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BSC6926 – Spring 2020

Final Project: Regression analysis and non-linear model fit of periphyton inorganic carbon content vs. water depth and TP, across regions

Introduction

The precipitation of calcium carbonate minerals (CaCO3) is a widespread process in aquatic ecosystem, key throughout our geological history by building soils, sedimentary rock (storing carbon) and by influencing global carbon fluxes. At the microscopic level, this precipitation occurs in various ways and is often caused by biological activity of cyanobacteria and other microbes in complex interactions with physical conditions and water chemistry (eg. pH, DOC). In the South FL Everglades, microbial mats (or periphyton) play important roles in the ecosystem, are highly productive and precipitate large amounts of CaCO3 on a landscape-scale, and thereby may drive carbon fluxes of the system. Conditions (eg. water chemistry, hydrology) vary over this low-nutrient, flood-pulsed wetland, so understanding how these variations influence CaCO3 precipitation is important to evaluating carbon fluxes. It may also be insightful into drivers of cyanobacterial calcification occurring in other oligotrophic, karstic systems which are comparable to ancient stromatolite-forming communities. Cyanobacterial calcification has great potential application for carbon capture and storage (CCS) technologies and is important to our understanding of carbon fluxes in freshwater and marine systems.

A regression analysis and non-linear model fitting will be performed to evaluate the influence of environmental variables on this important microbially-driven process. A hydrological variable, water depth, and water chemistry variable, total phosphorus (TP), will be evaluated against inorganic carbon content (ash weight, an approximate measure of calcium carbonate precipitation), since these variables can both influence the biological processes involved in CaCO3 precipitation. For instance, water depth may influence temperature, ion concentrations, irradiance thereby photosynthesis (increasing pH), and/or limit gas diffusion (CO2) into the water column possibly augmenting DOC equilibrium. Phosphorus availability also influences precipitation processes indirectly, for example by stimulating heterotrophic metabolism, which decreases pH encouraging CaCO3 breakdown. Both these variables, water depth and phosphorus, also shape the composition of the algal and/or microbial community itself, so changes in the calcifying species presence and activity may be influential. Another important variable, region, is spatially implicit and represents areas of different landscape features, hydrology, management regime/intensity, chemistry, and more. Many of these factors, which vary regionally, may influence carbonate precipitation processes, by augmenting the local conditions and biological interactions within the periphyton. Since this dataset is well resolved at the landscape scale, measurements are replicated per site, and periphyton samples are collected simultaneously, this gives us the opportunity to assess environmental drivers of a microbial process that sustains/drives ecosystem functioning. Furthermore, the Everglades water flow is heavily managed and large-scale modifications are ongoing to restore surface flow and improve ecosystem functioning, but the impacts of these efforts are still being studied. Evaluating how CaCO3 precipitation is influenced by these major environmental factors may help us predict consequences of hydrologic and water chemistry shifts on carbon budget and Everglades functioning. My hypothesis is that water depth and total phosphorus will both be good predictors of ash weight, and that region will have a strong relationship with water depth.

Methods & Data: Samples and environmental data collected annually for the Comprehensive Everglades Restoration Plan (CERP MAP) from approximately 130 sites across the Everglades landscape. Includes: periphyton and water samples analyzed in the lab for nutrients, biomass, community composition, & more. Environmental and water chemistry variables include water depth, total phosphorus, conductivity, pH, soil depth, soil type, and more. These measurements were replicated 3 times per site and averaged. Periphyton/microbial mat samples were analyzed in the lab for nutrients and biomass, including organic matter content (ash-free dry weight) and inorganic carbon (ash weight). Ash weight is used as a reliable estimate of calcium carbonate content/precipitation, since the inorganic portion of the periphyton is almost all calcium carbonate. Regression analyses to evaluate how hydrology and water chemistry predict inorganic carbon content (ash weight) will be performed: models will include ash weight vs. water depth (continuous), vs. TP (continuous) and water depth vs. region (categorical). Residual plots and normality tests performed to evaluate model assumptions. Log transformation is used on response variable (ash weight) to improve linear regression fit. Variance inflation factor used for collinearity measure and ANOVA to compare model fits. Bootstrap was attempted to evaluate the water depth or ash weight by region. A non-linear model, Weibull distribution function, was attempted to fit to the data.

