

*The effects of abiotic and biotic factors on periphyton and fish  
communities in Everglades National Park, Florida, USA*

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# Introduction

## Background

### Scientific Questions

We investigated three major biological questions: (1) What biological impact does water temperature and dissolved oxygen have on fish biomass and fish diversity? (2) How does plant cover and phosphorus impact periphyton cover? And (3) How does periphyton coverage impact fish biomass/composition of fish functional groups?

### Hypotheses and Predictions

#### *Hypothesis 1: Temperature and DO effects on fish*

To answer our first question, we put forth the hypothesis that water temperature and dissolved oxygen impacts fish biomass and diversity. Elevated temperatures and lower dissolved oxygen (DO) will decrease the carrying capacity of an aquatic environment to support a larger fish community. Further, under these conditions, more sensitive species would no longer be able to inhabit the area, and only hypoxia-tolerant species would remain (Farwell et al., 2006). Thus, we predicted that as temperature increases, and DO decreases, fish biomass and diversity will also decrease. Therefore, key variables of interest include temperature and DO, however other variables may affect fish biomass and species diversity. Chlorophyll- $\alpha$  and water depth are expected to influence fish communities, these variables change the nutrient level and spatial variation of aquatic habitats (Gelwick et al., 1997). Characteristics of water quality such as ammonia (NH<sub>4</sub>) concentrations are expected to influence fish biomass and species richness since with increasing NH<sub>4</sub> levels, comes an increased likelihood of NH<sub>4</sub> toxicity (Eddy, 2005). Salinity is an important environmental factor which can dictate the species that can inhabit the area, as well as the growth rate of individual fish. Salinity can impact fish growth rate, depending on the amount of energy required for osmoregulation, as well as constrain the species that can inhabit the area (Lisboa et al., 2015). Turbidity can similarly impact fish growth rate, but this is due to a decrease in feeding rate and feeding success (Rowe and Dean, 1998). Finally, water depth can determine spatial dynamics of an aquatic ecosystem and will likely impact both species richness and overall fish biomass (Menezes et al., 2013).

#### *Hypothesis 2: Plant coverage, TP, and DO impact on periphyton*

Periphyton is a critical food resource for many fish species, particularly herbivorous and omnivorous species. It is defined by the US Environmental Protection Agency as a complex assemblage of algae, cyanobacteria, microinvertebrates, their related secretions, and detritus attached to submerged surfaces (Brown and Wright, 2009). Periphyton coverage has been found

to be linked to nutrient loading (particularly total phosphorus (TP)) and other water quality parameters, such as DO (Kanavillil and Kurissery, 2013). Periphyton also requires a hard substrate to attach to and grow, such as rocks, logs, and aquatic plants. We predict that as plant coverage/density increases, periphyton coverage should also increase as it provides an increased substrate area for periphyton to attach to. With regards to water quality parameters, we predict that as TP and soluble reactive phosphorus (SRP) increases, and as DO decreases, periphyton coverage should increase. Other physical and chemical characteristics of the water are also expected to impact periphyton coverage. Nutrients in the water such as total nitrogen (TN), chlorophyll- $\alpha$ , dissolved inorganic nitrogen (DIN), nitrates (NO<sub>3</sub>) and total organic carbon (TOC) are additional abiotic variables, which impact the overall primary productivity of aquatic ecosystems (Singh et al., 2017).

### *Hypothesis 3: Periphyton coverage and its impact on fish*

Since some fish species, and many invertebrate species consume periphyton, we predict that as periphyton coverage increases, so too should net fish biomass (van Dam et al., 2002). We predict that increasing amounts of periphyton provides more food, particularly for species that are herbivorous and omnivorous in nature. Therefore, changes in periphyton coverage are predicted to have a stronger effect on these groups of fish species, more so than piscivore or apex carnivore species. We do however predict some effect of periphyton coverage on piscivore and carnivore fish species, due to a bottom-up effect through the food web. This bottom-up effect caused by the presence of periphyton is also expected to positively impact fish species richness, as a high rate of primary production is the building block of a healthy aquatic ecosystem. Water temperature, light and nutrients control the growth of periphyton (Mahdy et al., 2015). For this reason, we expect that characteristics of the water such as temperature, turbidity and DO would influence periphyton cover, and in turn, fish biomass and species richness. In addition, salinity has also been observed to influence periphyton cover (Arfi et al., 1997).

## Methods

### Study Area

Data was collected from two important areas of everglades National Park (ENP), the first of which is Shark River Slough (SRS), which is the dominant path for flow of water into ENP. It is a mixture of sawgrass marshes, tree islands and wet prairies, and it begins in Water Conservation Area 3, extends through ENP, and ends in Florida Bay. The second area covered by our dataset is Taylor Slough (TS), which is a smaller wetland system to the east of SRS, together these two areas form the principle natural drainages for the freshwater Everglades.

## The Data

### Collection (by other researchers)

The data set that we have chosen was collected as a part of the Florida Coastal Everglades Long-Term Ecological Research Program (FCE LTER). The data collected ranges from the years of 1996 to 2005 for various abiotic and biotic variables collected in SRS and TS, Everglades National Park (ENP) in the southern tip of Florida's coast. Specifically, we have chosen data that contains species presence/absence for fish, as well as their biomass and richness, where applicable. Habitat data consists of plant cover, plant height, and periphyton cover. Water quality data collected included surface and bottom measures of salinity, temperature, and dissolved oxygen, as well as nitrates, nitrites, ammonium, total nitrogen, dissolved inorganic and organic nitrogen, total phosphorus, soluble reactive phosphorus, alkaline phosphatase activity, chlorophyll- $\alpha$ , total organic carbon, silicon dioxide in the water and turbidity.

Detailed records of data collection protocols can be found in the repository folder named 'feild\_and\_lab\_protocols'. In brief, water samples were collected at each wetland site every 3-4 weeks. These samples were analysed for total phosphorus, total nitrogen, and salinity. Additionally, grab samples were collected and used to quantify inorganic nutrients in the water. Finally, water temperature and turbidity were also measured in the field.

The plant community was sampled using 1 m<sup>2</sup> throw traps, where emergent plant species were identified, and all samples were returned to the lab for further analysis including total species counts. Artificial blades, which act as seagrass were introduced to the water at each site and allowed to incubate for 2 months. The blades were collected, the periphyton was isolated and periphyton volume was determined. Additionally, periphytometers were similarly placed into the water at each site and left for 2 months to accumulate periphyton and this was used to estimate periphyton cover.

The fish community was observed via a stratified random sampling design, whereby each canal was divided into multiple sampling stations 200 m apart which were randomly selected in advance of sampling. Sampling was repeated 4 times per year, where electrofishing was used to collect, identify, and measure larger species. The number of all centrarchids, fundulus and smaller rare fish species were counted, but mollies, mosquitofish, flagfish, bluefins, sheepshead and silversides were not. Seine-fishing was used to collect smaller species, invertebrates, and other organisms.

### Processing (joining and cleaning)

#### Hypothesis 1

The data we obtained was divided into multiple research projects (one collecting fish data, another collecting abiotic variables, etc.), and therefore the data files lack a common field

(matching site ID) to enable data joining. To resolve this issue, the data was uploaded into QGIS in order to see the spatial distribution of all the sites sampled in ENP and create a common ID field. Upon doing this, we realized that the proximity of these sites to one another varied considerably. Therefore, we have decided to classify sampling location by general waterway, SRS in the northwest and TS in the southeast. Additionally, we discovered that the temperature and dissolved oxygen dataset only overlapped temporally with the biotic data for one year, so we added 'ENP\_WQ\_1996to2005.csv', which contains abiotic data collected by another research team and overlaps temporally with our biotic data.

In order to produce a complete data set which included fish and water quality data, we first compiled a list of the unique fish species names observed over the two data sets (one from 1996-2000 and one from 2000-2005). We omitted the data points where the fish could not be identified or could only be identified to its genus (e.g., "unidentified fish" or "*Lepomis* sp." were omitted). Then, we corrected the spelling of six different species, as they were recorded using two different spellings and inflated the total number of species included in the data set. After eliminating these duplicates, we found that there were 38 different species observed over the course of this study. Using a fish database (FishBase), as well as additional literature, we performed a literature search to determine which food items make up the highest proportion of each species' diet and assigned them a functional group accordingly. The functional groups were originally as follows: piscivore, planktivore, detritivore, insectivore, periphytivore, algivore and omnivore. However, based on a preliminary literature search, it appeared that fish who feed on insects typically also feed on other invertebrates such as crustaceans, therefore this functional group was modified to "invertivores". Second, we found no record that any of the species included in this study have been observed to feed on periphyton, rather, it is the invertebrates that likely feed on it most regularly. For this reason, periphytivore was eliminated as a functional group and instead, we used invertivores as our most direct link to the influence of periphyton on fish populations.

A similar literature search was completed to assign each species to one of four broad 'thermal guild' categories based on their respective thermal optima of a given species in its native habitat (i.e., if it is invasive to Florida, thermal guild was determined based on its native environment) : cold-water ( $< 19^{\circ}\text{C}$ ), cool-water ( $\geq 19^{\circ}\text{C}$  and  $< 22^{\circ}\text{C}$ ), warm-water ( $\geq 22^{\circ}\text{C}$ ), and cool/warm-water (significant overlap in the cool and warm categories). These category cut-offs were the same as those from Wehrly et al. (2003). With all functional/thermal groups assigned, the fish category data was merged with each fish data set to create two merged files.

## Hypothesis 2

Standard data joining and cleaning was carried out similarly to hypothesis 1. Missing values (-9999) were replaced with NAs, which were later removed, and water quality data was merged with the habitat data containing the plant and periphyton data.

### Hypothesis 3

Similar to the previous two hypotheses, joining of data sets were required before cleaning and analysis could be performed. In this case, the fish data and habitat data needed to be merged. The same functional groups included in hypothesis 1 were also included in this data set to investigate hypothesis 3. All NAs were removed before analysis could be completed. The fish data was aggregated to the “all fish” level in order to analyse the response of species richness to various biotic and abiotic explanatory variables.

### Statistical tests

Considering all of our hypotheses and predictions attempt to quantify the effect of one variable on another, regression analyses were performed, employing methodologies that best suited the respective datasets. That being said, all model construction was built on the premise of expert opinion modelling. In other words, in order to attempt to answer our questions, models were built not only including our key variables of interest as explanatory variables, but any other variables, based on our knowledge of the systems being evaluated, which we theorized to effect fish biomass, species richness, or periphyton cover, to some degree which warranted their inclusion. Expert opinion modelling is often used when the biological relevance of the constructed model is important, rather than its “pure” predictive power. The success of this modelling approach is based solely on the level of expertise of the model builders for the system they are analyzing and whether they introduce bias due to personal reasons, data availability or political pressures (Krueger et al., 2012; Vennix and Gubbels, 1992).

We will therefore note, while we attempted to include all explanatory variables, we thought may affect our response variable, while minimizing variable redundancy, we are not experts in the Florida everglade ecosystems, nor can we guarantee no bias in our variable selection, but that it was unconscious, if it did occur.

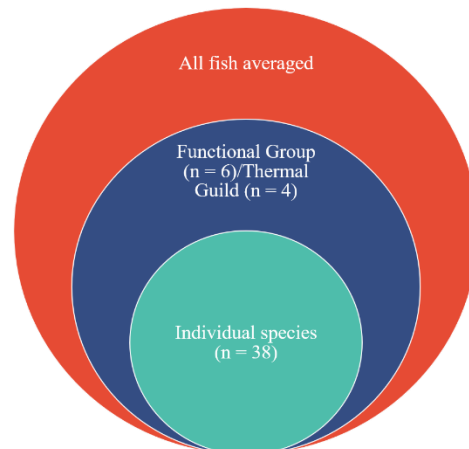
Variable collinearity was minimized by first generating Pearson correlation coefficients between all potential explanatory variable pairings. Any correlation coefficient greater than 0.7 (+/-) was evaluated further, and one of the two pairings was selected based on the one we agreed made more biological sense to remain in the model. In some instances, such as water depth and salinity, while highly correlated, were both kept in the models, as biologically both of these variables provide different information to the processes of the study system. Potential categorical variables that may affect our response variable were evaluated by building a preliminary full model (which included all observations and continuous explanatory variables), and then evaluating the boxplots of this model’s residuals grouped by one of these categorical variables. Potential categorical variables hypothesized to influence the response variables were area of data collection (SRS or TS), year and month of data collection (ranging from 1996 to 2005 and January to December, respectively), as well as thermal guild or functional group.

