treatment (y) and *A. valida* treatment (legend). The box plot shows a slight preference of *A.valida* to feed on eelgrass exposed to reduced pH and increased nutrients, when *A. valida* has also been exposed to reduced pH.

Statistical Analysis

```
#bulk area loss analysis

glmm2 <- lmer((area_change) ~ (pH_treat * nut_treat * tankpH_treat) +
              (1 | tank_ID:unit_ID) + (1 | plant_ID),
         data = choicedata[choicedata$AV_control == "AV",])
glmm2
```

```
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: (area_change) ~ (pH_treat * nut_treat * tankpH_treat) + (1 |
##     tank_ID:unit_ID) + (1 | plant_ID)
##    Data: choicedata[choicedata$AV_control == "AV", ]
## REML criterion at convergence: 30.4803
## Random effects:
##  Groups          Name        Std.Dev.
##  tank_ID:unit_ID (Intercept) 0.07206
##  plant_ID        (Intercept) 0.10636
##  Residual                    0.22697
```

```
## Number of obs: 120, groups:  tank_ID:unit_ID, 30; plant_ID, 24
## Fixed Effects:
##                                               (Intercept)
##                                                  -0.10425
##                                            pH_treatreduced
##                                                  -0.03782
##                                          nut_treatincreased
##                                                  -0.04193
##                                          tankpH_treatreduced
##                                                   0.07962
##                      pH_treatreduced:nut_treatincreased
##                                                   0.08195
##                      pH_treatreduced:tankpH_treatreduced
##                                                  -0.04103
##                    nut_treatincreased:tankpH_treatreduced
##                                                  -0.08669
## pH_treatreduced:nut_treatincreased:tankpH_treatreduced
##                                                  -0.15250
```

```
#use the shapiro test to see normality of data
shapiro.test(resid(glmm2)) #ns = good
```

```
##
##  Shapiro-Wilk normality test
##
## data:  resid(glmm2)
## W = 0.87359, p-value = 1.086e-08
```

```
#use the Kenward-Roger test to find degrees of freedom

summary(glmm2, ddf = "Kenward-Roger")
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: (area_change) ~ (pH_treat * nut_treat * tankpH_treat) + (1 |
##     tank_ID:unit_ID) + (1 | plant_ID)
##     Data: choicedata[choicedata$AV_control == "AV", ]
##
## REML criterion at convergence: 30.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -4.6654 -0.2039  0.2052  0.5152  2.0632
##
## Random effects:
##  Groups          Name        Variance Std.Dev.
##  tank_ID:unit_ID (Intercept) 0.005192 0.07206
##  plant_ID        (Intercept) 0.011312 0.10636
##  Residual                    0.051518 0.22697
## Number of obs: 120, groups:  tank_ID:unit_ID, 30; plant_ID, 24
##
## Fixed effects:
##                                                Estimate Std. Error
```

```
## (Intercept)                                                -0.10425   0.07694
## pH_treatreduced                                            -0.03782   0.10533
## nut_treatincreased                                         -0.04193   0.10558
## tankpH_treatreduced                                         0.07962   0.09077
## pH_treatreduced:nut_treatincreased                          0.08195   0.14953
## pH_treatreduced:tankpH_treatreduced                        -0.04103   0.12347
## nut_treatincreased:tankpH_treatreduced                     -0.08669   0.12200
## pH_treatreduced:nut_treatincreased:tankpH_treatreduced     -0.15250   0.17342
##                                                                 df t value
## (Intercept)                                                40.29616  -1.355
## pH_treatreduced                                            35.05382  -0.359
## nut_treatincreased                                         35.04796  -0.397
## tankpH_treatreduced                                       102.34402   0.877
## pH_treatreduced:nut_treatincreased                         34.75253   0.548
## pH_treatreduced:tankpH_treatreduced                        79.71632  -0.332
## nut_treatincreased:tankpH_treatreduced                     76.68212  -0.711
## pH_treatreduced:nut_treatincreased:tankpH_treatreduced     78.58825  -0.879
##                                                            Pr(>|t|)
## (Intercept)                                                   0.183
## pH_treatreduced                                               0.722
## nut_treatincreased                                            0.694
## tankpH_treatreduced                                           0.382
## pH_treatreduced:nut_treatincreased                            0.587
## pH_treatreduced:tankpH_treatreduced                           0.741
## nut_treatincreased:tankpH_treatreduced                        0.480
## pH_treatreduced:nut_treatincreased:tankpH_treatreduced        0.382
##
## Correlation of Fixed Effects:
##             (Intr) pH_trt nt_trt tnkpH_ pH_t:_ pH_:H_ nt_:H_
## pH_tretrdcd -0.687
## nt_trtncrsd -0.686  0.501
## tnkpH_trtrd -0.595  0.398  0.397
## pH_trtrdc:_  0.485 -0.705 -0.706 -0.281
## pH_trtrd:H_  0.401 -0.584 -0.292 -0.674  0.412
## nt_trtnc:H_  0.405 -0.296 -0.587 -0.680  0.415  0.500
## pH_trt:_:H_ -0.285  0.416  0.413  0.479 -0.587 -0.711 -0.705
```

```r
#ANOVA for hypothesis testing using the Kenward-roger method
anova(glmm2, type = 3, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                               Sum Sq   Mean Sq NumDF  DenDF F value  Pr(>F)
## pH_treat                    0.043616 0.043616     1 17.730  0.8466 0.36986
## nut_treat                   0.096228 0.096228     1 17.730  1.8679 0.18880
## tankpH_treat                0.010017 0.010017     1 26.825  0.1944 0.66278
## pH_treat:nut_treat          0.000115 0.000115     1 17.728  0.0022 0.96286
## pH_treat:tankpH_treat       0.094284 0.094284     1 78.448  1.8301 0.18000
## nut_treat:tankpH_treat      0.182230 0.182230     1 77.766  3.5372 0.06375
## pH_treat:nut_treat:tankpH_treat 0.039843 0.039843     1 78.588  0.7734 0.38186
##
## pH_treat
## nut_treat
## tankpH_treat
## pH_treat:nut_treat
```

```
## pH_treat:tankpH_treat
## nut_treat:tankpH_treat            .
## pH_treat:nut_treat:tankpH_treat
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Results

A Generalized Linear Mixed Model was used to include both fixed and random effects in the experiment. A Shapiro-wilks normality test showed that the data was not normal (p-value= 1.086e-08.) The "Kenward Roger's F test" method was used to calculate the degrees of freedom in the statistical analysis. I performed an ANOVA test with Kenward Roger's method and there were no significant interactions between treatments. There was one interaction that was had a slightly significant effect on *A.valida* consumption and that was between eelgrass nutrient treatment (ambient or reduced) and *A.valida* pH treatment (ambient or reduced), p-value= 0.06.

Discussion

This pilot experiment warrants future experimentation to determine *A.valida* feeding preferences under future stressor conditions. The interaction between eelgrass nutrient treatment (ambient or reduced) and *A.valida* pH treatment (ambient or reduced) was notable (p-value=0.06). We also saw a slightly elevated feeding preference when *A.valida* was exposed to reduced pH conditions, and eelgrass was exposed to reduced pH and increased nutrients. Moving forward, I would like to examine this further in my own thesis experiment to offer more insight into these questions. I also would like to incorporate size class as a variable for *A.valida* in my future study, as we saw only larger individuals consuming eelgrass tissue. This experiment will be pertinent to successful conservation and restoration of eelgrass meadows in San Francisco Bay.