Results

There is a strong effect of region on water depth (p<2e^-16), as the graph shows significant differences in the mean and variation between regions (Fig. 3). Scatterplots show the relationship between ash weight and both water depth (Fig. 1a) and TP (Fig. 2a) have a non-linear relationship which seems logarithmic, or in a Weibull distribution; after log transforming data, both display a more linear relationship (Figure 1&2b). Residual plots show that raw data is not normally distributed (QQ plots) and could be non-linear (Fig.4), which is supported by Shapiro tests (all p-values <0.0001; Fig. 6a). Normality of distribution was achieved in model m1 and m3 after log tranformation of the response variable, but the models still displayed some skewness (Fig. 5). Using simple linear models with raw ash weight calculated a weak linear trend with both water depth ($R^2 = 0.1535$) and TP (adj $R^2 = 0.189$). After log transforming the response variable (ash weight), both relationships were notably improved: water depth R^2 increased to 0.275, and the combined model with water depth + TP increased to adjusted R^2 of 0.532. Introducing region to the model did not improve the model fit (p<1.46e^-8) as much as TP (p<2e^-16). Combining water depth and TP against log transformed ash weight gave the best fit, with a negative linear relationship explaining more than half the variation (p-value<2e^-16, R = -0.642, Res.SE=1.684, df=604). An ANOVA test comparing models confirmed best fit was model LM2. Co-linearity between TP and water depth seemed like it could be an issue (p-value =0.00024; $R^2 = 0.029$), but these variables are influenced by some of the same factors in the field and are still functionally and dynamically independent of each other. Variation inflation factor test confirmed there is little inflation/collinearity between TP and water depth. Attempts to use a Weibull self-start function for a non-linear model were not successful. Initial values for this function included: the horizontal asymptote, drop, natural log of rate constant, and power x is raised. These values were chosen based on the scatterplot of data, but I couldn't figure out exactly how to calculate them by hand or with code or derive them from the graph (Fig. 7a). Graph of predicted values doesn't match the data, as the initial values didn't work or fit the model as they were chosen. Bootstrap non-parametric analysis was conducted for the models including ash weight vs. region, depth, and tp + depth. The t-values don't seem to make sense, as the analysis didn't generate bias or standard error values. I believe something was wrong with the response variable having NA or 0 values, but I couldn't get the bootstrap to work even after fixing this.

Discussion

In conclusion, my hypothesis that both water depth and TP were good predictors of periphyton ash weight (or inorganic carbon/calcium carbonate content), was supported by the analysis, but further investigation is needed. The influence of region is still not fully resolved, so analysis into variations between regional conditions will be prudent. Since both these variables (water depth and TP) effect the biological activity of microbial mats which cause calcification, exploring how they affect calcification on the meso- or microscopic scale is necessary to pinpoint important environmental and/or biological factors. A combination of laboratory microcosm and field mesocosm experiments on periphyton calcium carbonate precipitation would help answer questions about carbonate dynamics and cyanobacterial calcification in oligotrophic systems in general. Considering the environmental gradients of this vast wetland, analyses performed separated by region may be prudent since periphyton community, functioning and calcification may occur differently under regional conditions. The application of a Weibull distribution model may enable a better fit to the overall regression, as it is commonly used to model natural processes and seems to fit the data distribution of both influential variables (water depth and TP). Water depth can be related to many important physical conditions, like water column pressure that limit gas exchange and ionic content of the water, but also indirectly related to various factors since water depth helps shape the biological community, which in turn define the systems chemistry. Since the Everglades has been undergoing large scale dredging which increased groundwater input and modified ionic composition, evaluating the water's ionic content (against periphyton ash weight) could be informative. Other variables like conductivity, DOC, pH and various biological factors should be compared between regions or in other analyses. Experiments with periphyton using these variables, paired with landscape/observational studies may be very insightful into calcification drivers, carbon fluxes, and help resolve our understanding of processes at the landscape scale.

