ACR regeneration: Coral growth rate after filtering for worm damage

Ninah Munk

2024-05-17

GROWTH

Read in growth rate data and worm information. Combine data frames.

```
read.csv("/Users/ninahmunk/Desktop/Projects/Acropora_Regeneration-main/Growth/Output/growthrates.csv")
read.csv("/Users/ninahmunk/Desktop/Projects/Acropora_Regeneration-main/Wound_Closure/Data/worm_damage.c

growth%>%left_join(worm%>%select(coral_id, affected, percent_affected), by = "coral_id") -> growthdata
```

Turn variables into factors

```
growthdata%>%mutate(genotype = as.factor (genotype),
                    wound = as.factor(wound),
                    temp = as.factor(temp))-> growthdata
```

FULL MODEL

Here I am incorporating worm damage 'percent_affected' into my full model for growth and it turns out to be highly significant p = 0.0005575. The three-way interaction is highly non-significant so I removed it. The interaction of temperature and worm damage was also highly non-significant, so I remove that and keep only the interaction of my main effects (temp and wound) and the interaction of wound and percent affected. In the reduced model worm damage is still highly significant (p = 0.0001522) and the interactions are moderately non-significant (p> 0.5) so this is the final model. The interaction of wound and temp is almost significant, p = 0.0567.

```
#full model
full<- lmer(growthrate ~ wound*temp*percent_affected + (1|genotype), data = growthdata)
anova(full)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                          Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## wound                   0.49079 0.24539     2 83.818  1.3848 0.2560362
## temp                    0.26433 0.26433     1 84.352  1.4917 0.2253570
## percent_affected        2.80248 2.80248     1 24.034 15.8151 0.0005575 ***
## wound:temp              0.76955 0.38477     2 83.660  2.1714 0.1204045
## wound:percent_affected  0.44918 0.22459     2 85.798  1.2674 0.2867764
## temp:percent_affected   0.00957 0.00957     1 86.099  0.0540 0.8168262
```

```
## wound:temp:percent_affected 0.06253 0.03127     2 85.159  0.1764 0.8385559
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#reduced model
reduced<- lmer(growthrate ~ wound*temp+wound*percent_affected + (1|genotype), data = growthdata)
anova(reduced)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                       Sum Sq Mean Sq NumDF  DenDF F value     Pr(>F)
## wound                 0.5108  0.2554     2 86.933  1.4820 0.2328702
## temp                  0.3450  0.3450     1 86.031  2.0017 0.1607347
## percent_affected      3.4977  3.4977     1 23.550 20.2939 0.0001522 ***
## wound:temp            1.0223  0.5111     2 86.679  2.9656 0.0567800 .
## wound:percent_affected 0.4757 0.2378     2 88.185  1.3799 0.2569831
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

FILTERING DATA TO REMOVE WORM EFFECT

Here I filter the data to use only the corals that had low worm damage. I systematically filtered the data
by lowering the threshold for worm damage until it was not significant in the full model. This threshold is
$\leq 10\%$ worm damage. The remaining sample size is N = 71

```
growthdata.filtered = subset(growthdata, percent_affected <= 10)
```

FULL MODEL WITH FILTERED DATA

Here I am running the models again with filtered data. The three way interaction in the full model has a
p-value of 0.37 which is moderately unsignificant? I think I should use the full model because the three way
interaction is not highly non-significant aka p<0.5. In this model the interaction of wound and temperature
is significant, p = 0.03540. (do not interpret temp*)

```
#full model, percent affected is no longer significant
full.filtered<- lmer(growthrate ~ wound*temp*percent_affected + (1|genotype), data = growthdata.filtere
anova(full.filtered)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                          Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
## wound                    0.50094 0.25047     2 56.580  1.5579 0.21947
## temp                     0.67264 0.67264     1 58.461  4.1839 0.04532 *
## percent_affected         0.27883 0.27883     1 56.686  1.7344 0.19315
## wound:temp               1.13945 0.56973     2 57.266  3.5438 0.03540 *
## wound:percent_affected   0.52020 0.26010     2 57.815  1.6178 0.20717
## temp:percent_affected    0.63420 0.63420     1 58.997  3.9448 0.05167 .
## wound:temp:percent_affected 0.32159 0.16080  2 58.527  1.0002 0.37402
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
reduced.filtered<- lmer(growthrate ~ wound*temp + temp*percent_affected + (1|genotype), data = growthda
anova(reduced.filtered)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                       Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
## wound                0.17998 0.08999     2 60.077  0.5468 0.58164
## temp                 0.55851 0.55851     1 62.426  3.3938 0.07019 .
## percent_affected     0.11419 0.11419     1 58.586  0.6939 0.40823
## wound:temp           0.81856 0.40928     2 60.396  2.4870 0.09164 .
## temp:percent_affected 0.46781 0.46781     1 63.000  2.8426 0.09674 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