For hypothesis 1 and 3, modelling methods were essentially the same, except for the inclusion of periphyton and plant cover as explanatory variables in hypothesis 3, and the grouping of fish species to either thermal guild (hypothesis 1) or functional group (hypothesis 3), based on the key variables of focus for a given hypothesis (Fig. 1). For all hypotheses, two models were created (full and subset) using the all-fish-level or periphyton and water quality aggregated datasets for both log(biomass) and species richness (hypothesis 1 and 3), or periphyton percent cover (hypothesis 2) as the response variable. A full model consisted of all explanatory variables, whereas the subset model was the same as the full model, except for 1 to 4 explanatory variables being removed. These removed explanatory variables were the key variables of interest for a given hypothesis (see Results).

The log(biomass) models for hypotheses 1 and 3 were multivariate linear regressions, whereas the species richness models were generalized linear models with a Poisson error distribution and logistic link function. This distinction was made considering that species richness is not a continuous response variable, but discrete count data.

While analysis of model residuals grouped by year, month, and area did not show distinct patterns in the all-fish-level aggregated dataset, which may have indicated a different effect size of these variables on the response variables; this was not the case for hypothesis 2. In this instance, year appeared to have a fluctuating effect on percent periphyton cover, therefore a mixed effects model was built for hypothesis 2, where year was included as a random effect (see Results).

Finally, for hypothesis 1 and 3, a general linear regression with interactions was created for the data aggregated to thermal guild or functional group level for the response variable log(biomass) (Fig. 1; see Results).



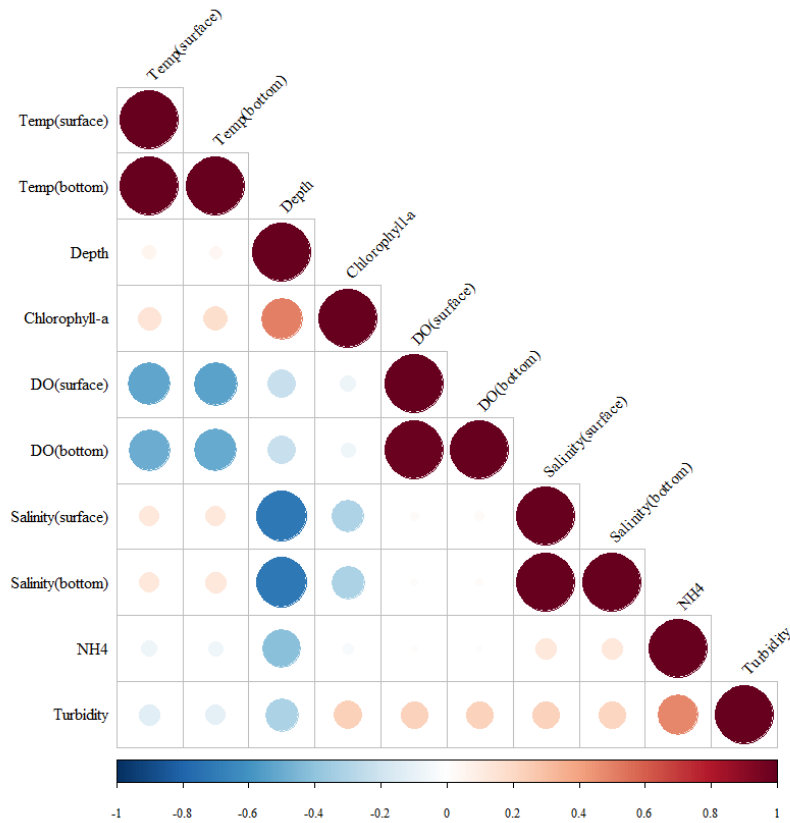
**Figure 1.** Diagram illustrating the three levels of data aggregation that occurred based on differing characteristics of fish caught. For simplicity of data analysis, the species-level dataset was not analyzed.

# Results

## Diagnostics

### Hypothesis 1

Based on the results of the correlation matrix (Fig. 2), we found that surface and bottom measurements for temperature, DO and salinity were highly correlated, with a correlation coefficient of approximately 1. Given this, we decided to include only the bottom measurements in our model (Table 1), since these are more biologically relevant to the many species of fish who reside mainly in the lower half of the water column. This same principle was also applied to hypothesis 3.



**Figure 2.** Correlation matrix of potential explanatory variables for the hypothesis 1 models. Colours range from dark blue to dark red. Blue indicates a negative Pearson correlation coefficient, red a positive, where the darkness of the colour indicates a correlation coefficient closer to 1 (+/-). Size of circles indicates the significance of the correlation coefficient (i.e.,  $p$ -value), where the larger the circle, the lower the  $p$ -value.

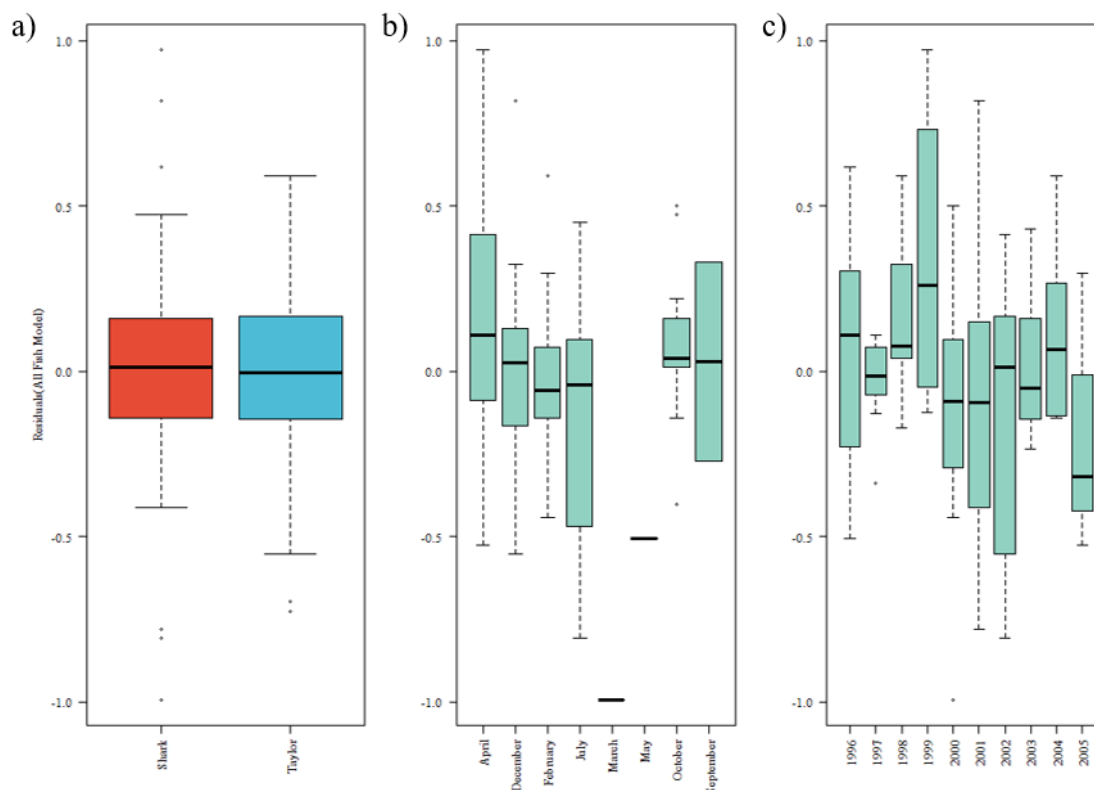


Further, a histogram was used to check the distribution of the response variable for approximate normality. After which, biomass was log-transformed to improve the fit of the linear regression.

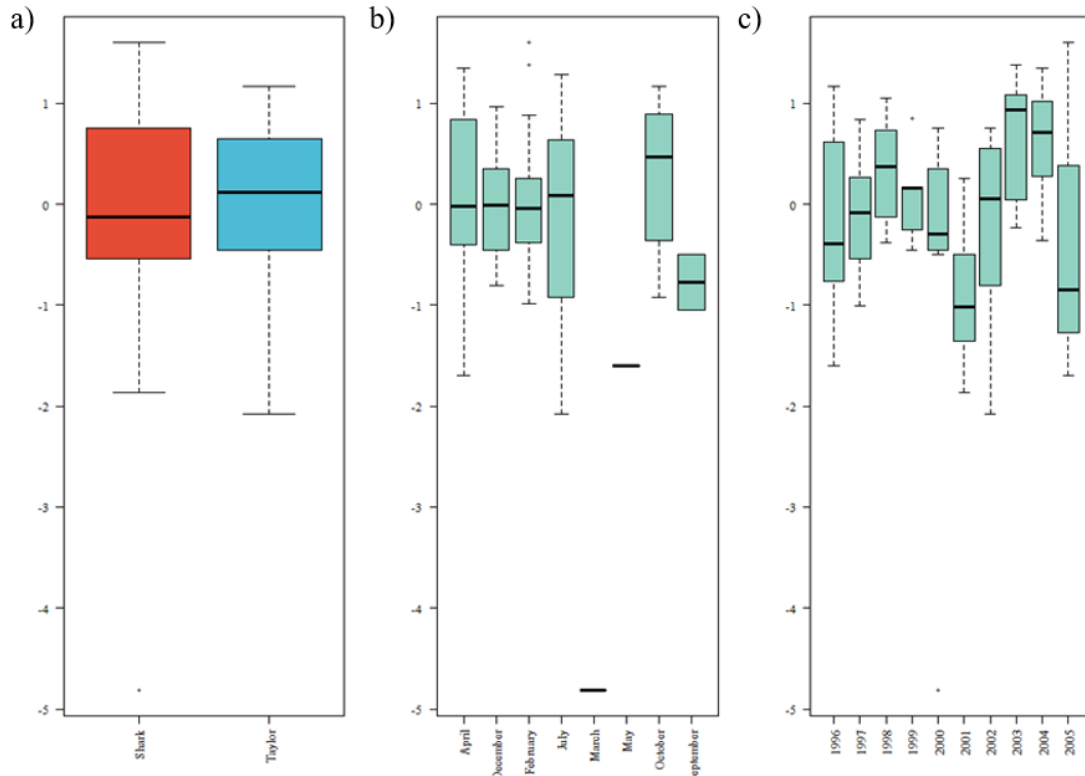
The residuals of the all-fish model were evaluated with boxplots broken down into potentially influential categorical variables, i.e., area, month, and year (Fig. 3; Fig. 4). All three of the categorical variables evaluated were found to have no differential effect on the residuals. March appeared at first to be an outlier, but this was explained by there being only one observation in this category. Based on this result, none of the mentioned categorical variables were included in the model.

**Table 1.** Selected explanatory variables for hypothesis 1 “full” models. The response variables = log(biomass) and species richness. \* = key variable of interest removed from the “subset” model.

Explanatory variable	Variable name	Units (unscaled)	Hypothesized relationship with response variable	Reference
Temperature*	TEMP_B	°C	positive/negative (species specific temperature optima)	Duque et al. (2020)
DO*	DO_B	mg/L	positive	Duque et al. (2020)
Salinity	SAL_B	PSU	negative	Duque et al. (2020)
NH <sub>4</sub>	NH4	μM/L	negative	Duque et al. (2020)
Chl-α	CHLA	μg/L	positive/negative (system dependent based on energy-mass balance)	McQueen et al. (1989)
Turbidity	TURB	NTU	negative	Henley et al. (2000)
Depth	Depth	m	positive (if biomass is measured volumetrically)	Fernandes et al. (2010); Jeppesen et al. (1997)