Figure 1: top graphs inorganic carbon/ash weight against water depth with no transformation. Bottom graph shows the same data with ash weight on log transformed scale. Regions are indicated by colored dots.

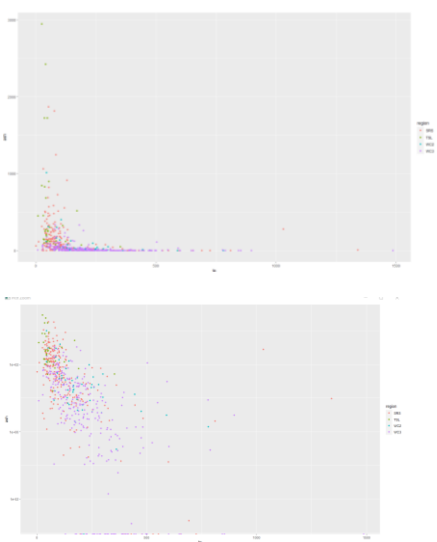
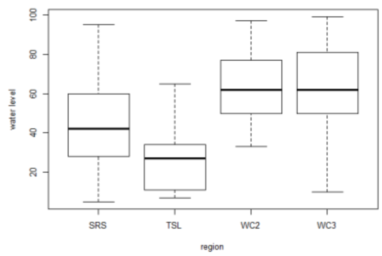


Figure 2: top graphs inorganic carbon/ash weight against total phosphorus with no transformation. Bottom graph shows the same data with ash weight on log transformed scale. Regions are indicated by colored dots.

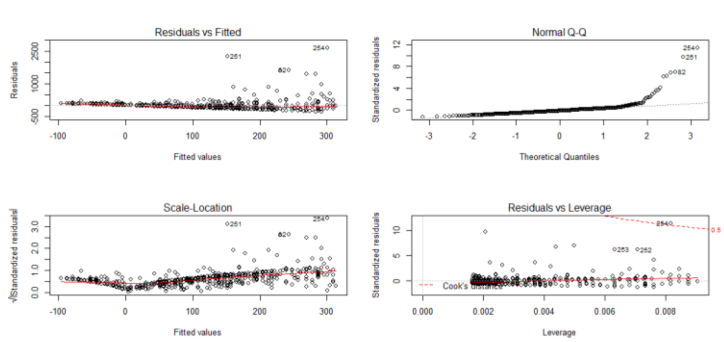


a.

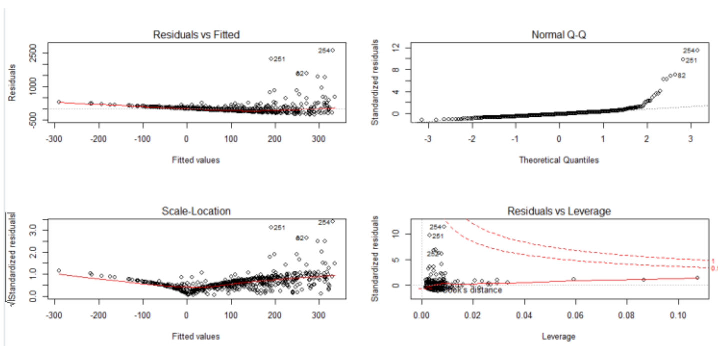
b.

```
> plot(an$region, an$depth,ylab="water level", xlab="region")
> summary(aov(an$depth~an$region))
             Df Sum Sq Mean Sq F value Pr(>F)
an$region     3  78918   26306   66.01 <2e-16 ***
Residuals   602 239890     398
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> (aov(an$depth~an$region))
Call:
   aov(formula = an$depth ~ an$region)

Terms:
                an$region Residuals
Sum of Squares   78917.58 239890.14
Deg. of Freedom         3       602

Residual standard error: 19.96218
Estimated effects may be unbalanced
> |
```