MAKE PAIRWISE COMPARISONS

```
emmeans <- emmeans(full.filtered, ~ wound*temp*percent_affected)
# Display the EMMs
print(emmeans)
```

```
##  wound temp percent_affected emmean   SE    df lower.CL upper.CL
## 0     A                2.89   1.75 0.129 37.9     1.49     2.01
## 1     A                2.89   1.44 0.123 37.8     1.19     1.69
## 2     A                2.89   1.77 0.130 37.6     1.51     2.04
## 0     H                2.89   1.48 0.120 32.4     1.24     1.73
## 1     H                2.89   1.66 0.129 36.6     1.40     1.92
## 2     H                2.89   1.56 0.125 35.4     1.31     1.81
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
```

```
# Pairwise comparisons for the levels within each predictor
pairwise_results <- pairs(emmeans)
# Display the pairwise comparisons
print(pairwise_results)
```

```
##  contrast
##  wound0 A percent_affected2.88732394366197 - wound1 A percent_affected2.88732394366197
##  wound0 A percent_affected2.88732394366197 - wound2 A percent_affected2.88732394366197
##  wound0 A percent_affected2.88732394366197 - wound0 H percent_affected2.88732394366197
##  wound0 A percent_affected2.88732394366197 - wound1 H percent_affected2.88732394366197
##  wound0 A percent_affected2.88732394366197 - wound2 H percent_affected2.88732394366197
##  wound1 A percent_affected2.88732394366197 - wound2 A percent_affected2.88732394366197
##  wound1 A percent_affected2.88732394366197 - wound0 H percent_affected2.88732394366197
##  wound1 A percent_affected2.88732394366197 - wound1 H percent_affected2.88732394366197
##  wound1 A percent_affected2.88732394366197 - wound2 H percent_affected2.88732394366197
##  wound2 A percent_affected2.88732394366197 - wound0 H percent_affected2.88732394366197
##  wound2 A percent_affected2.88732394366197 - wound1 H percent_affected2.88732394366197
##  wound2 A percent_affected2.88732394366197 - wound2 H percent_affected2.88732394366197
##  wound0 H percent_affected2.88732394366197 - wound1 H percent_affected2.88732394366197
```

```
##  wound0 H percent_affected2.88732394366197 - wound2 H percent_affected2.88732394366197
##  wound1 H percent_affected2.88732394366197 - wound2 H percent_affected2.88732394366197
##  estimate    SE   df t.ratio p.value
##    0.3131 0.169 56.0   1.848  0.4442
##   -0.0218 0.175 56.4  -0.125  1.0000
##    0.2706 0.167 55.5   1.622  0.5876
##    0.0909 0.173 55.5   0.525  0.9949
##    0.1910 0.170 55.1   1.125  0.8690
##   -0.3350 0.170 56.5  -1.971  0.3713
##   -0.0425 0.162 55.8  -0.263  0.9998
##   -0.2223 0.168 55.9  -1.319  0.7732
##   -0.1221 0.166 56.3  -0.735  0.9767
##    0.2924 0.167 55.7   1.751  0.5047
##    0.1127 0.173 55.7   0.652  0.9863
##    0.2129 0.171 56.3   1.243  0.8139
##   -0.1797 0.166 55.2  -1.085  0.8854
##   -0.0796 0.163 55.7  -0.487  0.9965
##    0.1002 0.170 55.9   0.589  0.9913
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 6 estimates
```