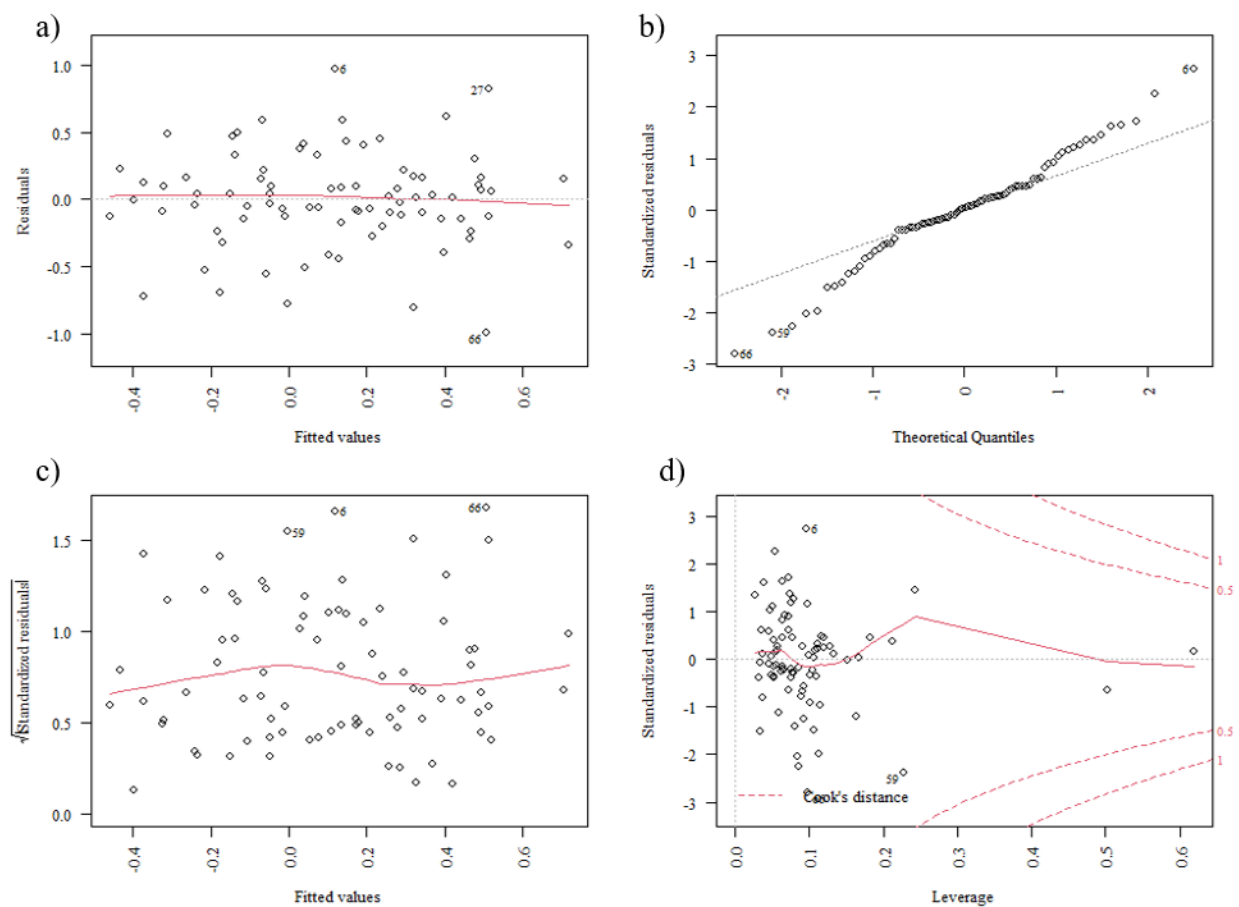


**Figure 3.** Boxplots of the “full” model’s residuals for hypothesis 1, where the response variable is  $\log(\text{biomass})$ , using the all-fish-level aggregated dataset. Boxplots are grouped by the categorical variable a) Area, b) Month, and c) Year; in order to evaluate whether these variables disproportionately affect the response variable.

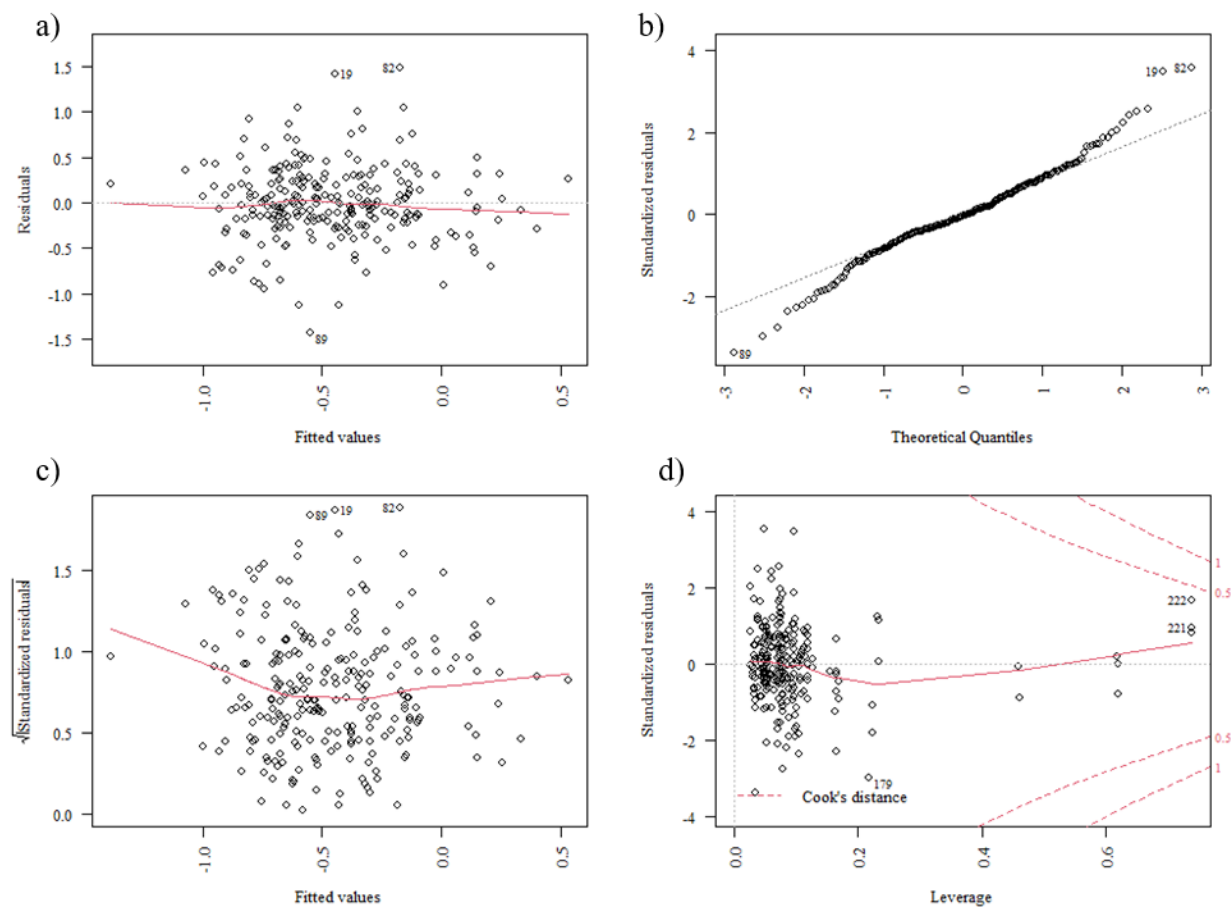


**Figure 4.** Boxplots of the “full” model’s residuals for hypothesis 1, where the response variable is species richness, using the all-fish-level aggregated dataset. Boxplots are grouped by the categorical variable a) Area, b) Month, and c) Year; in order to evaluate whether these variables disproportionately affect the response variable.

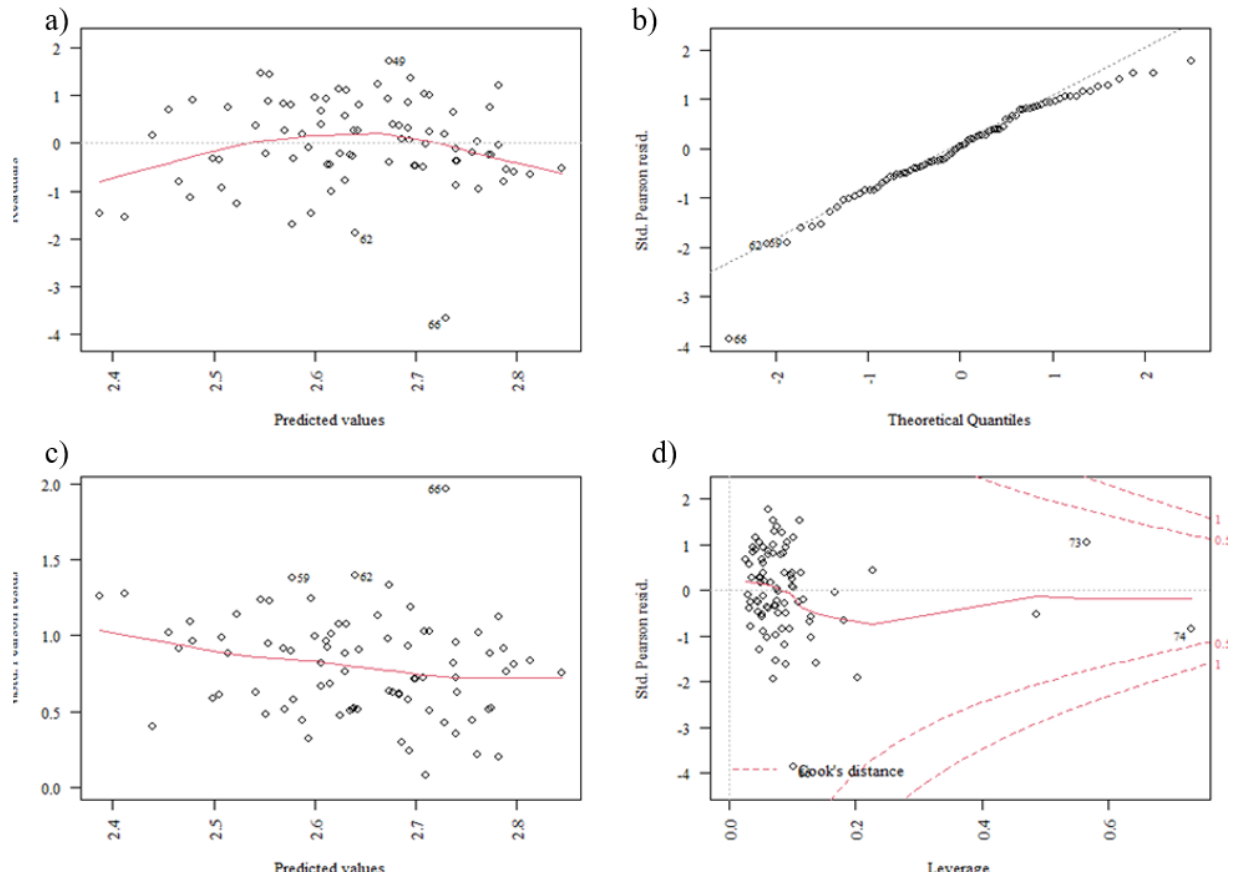
The diagnostic plots for the “all fish” model (Fig. 5), the thermal guild level model (Fig. 6) and the species richness model (Fig. 7) did not display significant violations of any assumptions. For all three however, there are slight tails at the lowest and highest extremes of the theoretical quantiles. Select points on each leverage plot were omitted if they deviated significantly from the rest or ones with a leverage of 1 (all fish model: observation 74, thermal guild model: observations 4, 101, 235 and 240, species richness model: no observations removed).



**Figure 5.** Diagnostic plots for the “full” log(biomass) model for hypothesis 1. Plots show a) residuals versus fitted values, b) a normal Q-Q plot, c) a scale-location plot, and d) residuals versus leverage plot for the given regression.