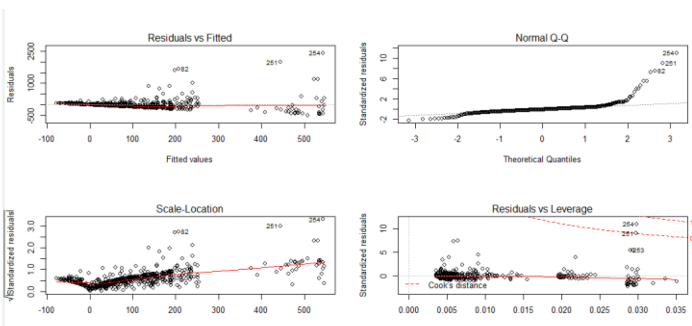
Figure 3: Water depths by region.



a.

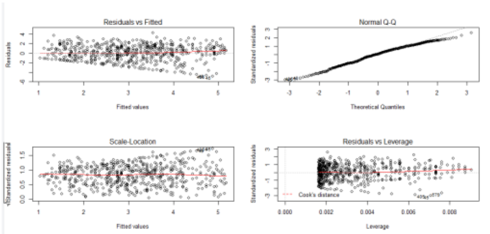


b.

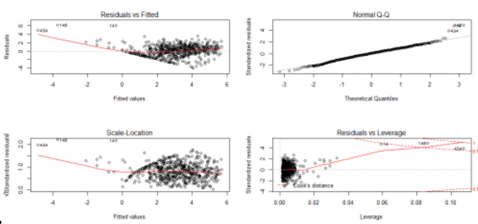


c.

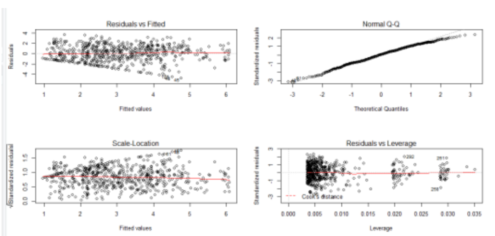
Figure 4: Residual plots for evaluating model assumptions, models being plotted are a) ash weight vs. depth, b) vs. depth + TP, c) vs. depth + region.



a.

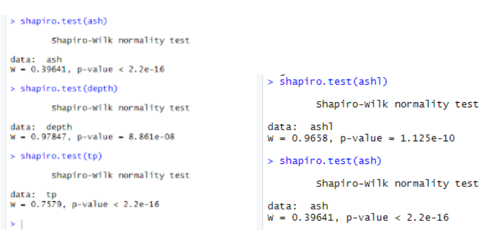


b.



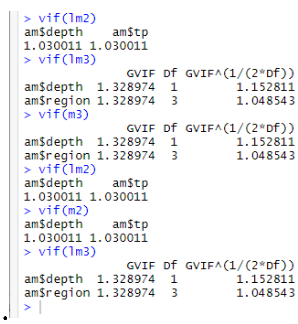
c.

Figure 5: Residual plots after ash weight was log transformed. models being plotted are a) ash weight vs. depth, b) vs. depth + TP, c) vs. depth + region.

a. 

```
> shapiro.test(ash)

        Shapiro-Wilk normality test

data:  ash
W = 0.39641, p-value < 2.2e-16

> shapiro.test(depth)

        Shapiro-Wilk normality test

data:  depth
W = 0.97847, p-value = 8.861e-08

> shapiro.test(tp)

        Shapiro-Wilk normality test

data:  tp
W = 0.7579, p-value < 2.2e-16

>
```

```
> shapiro.test(ashl)

        Shapiro-Wilk normality test

data:  ashl
W = 0.9658, p-value = 1.125e-10

> shapiro.test(ash)

        Shapiro-Wilk normality test

data:  ash
W = 0.39641, p-value < 2.2e-16
```

b. 

```
> vif(lm2)
am$depth     am$tp
1.030011 1.030011
> vif(lm3)
              GVIF Df GVIF^(1/(2*Df))
am$depth  1.328974  1        1.152811
am$region 1.328974  3        1.048543
> vif(m3)
              GVIF Df GVIF^(1/(2*Df))
am$depth  1.328974  1        1.152811
am$region 1.328974  3        1.048543
> vif(lm2)
am$depth     am$tp
1.030011 1.030011
> vif(m2)
am$depth     am$tp
1.030011 1.030011
> vif(lm3)
              GVIF Df GVIF^(1/(2*Df))
am$depth  1.328974  1        1.152811
am$region 1.328974  3        1.048543
>
```