VISUALIZE GROWTH BASED ON RESULTS OF FULL MODEL WITH FILTERED DATA

In the second figure there is a growth reduction at warmer temp for control corals. Similar pattern for abrased corals. Overall growth lowered for fragmented corals but in an opposite pattern with respect to temperature.

```
ggplot(data = growthdata.filtered, aes(x = wound, growthrate))+ geom_boxplot()+facet_wrap(~temp)
```
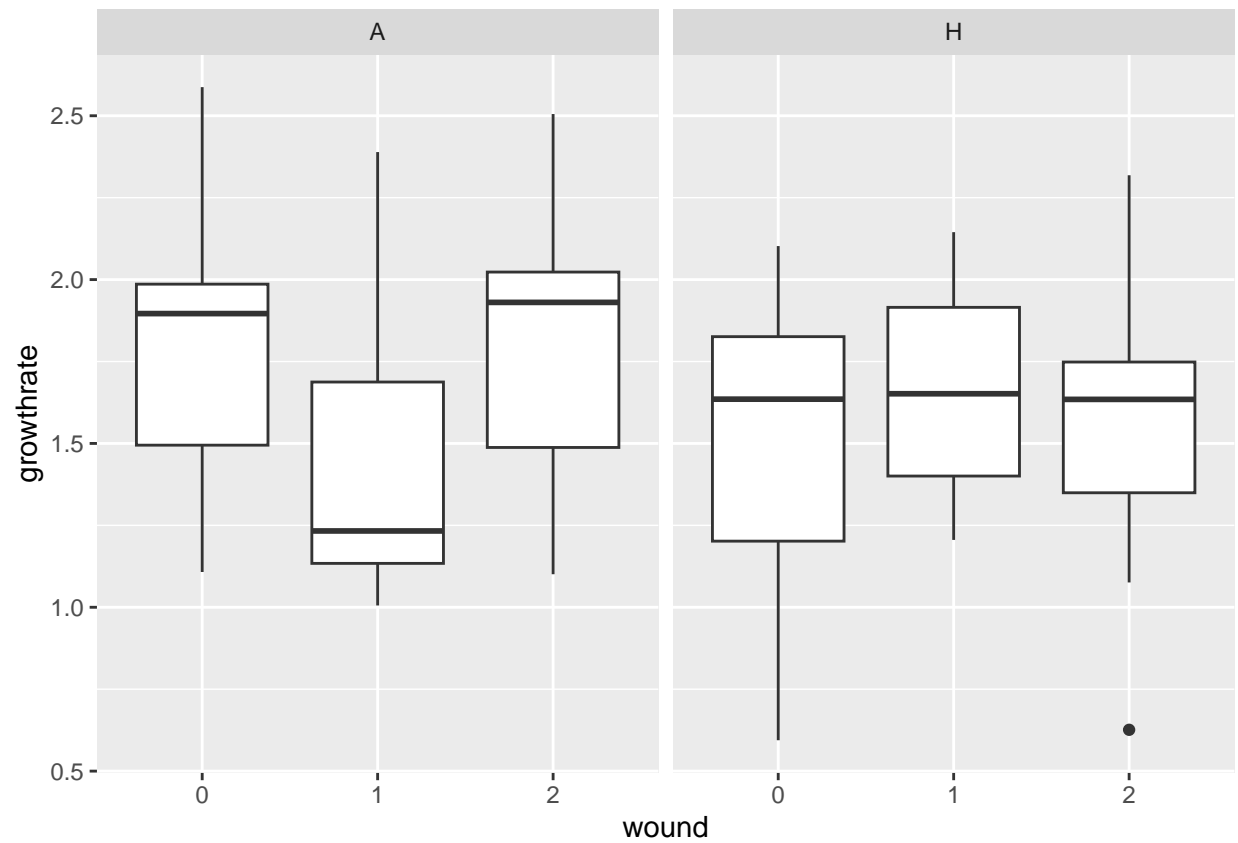
```
ggplot(data = growthdata.filtered, aes(x = temp, growthrate))+ geom_boxplot()+facet_wrap(~wound)
```
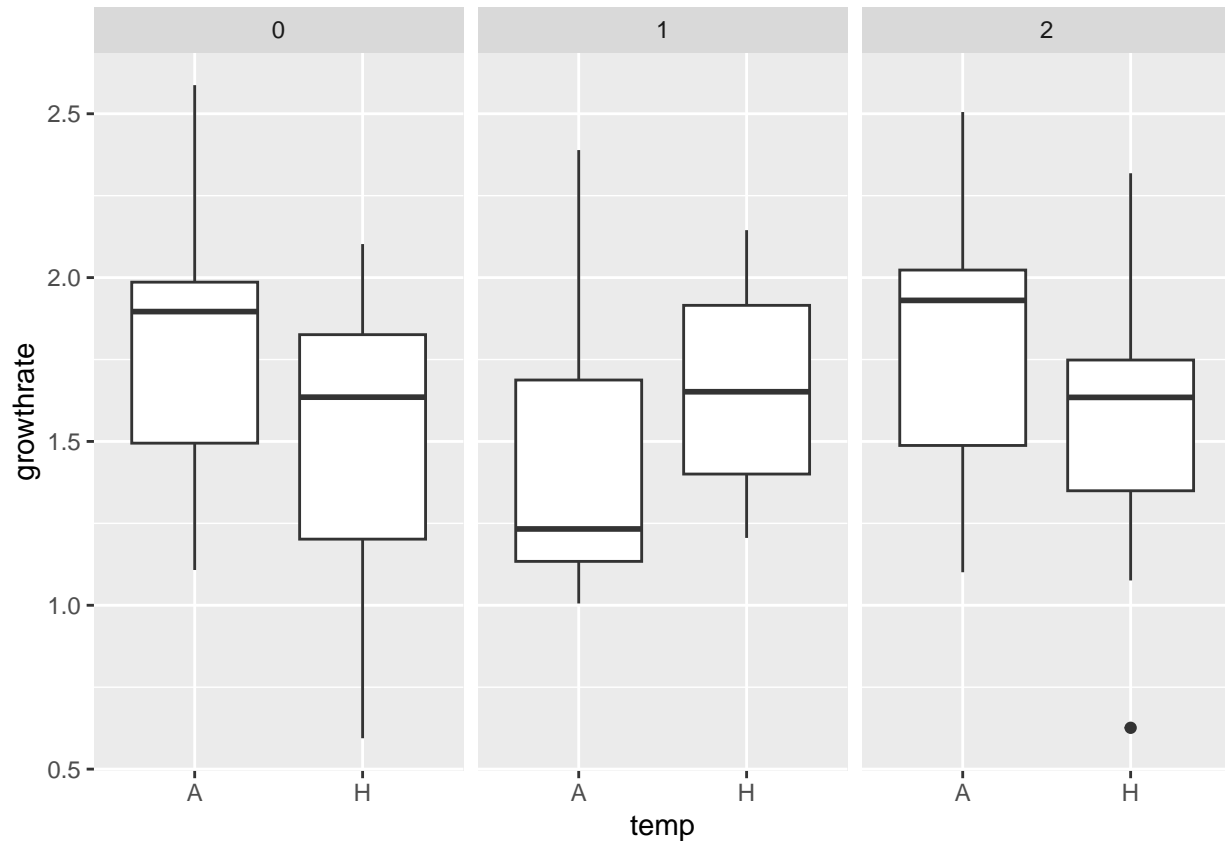
Run models within each wound treatment. There is a significant difference of growth rate between ambient and elevated control corals, where growth is reduced for unwounded corals at warmer temperature.

```
anova(control<- lmer(growthrate ~ temp*percent_affected + (1|genotype), data = growthdata.filtered%>%fi
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                       Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
## temp                 0.98513 0.98513     1 20.363  6.7447 0.01708 *
## percent_affected     0.02362 0.02362     1 20.716  0.1617 0.69168
## temp:percent_affected 0.43247 0.43247     1 19.744  2.9609 0.10093
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(fragment<- lmer(growthrate ~ temp*percent_affected + (1|genotype), data = growthdata.filtered%>%fi
```

```
## boundary (singular) fit: see help('isSingular')
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                        Sum Sq  Mean Sq NumDF DenDF F value Pr(>F)
## temp                 0.180706 0.180706     1    19  1.1178 0.3036
## percent_affected     0.052619 0.052619     1    19  0.3255 0.5750
## temp:percent_affected 0.000619 0.000619     1    19  0.0038 0.9513
```

```
anova(abrasion<- lmer(growthrate ~ temp*percent_affected + (1|genotype), data = growthdata.filtered%>%f
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                       Sum Sq Mean Sq NumDF  DenDF F value Pr(>F)
## temp                 0.51465 0.51465     1 18.585  2.9495 0.1025
## percent_affected     0.51496 0.51496     1 18.834  2.9513 0.1022
## temp:percent_affected 0.24972 0.24972    1 18.198  1.4312 0.2469
```