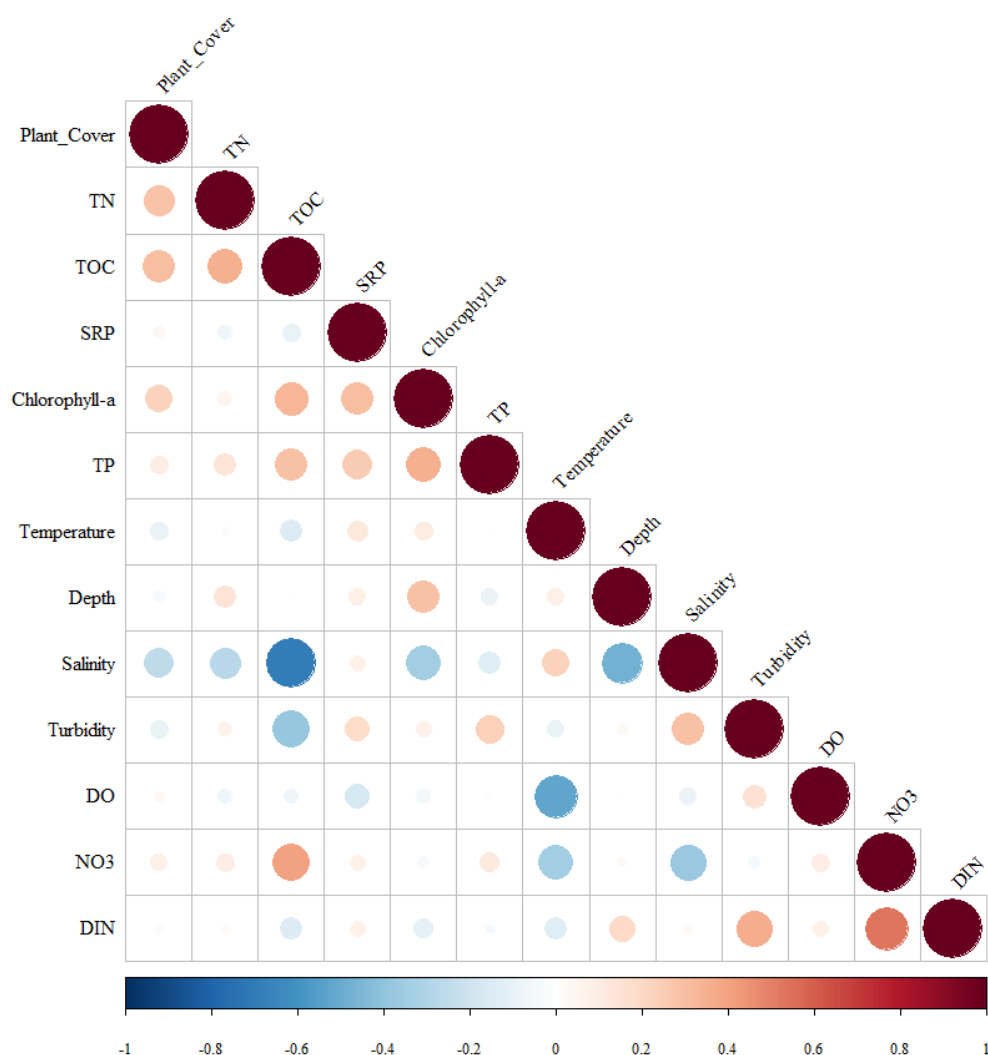
**Figure 6.** Diagnostic plots for the log(biomass) model for hypothesis 1 separated by thermal guild. Plots show a) residuals versus fitted values, b) a normal Q-Q plot, c) a scale-location plot, and d) residuals versus leverage plot for the given regression.



**Figure 7.** Diagnostic plots for the “full” species richness model for hypothesis 1. Plots show a) residuals versus fitted values, b) a normal Q-Q plot, c) a scale-location plot, and d) residuals versus leverage plot for the given generalized linear regression.

## Hypothesis 2

Based on the correlation matrix of potential explanatory variables (Fig. 8), salinity and TOC appeared to be highly correlated. Biologically however, these parameters both could provide information about the processes of the system of interest, therefore they were both retained in the model. A full list of explanatory variables included in the “full” model can be seen in Table 2.



**Figure 8.** Correlation matrix of potential explanatory variables for the hypothesis 2 models. Colours range from dark blue to dark red. Blue indicates a negative Pearson correlation coefficient, red a positive, where the darkness of the colour indicates a correlation coefficient closer to 1 (+/-). Size of circles indicates the significance of the correlation coefficient (i.e.,  $p$ -value), where the larger the circle, the lower the  $p$ -value.

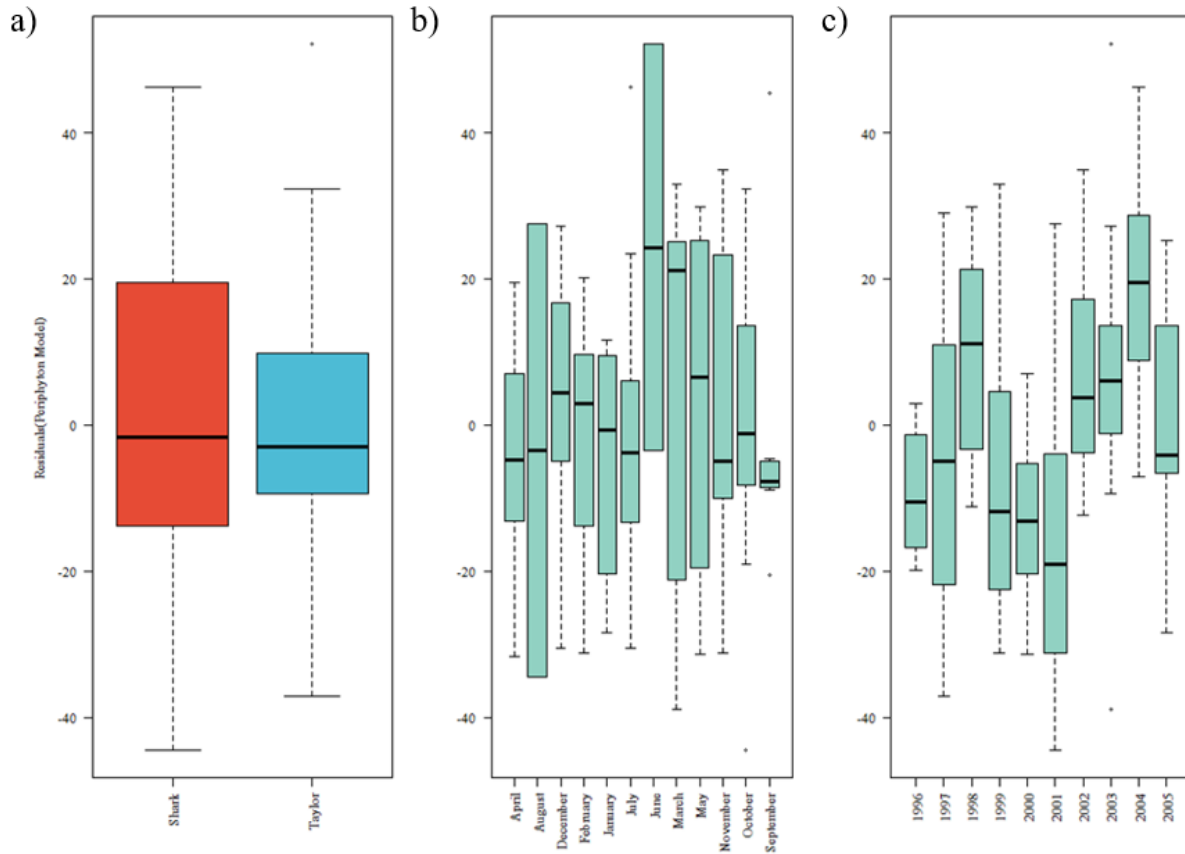
**Table 2.** Selected explanatory variables for hypothesis 2 “full” model. The response variable in this model is % periphyton cover. \* = key variable of interest removed from the “subset” model.

Explanatory variable	Variable name	Units (unscaled)	Hypothesized relationship with response variable	Reference
Temperature	TEMP_B	°C	negative	Kanavillil and Kurissery (2013) <sup>a</sup> .
DO*	DO_B	mg/L	negative	Kanavillil and Kurissery (2013) <sup>a</sup> .
Salinity	SAL_B	PSU	negative	Kanavillil and Kurissery (2013) <sup>a</sup> .
NO <sub>3</sub>	NO3	μM/L	negative	Kanavillil and Kurissery (2013) <sup>a</sup> .
Chl- <i>α</i>	CHLA	μg/L	positive	Kanavillil and Kurissery (2013) <sup>a</sup> .
Turbidity	TURB	NTU	negative	Uehlinger et al. (2010) <sup>b</sup> .
TN	TN	μM/L	negative/neutral	Iwaniec et al. 2006) <sup>b</sup> ; Hillebrand and Kahlert (2001) <sup>c</sup> .
DIN	DIN	μM/L	positive	Hillebrand and Kahlert (2001) <sup>c</sup> .
TP*	TP	μM/L	negative/neutral	Kanavillil and Kurissery (2013) <sup>a</sup> ; Iwaniec et al. (2006) <sup>b</sup> ; Hillebrand and Kahlert (2001) <sup>c</sup> .
SRP*	SRP	μM/L	positive	Hill and Fanta (2008) <sup>d</sup> .
TOC	TOC	μM/L	positive	Furnish and Keller (2020) <sup>d</sup> ; Tarkowska-Kukuryk and Mieczan (2012) <sup>e</sup> .
Depth	AvgWaterDepth	cm	negative	Iwaniec et al. (2006) <sup>b</sup> .
% Plant cover*	Avg.PlantCover	%	negative/positive (debated in the literature)	Jones et al. (2002, 2000) <sup>a</sup> ; Wetzel (1983) <sup>b</sup> .

<sup>a</sup> Response = density; <sup>b</sup> Response = biomass; <sup>c</sup> Response = biovolume; <sup>d</sup> Response = productivity/growth rate; <sup>e</sup> Response = diversity

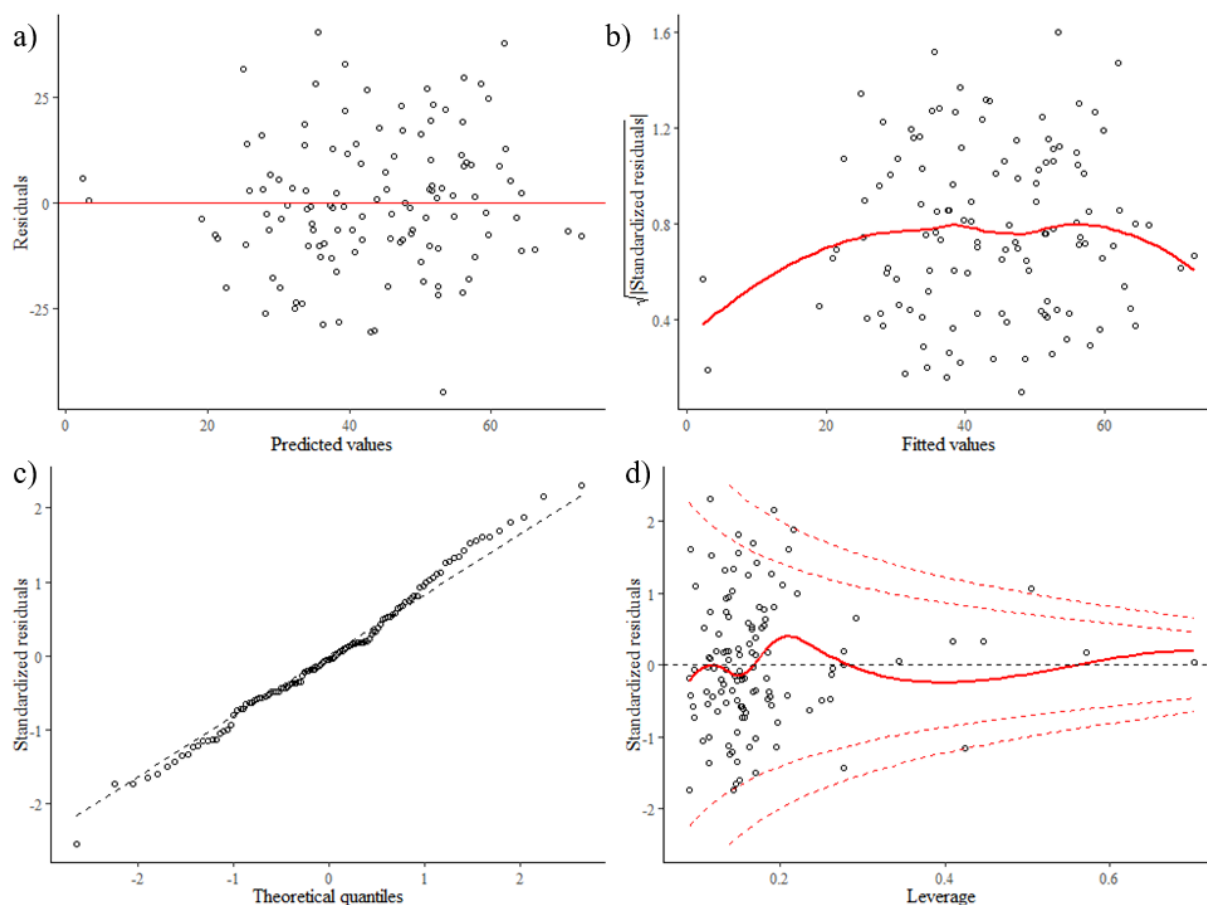


Boxplots of residuals grouped by the categorical variables area and month showed no distinct pattern of effect on percent periphyton cover, however year appeared to fluctuate up and down over the study's time period (Fig. 9 c)). Due to this, year was included as a random effect in the model.



**Figure 9.** Boxplots of the “full” model’s residuals for hypothesis 2, where the response variable is % periphyton cover, using the periphyton and water quality parameters dataset. Boxplots are grouped by the categorical variable a) Area, b) Month, and c) Year; in order to evaluate whether these variables disproportionately affect the response variable.