Figure 6: a. Shapiro tests all failed to reject the null hypothesis that data was normally distributed, for all variables. Some p-values were much lower than others, which could mean the larger p-values were from datasets closer to normally distributed. B. Variance inflation factor shows there isn't high collinearity between variables.

```
Estimated effects may be unbalanced
> summary(aov(m1))
             Df   Sum Sq Mean Sq F value Pr(>F)
am$depth      1  6055840 6055840   110.7 <2e-16 ***
Residuals   604 33032527   54690
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(aov(m2))
             Df   Sum Sq Mean Sq F value   Pr(>F)
am$depth      1  6055840 6055840  115.58  < 2e-16 ***
am$tp         1  1438611 1438611   27.46 2.22e-07 ***
Residuals   603 31593916   52395
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(aov(m3))
             Df   Sum Sq Mean Sq F value   Pr(>F)
am$depth      1  6055840 6055840  122.33  < 2e-16 ***
am$region     3  3279341 1093114   22.08 1.42e-13 ***
Residuals   601 29753186   49506
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> summary(lm(depth~tp))

Call:
lm(formula = depth ~ tp)

Residuals:
    Min      1Q  Median      3Q     Max
-66.689 -16.620   0.346  17.391  44.420

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 49.235532   1.381870  35.630  < 2e-16 ***
tp           0.023743   0.005577   4.258  2.4e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 22.64 on 604 degrees of freedom
Multiple R-squared:  0.02914,	Adjusted R-squared:  0.02753
F-statistic: 18.13 on 1 and 604 DF,  p-value: 2.396e-05
```

```
> plot(ashl)
> summary(aov(lm1))
             Df Sum Sq Mean Sq F value Pr(>F)
am$depth      1  622.7   622.7   229.3 <2e-16 ***
Residuals   604 1640.1     2.7
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(aov(lm2))
             Df Sum Sq Mean Sq F value Pr(>F)
am$depth      1  622.7   622.7   355.6 <2e-16 ***
am$tp         1  584.1   584.1   333.5 <2e-16 ***
Residuals   603 1056.0     1.8
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(aov(lm3))
             Df Sum Sq Mean Sq F value Pr(>F)
am$depth      1  622.7   622.7  261.25 <2e-16 ***
am$region     3  207.6    69.2   29.03 <2e-16 ***
Residuals   601 1432.5     2.4
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> anova(m1,m2)
Analysis of Variance Table

Model 1: am$ash ~ am$depth
Model 2: am$ash ~ am$depth + am$tp
  Res.Df      RSS Df Sum of Sq      F    Pr(>F)
1    604 33032527
2    603 31593916  1   1438611 27.457 2.224e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> anova(m2,m3)
Analysis of Variance Table

Model 1: am$ash ~ am$depth + am$tp
Model 2: am$ash ~ am$depth + am$region
  Res.Df      RSS Df Sum of Sq      F    Pr(>F)
1    603 31593916
2    601 29753186  2   1840730 18.591 1.465e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

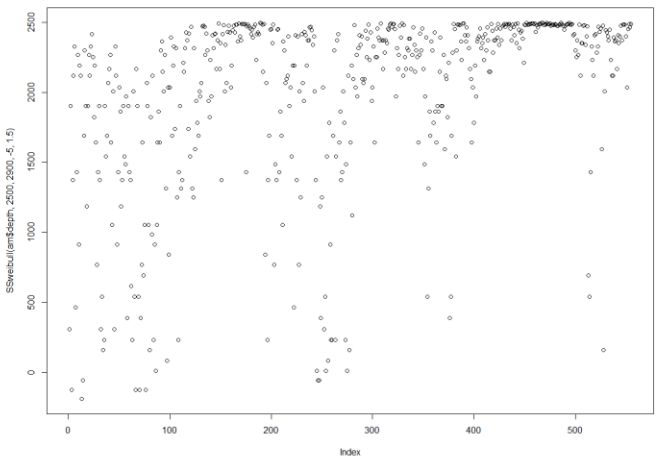
```
> anova(m1,m2)
Analysis of Variance Table