BREAK DOWN OF SAMPLE SIZE WITHIN TREATMENTS

First I make a histogram to look at how genotype (i.e., parental colony) is represented by worm damage. The most heavily affected corals were from parental colonies: 68, 69, and 70. Genotype was originally represented by an n of 3 within each treatment (genotypeXwoundXtemp, n = 3), but now there are several cases n = 1. Next I check the sample sizes of the woundxtemperature treatments disregarding genotype. These sample sizes range between n = 11-13.

```
ggplot(data = worm, aes(x = percent_affected))+geom_histogram()+facet_wrap(~genotype)
```

```
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
```
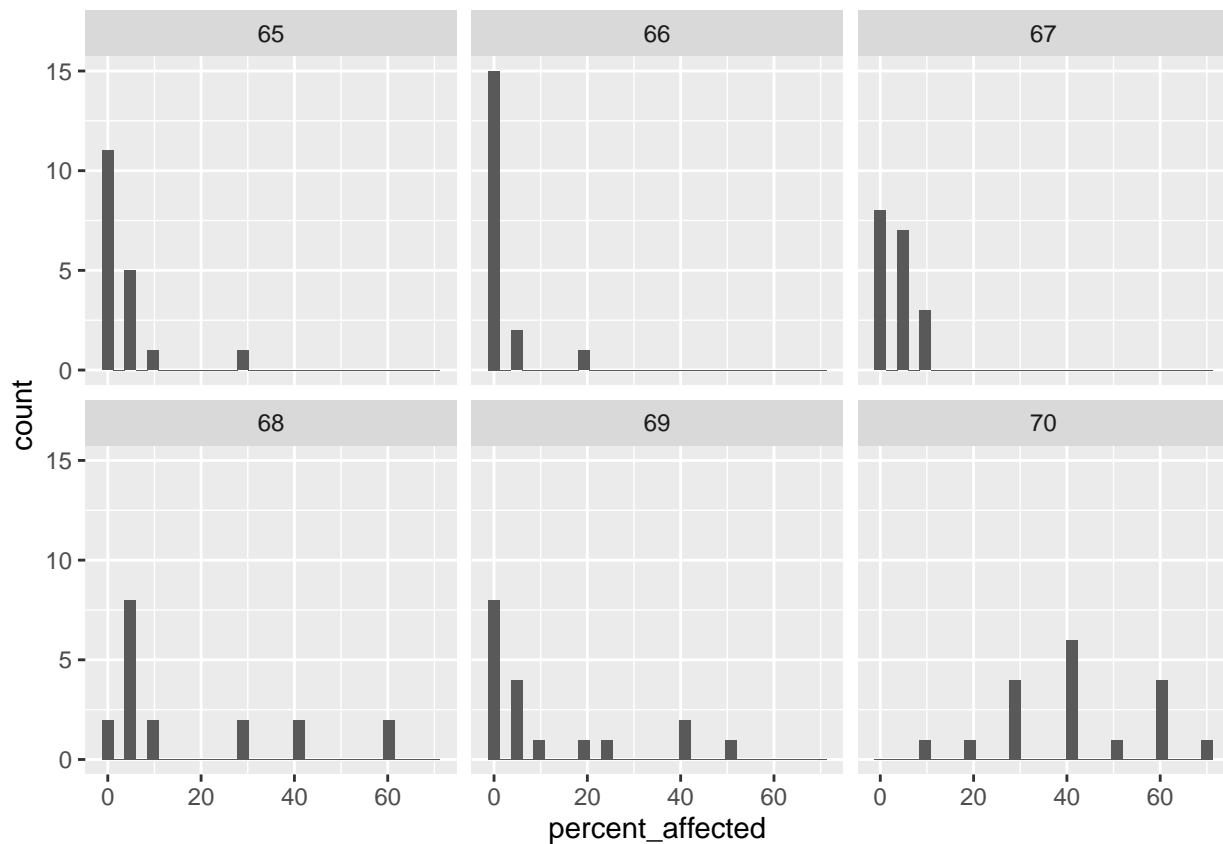


```
summarySE(data = growthdata.filtered, measurevar = "growthrate", groupvars = c("wound", "temp", "genotyp
          na.rm = FALSE, conf.interval = 0.95, .drop = TRUE)
```

```
## Warning in qt(conf.interval/2 + 0.5, datac$N - 1): NaNs produced
```

```
##     wound temp genotype N growthrate         sd         se        ci
## 1       0    A       65 3   1.933019 0.66994425 0.38679249 1.6642338
## 2       0    A       66 3   1.633389 0.45590318 0.26321583 1.1325263
## 3       0    A       67 3   2.027750 0.07105983 0.04102641 0.1765224
## 4       0    A       68 1   1.278185         NA         NA       NaN
## 5       0    A       69 2   1.693037 0.17843160 0.12617019 1.6031443
## 6       0    H       65 3   1.589039 0.35833495 0.20688478 0.8901534
## 7       0    H       66 3   1.010688 0.36859124 0.21280625 0.9156314
## 8       0    H       67 3   1.762085 0.20531976 0.11854142 0.5100426
## 9       0    H       68 2   1.964122 0.19546838 0.13821702 1.7562138
## 10      0    H       69 2   1.313504 0.28941806 0.20464947 2.6003181
## 11      1    A       65 2   1.244077 0.19746600 0.13962955 1.7741616
## 12      1    A       66 3   1.528598 0.32779383 0.18925186 0.8142850
## 13      1    A       67 3   1.414264 0.56066591 0.32370061 1.3927713
## 14      1    A       68 1   1.005428         NA         NA       NaN
## 15      1    A       69 2   1.786273 0.85263190 0.60290180 7.6605937
## 16      1    A       70 1   1.282511         NA         NA       NaN
## 17      1    H       65 3   2.054118 0.08271551 0.04775582 0.2054767
## 18      1    H       66 2   1.487629 0.19596958 0.13857142 1.7607168
## 19      1    H       67 3   1.568359 0.32913047 0.19002357 0.8176054
## 20      1    H       68 1   1.346677         NA         NA       NaN
## 21      1    H       69 2   1.554314 0.14477553 0.10237176 1.3007565
## 22      2    A       65 3   2.003738 0.05813434 0.03356388 0.1444137
## 23      2    A       66 2   2.417805 0.12364478 0.08743006 1.1109043
## 24      2    A       67 3   1.300928 0.17350644 0.10017399 0.4310139
## 25      2    A       68 2   1.818989 0.15758066 0.11142636 1.4158061
## 26      2    A       69 1   1.566465         NA         NA       NaN
## 27      2    H       65 2   1.871646 0.18853223 0.13331242 1.6938949
## 28      2    H       66 2   0.850943 0.31798929 0.22485238 2.8570204
## 29      2    H       67 3   1.540121 0.35574008 0.20538663 0.8837074
## 30      2    H       68 2   1.870354 0.63372802 0.44811338 5.6938203
## 31      2    H       69 3   1.609048 0.04434628 0.02560334 0.1101623
```

```
print(growthdata.filtered %>% group_by(wound, temp) %>% reframe(samp.size=n()))
```

```
## # A tibble: 6 x 3
##   wound temp  samp.size
##   <fct> <fct>     <int>
## 1 0     A            12
## 2 0     H            13
## 3 1     A            12
## 4 1     H            11
## 5 2     A            11
## 6 2     H            12
```