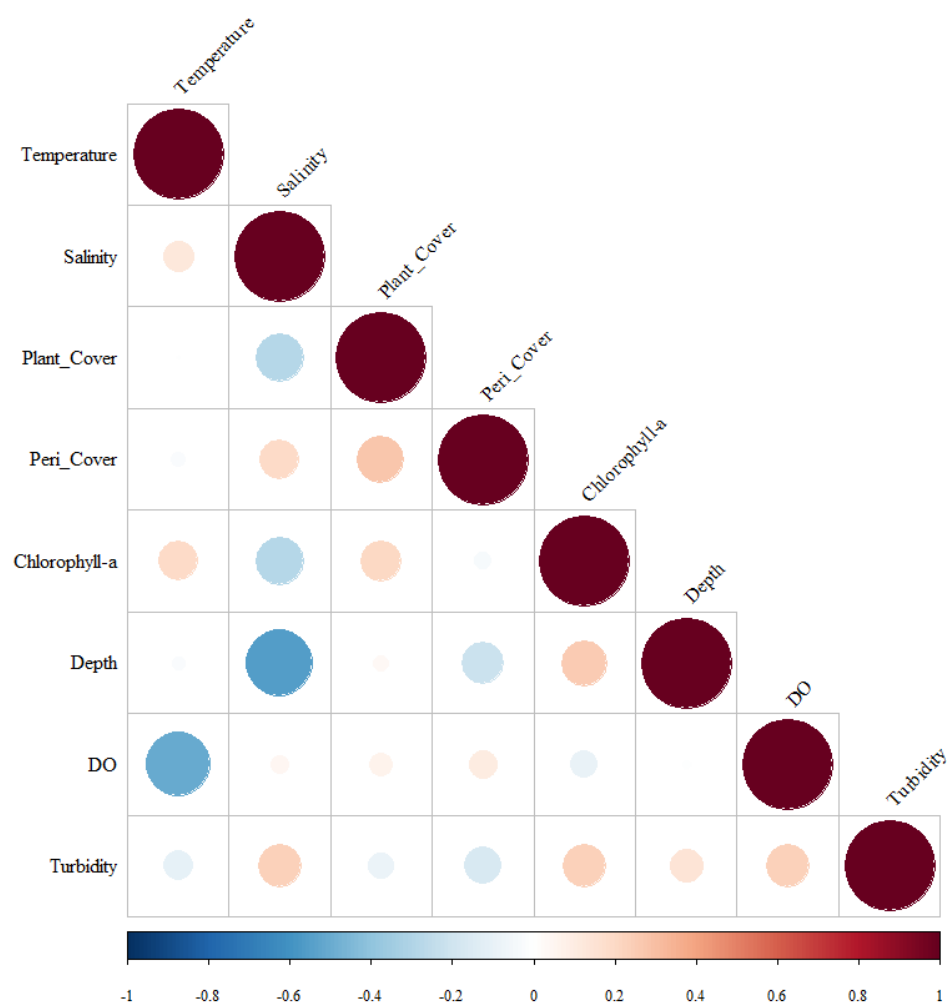
The diagnostic plots for the “full” model, once year was included as a random effect, indicated that the constructed model does not have any significant assumption violations (Fig. 10). There are a few observations that appear to leverage the resulting standardized residuals (Fig. 10 d)), however, they were determined to not affect the results enough to warrant their removal.



**Figure 10.** Diagnostic plots for the “full” % periphyton cover mixed effects model for hypothesis 2. Plots show a) residuals versus fitted values, b) a scale-location plot, c) a normal Q-Q plot, and d) residuals versus leverage plot for the given regression.

### Hypothesis 3

As was mentioned in the Methods, the correlation matrix of the selected explanatory variables for hypothesis 3 show that depth and salinity are correlated, but were retained in the “full” model, due to their biological relevance. The high correlation between temperature and DO is to be expected, given their established negative relationship with one another (Fig. 11). The full list of selected explanatory variables can be found in Table 3.

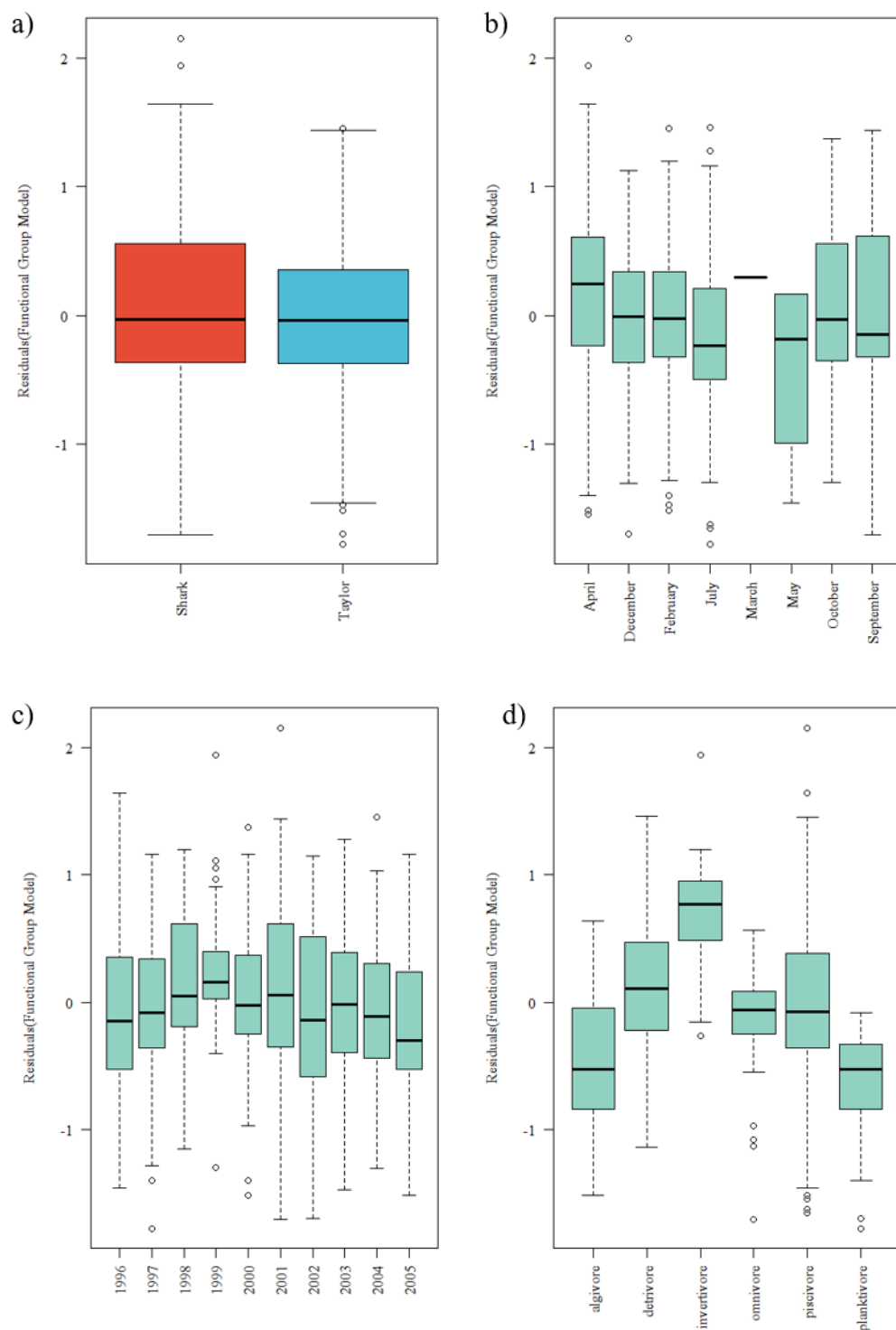


**Figure 11.** Correlation matrix of potential explanatory variables for the hypothesis 3 models. Colours range from dark blue to dark red. Blue indicates a negative Pearson correlation coefficient, red a positive, where the darkness of the colour indicates a correlation coefficient closer to 1 (+/-). Size of circles indicates the significance of the correlation coefficient (i.e.,  $p$ -value), where the larger the circle, the lower the  $p$ -value.

**Table 3.** Selected explanatory variables for hypothesis 3 “full” models. The response variables = log(biomass) and species richness. Nutrient explanatory variables were not included, as they were assumed to be accounted for by the average growth of the primary producers included in the model instead. \* = key variable of interest removed from the “subset” model.

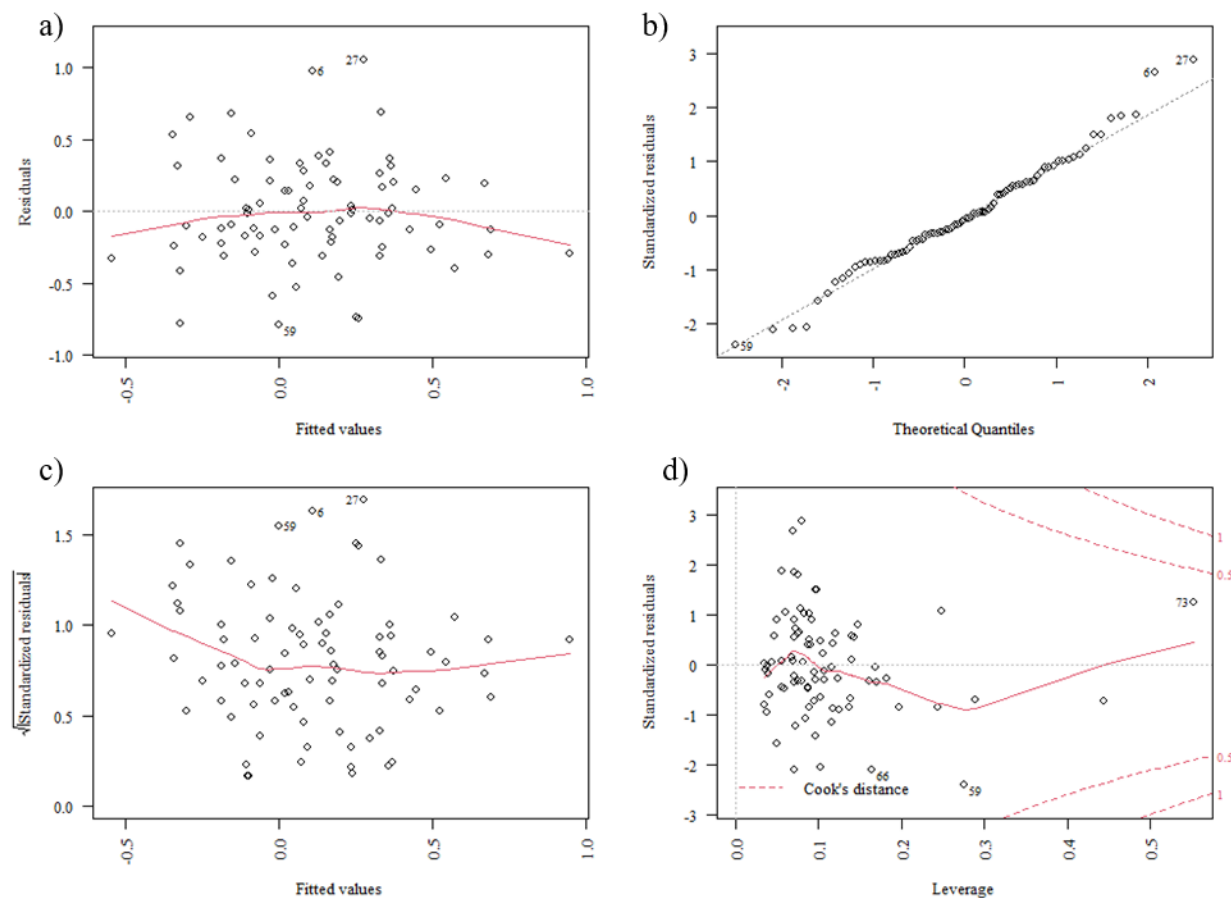
<b>Explanatory variable</b>	<b>Variable name</b>	<b>Units (unscaled)</b>	<b>Hypothesized relationship with response variable</b>	<b>Reference</b>
Temperature	TEMP_B	°C	positive/negative (species specific temperature optima)	Duque et al. (2020)
DO	DO_B	mg/L	positive	Duque et al. (2020)
Salinity	SAL_B	PSU	negative	Duque et al. (2020)
Chl- $\alpha$	CHLA	$\mu\text{g/L}$	positive/negative (system dependent based on energy-mass balance)	McQueen et al. (1989)
Turbidity	TURB	NTU	negative	Henley et al. (2000)
Depth	AvgWaterDepth	cm	positive (if biomass is measured volumetrically)	Jeppesen et al. (1997); Fernandes et al. (2010)
% Periphyton cover*	Avg.PeriphytonCover	%	positive	van Dam et al. (2002)
% Plant cover*	Avg.PlantCover	%	positive	van Dam et al. (2002)

Boxplots of residuals grouped by the categorical variables area, month, and year showed no distinct pattern of effect on log(biomass), however functional group did appear to have a differential effect, therefore an interaction model using this categorical variable was created as well (Fig. 12).