Model 1: am$ash ~ am$depth
Model 2: am$ash ~ am$depth + am$tp
  Res.Df      RSS Df Sum of Sq      F    Pr(>F)
1    604 33032527
2    603 31593916  1   1438611 27.457 2.224e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> anova(m1,m5)
Analysis of Variance Table

Model 1: am$ash ~ am$depth
Model 2: am$ash ~ am$depth + am$cond
  Res.Df      RSS Df Sum of Sq      F  Pr(>F)
1    604 33032527
2    603 32680739  1    351789 6.4909 0.01109 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

```
> cor(depth,ash)
[1] -0.3936076
> cor(depth+tp,ash)
[1] -0.3010672
> cor(tp,ash)
[1] -0.2562148
> cor(cond,ash)
[1] -0.1438681
>
> cor(depth,ashl)
[1] -0.5245826
> cor(tp,ashl)
[1] -0.5901461
> cor(depth+tp,ashl)
[1] -0.6420078
> cor(cond,ashl)
[1] -0.03920201
>
```

Figure 7: Anova tables for models (M1 ash weight vs. depth, M2 ash weight vs. depth + TP, M3 ash weight vs. depth +region; LM1 log(ash weight + 1) vs. depth, LM2 log(ash weight + 1) vs. depth + TP, LM3 log(ash weight + 1) vs. depth +region.

a.

```
+ clm = lm(am$ash~am$depth+am$tp, data = am)
+ clms = summary(clm)$r.square
+ rel  = c(dlms,plms,clms)
+ return(rel)
+ }
> library(boot)
>
> results = boot(data = am, statistic = ash_estimate, R = 1000)
> print(results)

ORDINARY NONPARAMETRIC BOOTSTRAP


Call:
boot(data = am, statistic = ash_estimate, R = 1000)


Bootstrap Statistics :
     original  bias    std. error
t1* 0.2482070       0           0
t2* 0.1902874       0           0
t3* 0.1911643       0           0
> plot(results, index = 1)
[1] "All values of t* are equal to  0.248206970908555"
>
>
> confidence_interval_H = boot.ci(results, index = 1, conf = 0.95)
[1] "All values of t are equal to  0.248206970908555 \n Cannot calculate confidence intervals"
> print(confidence_interval_H)
NULL
>
```



b.

```
> getInitial(am$depth ~ SSweibull(am$depth, Asym, Drop, lrc, pwr), data = am)
Error in nls(y ~ cbind(1, -exp(-exp(lrc) * x^pwr)), data = xy, algorithm = "plinear",
:
  step factor 0.000488281 reduced below 'minFactor' of 0.000976562
> y=am$ash
> summary(aov(am$depth~am$region))
             Df Sum Sq Mean Sq F value Pr(>F)


> ### nls models
> ## Initial values are in fact the converged values
> fm1 <- nls(y ~ SSweibull(am$depth, 3000, 2900, -5, 1.5), data = am)
Error in lm.fit(x, y, offset = offset, singular.ok = singular.ok, ...) :
  0 (non-NA) cases
In addition: Warning message:
In log((Rasym - y)/(Rasym - Lasym)) : NaNs produced
> summary(fm1)
Error in summary(fm1) : object 'fm1' not found
```

Figure 8: a. Bootstrap results for three models, ash weight vs. region; depth; and tp + depth. The t-values don't seem to make sense, as the analysis didn't generate bias or standard error values. b. Non-linear model with a Weibull distribution function. Graph of predicted values doesn't match the data, as the initial values didn't work or fit the model as they were chosen.

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