HOW MANY OF THE REMAINING CORALS DO I ALSO HAVE REGENERATION DATA FOR?

Out of the 71 corals I have growth data on, I have regeneration data for 14 of those. Within each time-point/wound/temp treatment there is a sample size of 3-4. I will have to check how worm damage acts as a

covaiate in the regeneration data.

```
read.csv("/Users/ninahmunk/Desktop/Projects/Acropora_Regeneration-main/Wound_Closure/Data/all.wounds.csv

growthdata.filtered%>%left_join(regen, by = "coral_id")%>%drop_na() -> growthregen

print(growthregen %>% group_by(coral_id) %>% reframe(samp.size=n()))
```

```
## # A tibble: 14 x 2
##     coral_id samp.size
##        <int>     <int>
## 1         10         4
## 2         13         4
## 3         38         4
## 4         39         4
## 5         48         4
## 6         65         4
## 7         66         4
## 8         72         4
## 9         79         4
## 10        80         4
## 11        82         4
## 12        88         4
## 13       102         4
## 14       105         4
```

```
print(growthregen %>% group_by(time_point, wound, temp) %>% reframe(samp.size=n()))
```

```
## # A tibble: 16 x 4
##     time_point wound temp  samp.size
##     <chr>      <fct> <fct>     <int>
## 1 24hr         1     A             3
## 2 24hr         1     H             4
## 3 24hr         2     A             3
## 4 24hr         2     H             4
## 5 day10        1     A             3
## 6 day10        1     H             4
## 7 day10        2     A             3
## 8 day10        2     H             4
## 9 final        1     A             3
## 10 final       1     H             4
## 11 final       2     A             3
## 12 final       2     H             4
## 13 initial     1     A             3
## 14 initial     1     H             4
## 15 initial     2     A             3
## 16 initial     2     H             4
```