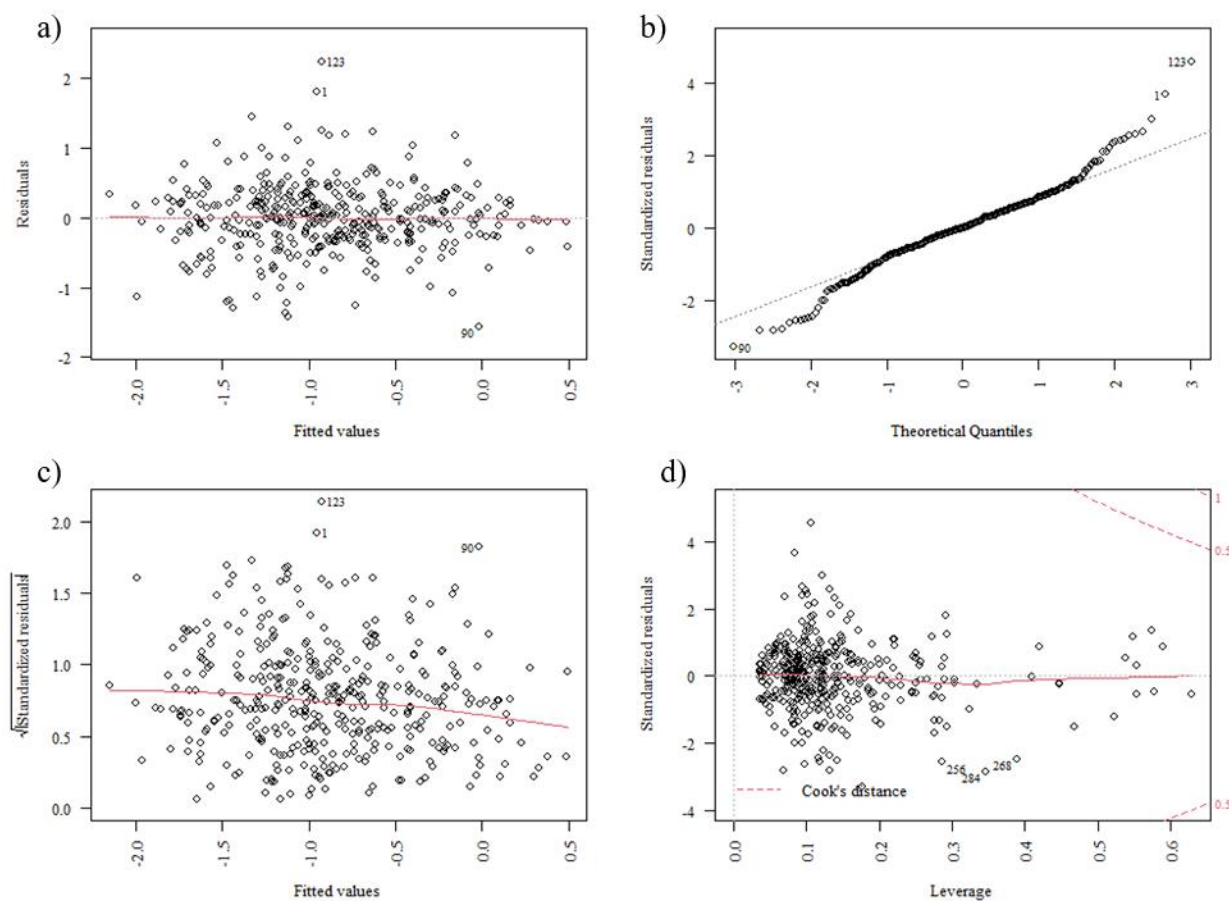


**Figure 12.** Boxplots of the “full” model’s residuals for hypothesis 3, where the response variable is  $\log(\text{biomass})$ , using the all-fish-level aggregated dataset. Boxplots are grouped by the categorical variable a) Area, b) Month, c) Year, and d) Functional Group; in order to evaluate whether these variables disproportionately affect the response variable.

The diagnostic plots for the “full” model and the functional group separated model, with  $\log(\text{biomass})$  as the response variable, indicated that the constructed model does not have any significant assumption violations (Fig. 13 and Fig. 14, respectively).

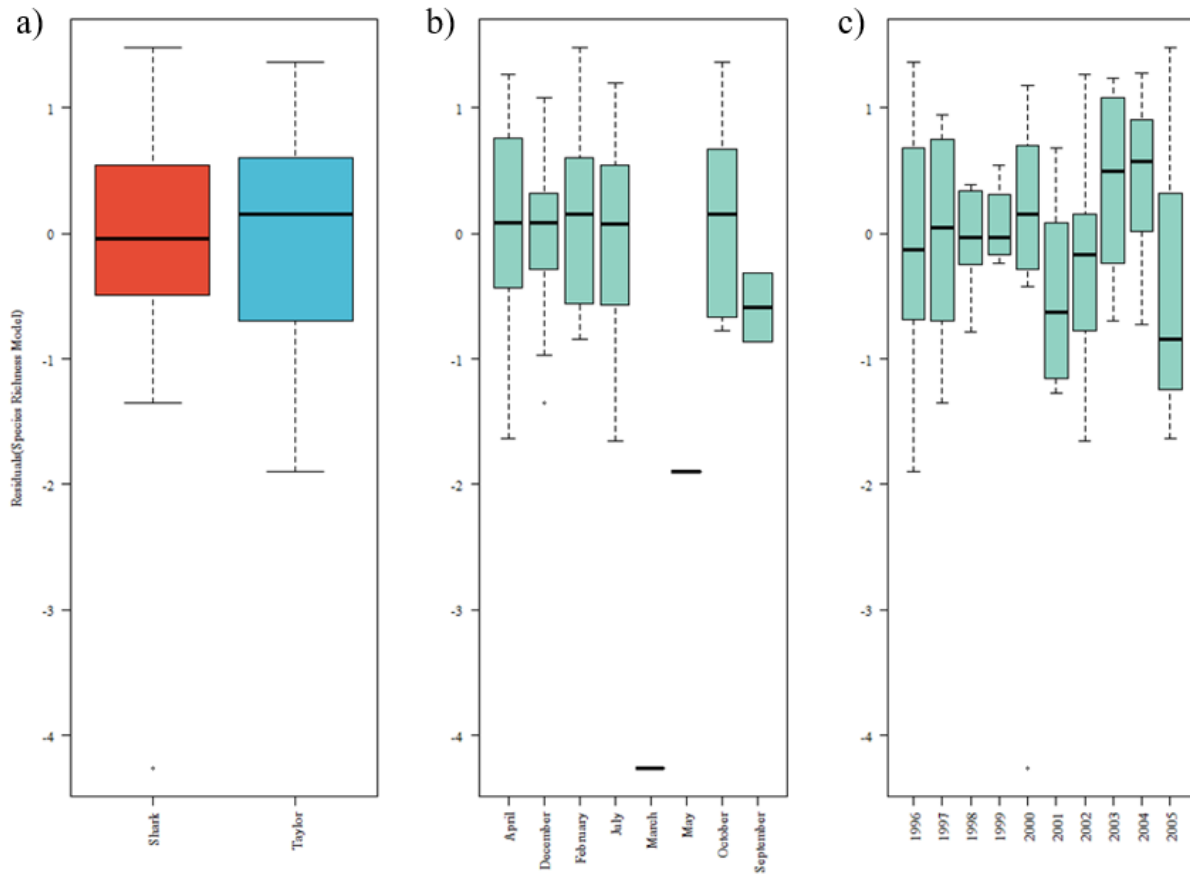


**Figure 13.** Diagnostic plots for the “full”  $\log(\text{biomass})$  model for hypothesis 3. Plots show a) residuals versus fitted values, b) a normal Q-Q plot, c) a scale-location plot, and d) residuals versus leverage plot for the given regression.



**Figure 14.** Diagnostic plots for the log(biomass) model for hypothesis 3, separated by functional group. Plots show a) residuals versus fitted values, b) a normal Q-Q plot, c) a scale-location plot, and d) residuals versus leverage plot for the given regression.

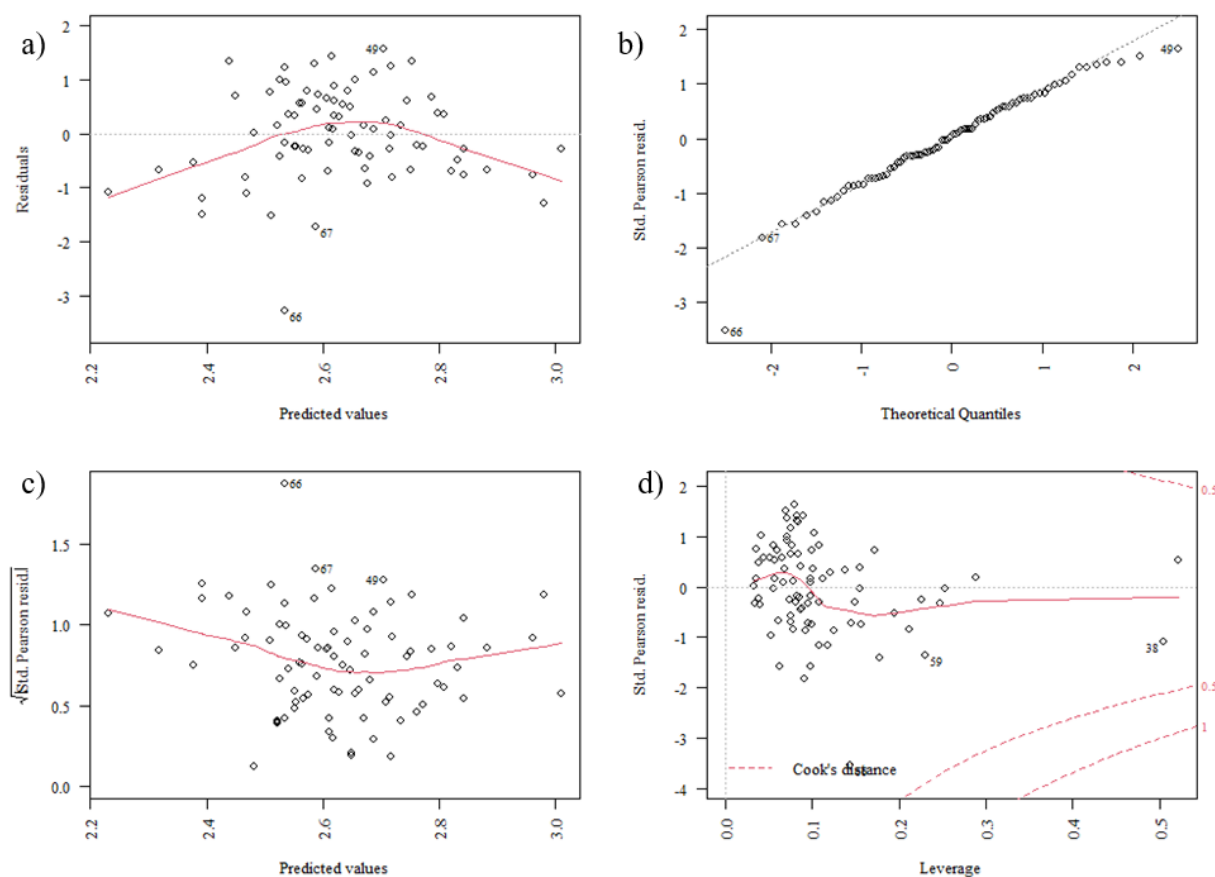
Boxplots of residuals grouped by the categorical variables area, month, and year showed no distinct pattern of effect on species richness, therefore they were not included in the model (Fig. 15).



**Figure 15.** Boxplots of the “full” model’s residuals for hypothesis 3, where the response variable is species richness, using the all-fish-level aggregated dataset. Boxplots are grouped by the categorical variable a) Area, b) Month, and c) Year; in order to evaluate whether these variables disproportionately affect the response variable.

The diagnostic plots for the “full” model with species richness as the response variable, indicated that the constructed model does not have any significant assumption violations (Fig. 16).





**Figure 16.** Diagnostic plots for the species richness generalized linear model for hypothesis 3. Plots show a) residuals versus fitted values, b) a normal Q-Q plot, c) a scale-location plot, and d) residuals versus leverage plot for the given regression.

## Plots and Summaries

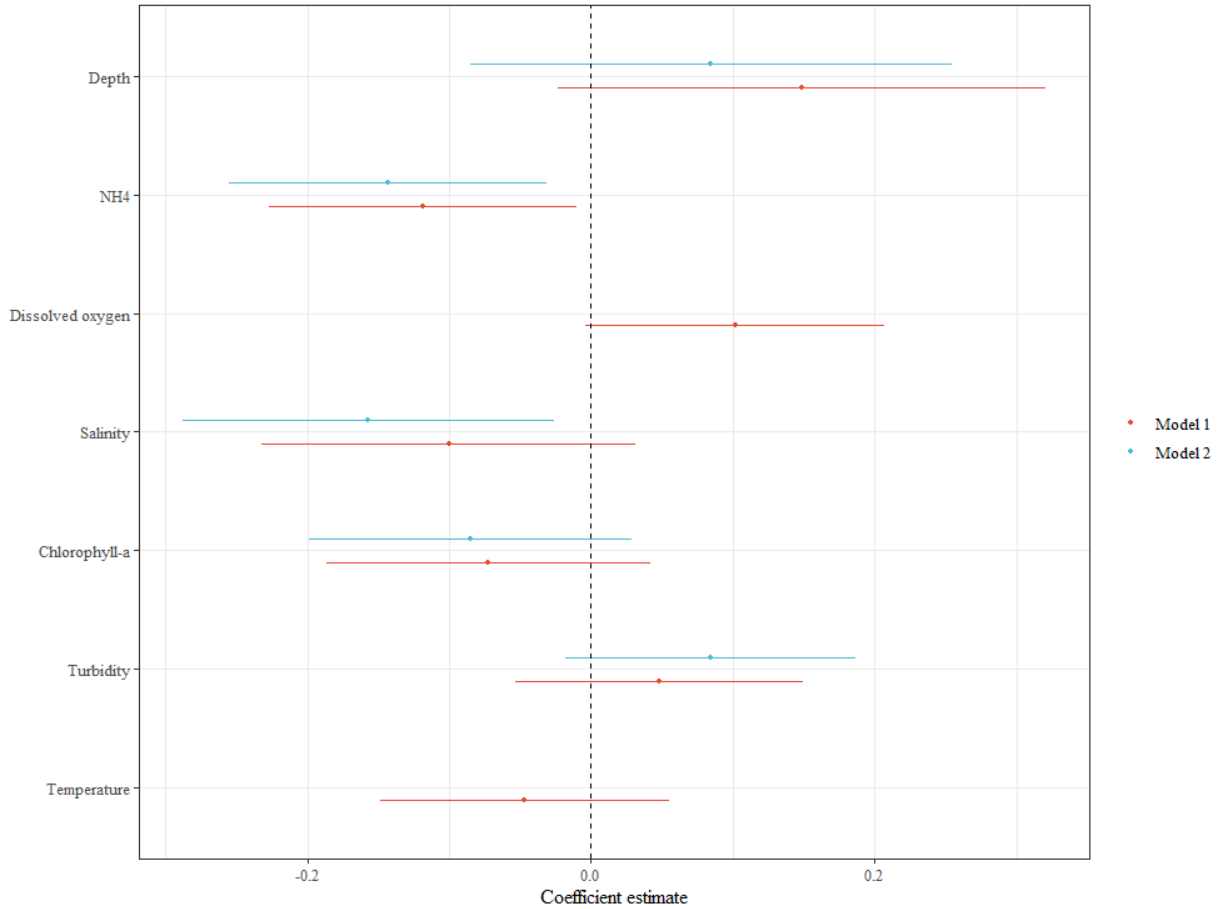
### Hypothesis 1

The coefficient plot of the full, all-fish model (Fig. 17) demonstrated that depth had the largest positive effect on fish log(biomass) and ammonia had the largest negative effect. It was observed that ammonia, salinity, chlorophyll- $\alpha$  and temperature all had a negative effect on fish log(biomass), whereas depth, DO and turbidity had an overall positive effect (Table 4).

**Table 4.** All fish model summary for hypothesis 1 ( $R^2 = 0.384$ ,  $F = 6.586$ ,  $p < 0.0001$ , response variable =  $\log(\text{biomass})$ ).

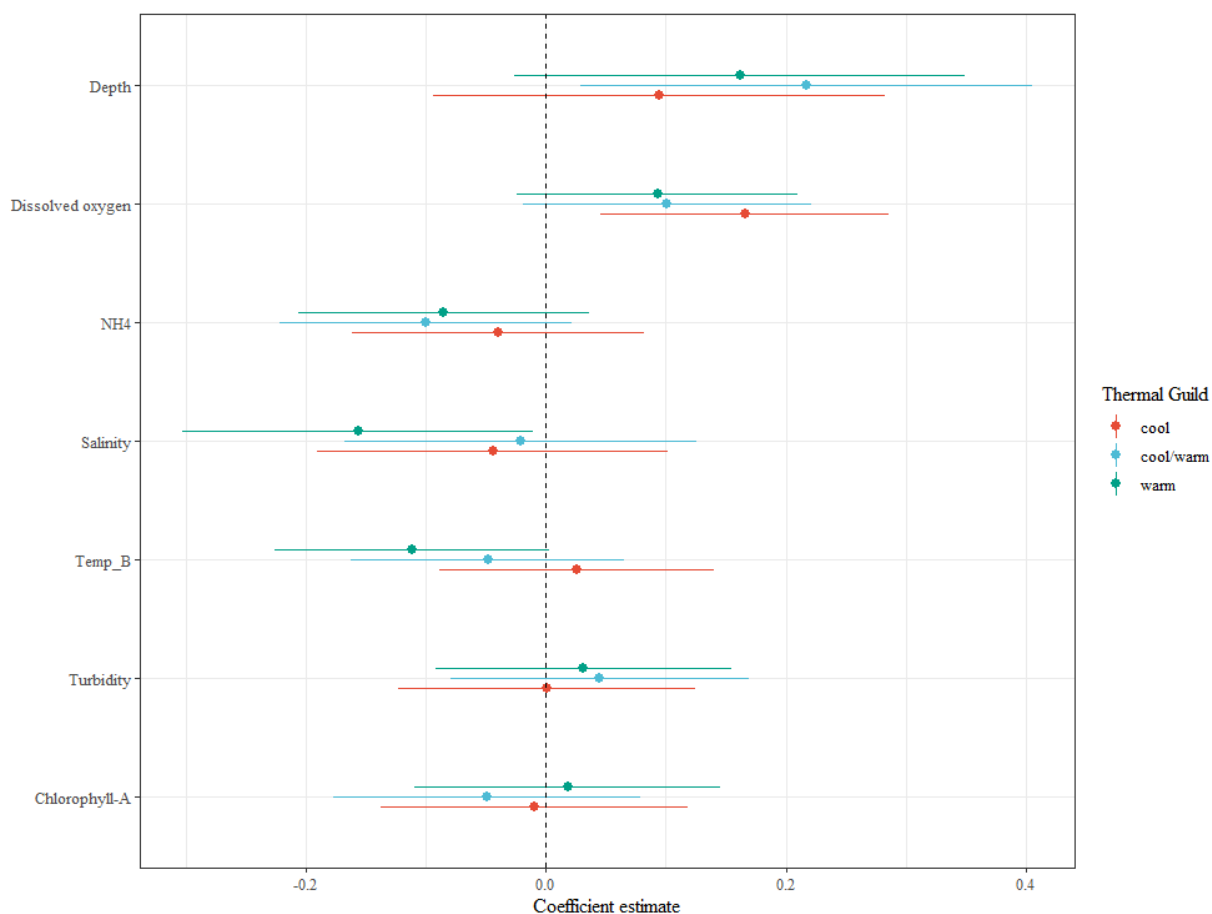
Model Parameter	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Intercept)	-0.999	1.163	0.114	-0.859	0.393	-3.316	1.317
Depth	0.593	0.345	0.149	1.718	0.090	-0.095	1.280
NH <sub>4</sub>	-0.095	0.044	-0.119	-2.170	0.033	-0.183	-0.008
Chl- $\alpha$	-0.048	0.038	-0.072	-1.259	0.212	-0.125	0.028
Salinity	-0.011	0.007	-0.100	-1.514	0.134	-0.025	0.003
Temperature	-0.013	0.015	-0.047	-0.911	0.365	-0.043	0.016
DO	0.084	0.044	0.102	1.923	0.058	-0.003	0.171
Turbidity	0.013	0.014	0.048	0.941	0.350	-0.014	0.040

In the subset model, which excluded temperature and DO, we observed this same effect for each of the remaining variables. When comparing the two models using an ANOVA, there was a significant difference between the two models, indicating that temperature and DO account for a significant impact on fish biomass ( $F = 4.199$ ,  $p = 0.019$ ).



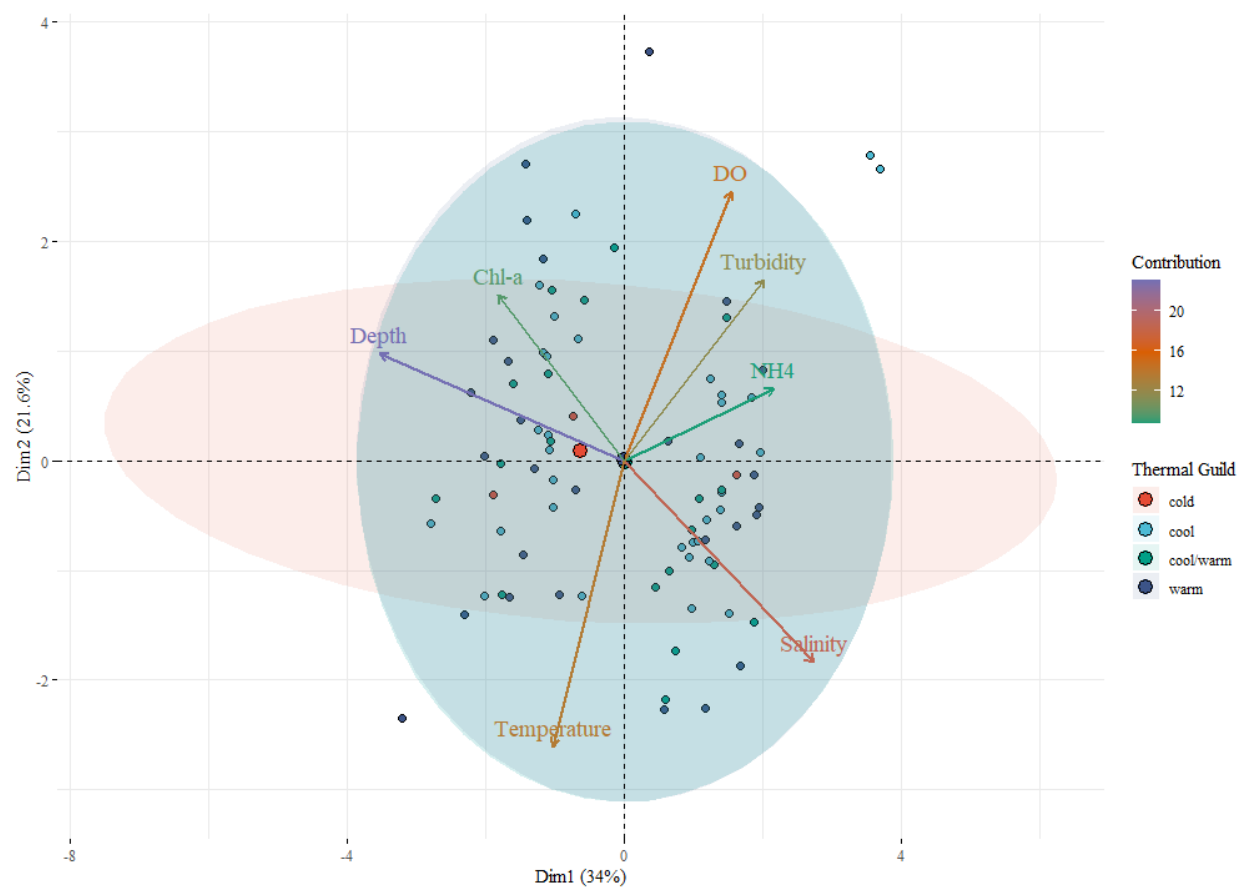
**Figure 17.** Coefficient plot of scaled coefficient estimates for the “full” and “subset” models for hypothesis 1. Response variable =  $\log(\text{biomass})$ ; Full model = Model 1; Subset model = Model 2 (missing DO and temperature).

The coefficient plot for the thermal guild level model (Fig. 18) demonstrated that depth and DO both had the largest effect on fish  $\log(\text{biomass})$ . Interguild differences in effect size of a given variable were also observed. Temperature, for example, had a positive effect on fish in the cool guild, whereas in cool/warm and warm guilds, temperature has a negative effect, but these effects were not significant (Table 5).



**Figure 18.** Coefficient plot of scaled coefficient estimates for hypothesis 1, broken down by thermal guild. Response variable = log(biomass).

The results of the PCA analysis (Fig. 19) show that depth had the greatest contribution, followed by DO, salinity and temperature which had moderate contribution, and finally, chlorophyll- $\alpha$  and ammonia, which had the lowest contribution. Similar to our regressions, cold thermal guild species seemed to differ from the other guilds, based on depth and DO.



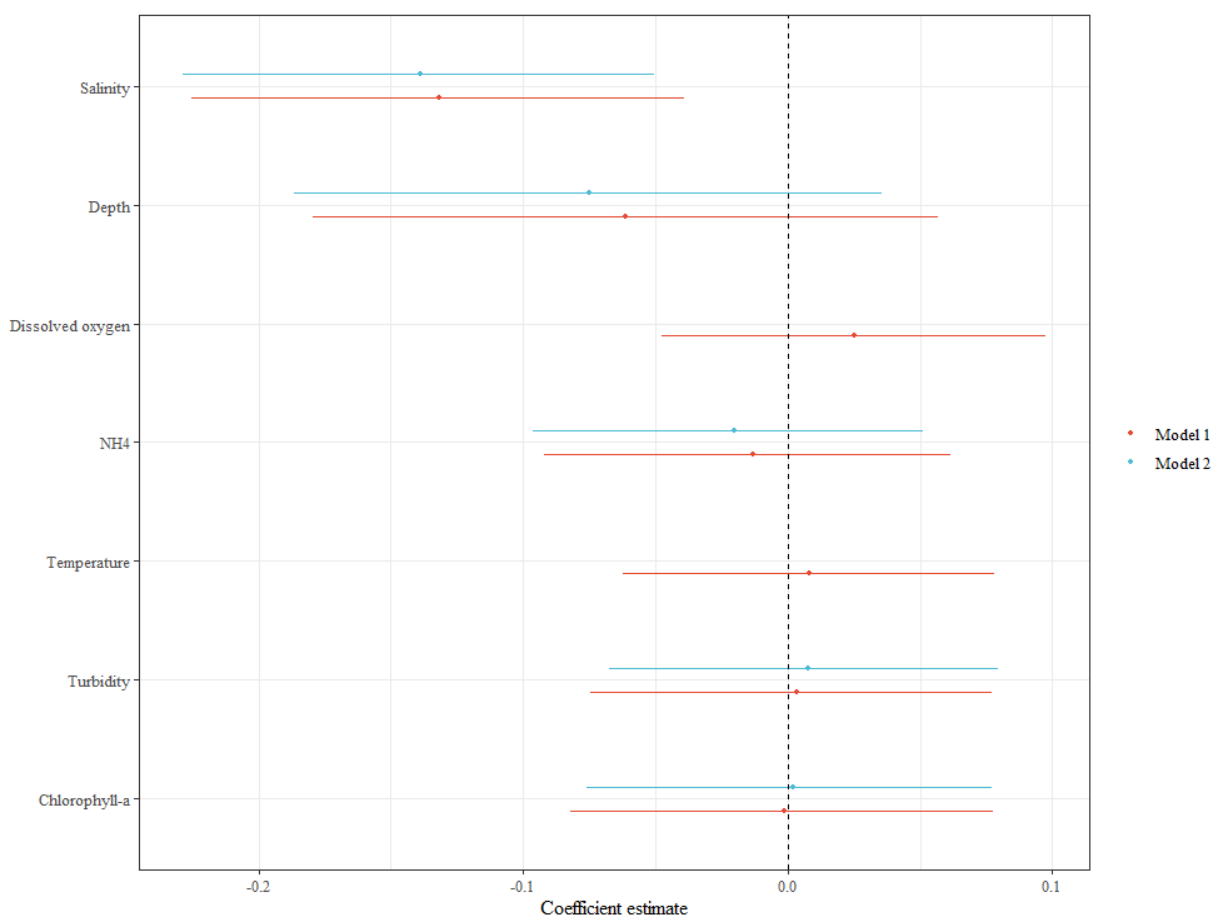
**Figure 19.** Principal component analysis plot for hypothesis 1, broken down by thermal guild. Response variable = log(biomass). Colour of the arrows indicates contribution level; colour of points and ellipses indicates thermal guild.

**Table 5.** Thermal guild level model summary ( $R^2 = 0.65$ ,  $F = 17.22$ ,  $p < 2.2e-16$ , response variable =  $\log(\text{biomass})$ ). Note: Cold guild missing due to there being only 4 observations in the data and therefore were removed, due to leverage.

Model Parameter	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Cool guild	-2.307	1.310	-0.568	-1.762	0.080	-4.888	0.274
Cool/warm guild	-2.493	1.310	-0.483	-1.904	0.058	-5.074	0.088
Warm guild	-1.122	1.303	-0.348	-0.861	0.390	-3.689	1.446
Cool:Depth	0.379	0.382	0.095	0.990	0.323	-0.375	1.133
Cool/warm:Depth	0.870	0.382	0.217	2.274	0.024	0.116	1.624
Warm:Depth	0.648	0.382	0.162	1.697	0.091	-0.105	1.401
Cool:NH <sub>4</sub>	-0.019	0.030	-0.040	-0.642	0.522	-0.078	0.040
Cool/warm:NH <sub>4</sub>	-0.048	0.030	-0.100	-1.623	0.106	-0.107	0.010
Warm: NH <sub>4</sub>	-0.041	0.030	-0.085	-1.380	0.169	-0.100	0.018
Cool:Chl- $\alpha$	-0.006	0.043	-0.010	-0.149	0.881	-0.091	0.079
Cool/warm:Chl- $\alpha$	-0.033	0.043	-0.049	-0.755	0.451	-0.118	0.052
Warm:Chl- $\alpha$	0.012	0.043	0.018	0.281	0.779	-0.072	0.096
Cool:Salinity	-0.005	0.008	-0.044	-0.594	0.553	-0.021	0.011
Cool/warm:Salinity	-0.002	0.008	-0.021	-0.283	0.777	-0.018	0.014
Warm:Salinity	-0.017	0.008	-0.157	-2.114	0.036	-0.033	-0.001
Cool:Temperature	0.007	0.017	0.026	0.444	0.658	-0.026	0.040
Cool/warm:Temperature	-0.014	0.017	-0.048	-0.836	0.404	-0.047	0.019
Warm:Temperature	-0.032	0.017	-0.111	-1.924	0.056	-0.065	0.0008
Cool:DO	0.139	0.051	0.166	2.723	0.007	0.039	0.240
Cool/warm:DO	0.085	0.051	0.101	1.654	0.010	-0.016	0.186
Warm:DO	0.080	0.050	0.093	1.565	0.119	-0.020	0.177
Cool:Turbidity	0.000	0.016	0.001	0.016	0.988	-0.031	0.031
Cool/warm:Turbidity	0.011	0.016	0.045	0.715	0.475	-0.020	0.042

Model Parameter	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Warm:Turbidity	0.008	0.016	0.031	0.502	0.616	-0.023	0.039

The coefficient plot of the species richness model demonstrated that salinity and depth had the largest negative effect on species richness (Fig. 20). Turbidity, DO, temperature, ammonia, and chlorophyll- $\alpha$  each had little effect on species richness. In the subset model, which excluded DO and temperature, demonstrated the same effect for each of the remaining variables (Table 6). When comparing the deviance of the two models using an ANOVA, it is evident that dissolved oxygen and temperature have little effect on overall fish species richness (Deviance = -0.474,  $p = 0.789$ ).



**Figure 20.** Coefficient plot of scaled coefficient estimates for the “full” and “subset” models for hypothesis 1. Response variable = species richness; Full model = Model 1; Subset model = Model 2 (missing DO and temperature).

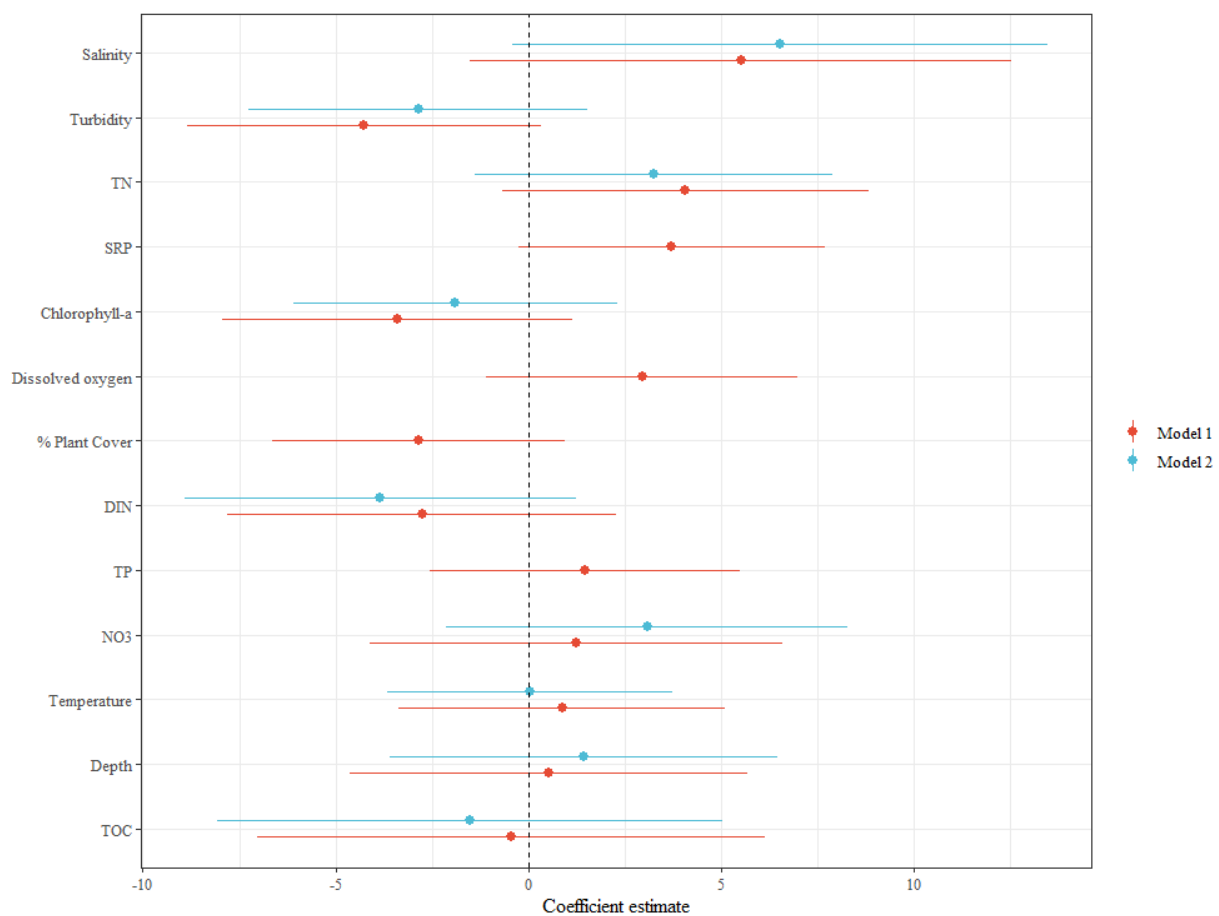
**Table 6.** Species richness generalized linear model summary for hypothesis 1 (McFadden's pseudo- $R^2 = 0.129$ , AIC = 462.58, response variable = species richness, distribution = Poisson, link function = logistic).

Model Parameter	Unstandardized Coefficients		Standardized Coefficients	z	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Intercept)	3.329	0.812	2.644	4.100	0.000	1.736	4.920
Depth	-0.244	0.241	-0.061	-1.014	0.311	-0.717	0.227
NH <sub>4</sub>	-0.006	0.019	-0.013	-0.339	0.734	-0.044	0.030
ChlA	-0.001	0.027	-0.001	-0.032	0.975	-0.054	0.051
Salinity	-0.014	0.005	-0.132	-2.766	0.006	-0.024	-0.004
DO	0.021	0.031	0.025	0.675	0.500	-0.039	0.081
Temperature	0.002	0.010	0.008	0.227	0.821	-0.018	0.023
Turbidity	0.001	0.010	0.003	0.082	0.934	-0.019	0.019

## Hypothesis 2

The coefficient plot of the full and subset models for hypothesis 2 (Fig. 21) demonstrated that salinity had the largest positive effect on percent periphyton cover and turbidity had the largest negative effect. In general, it was observed that chlorophyll- $\alpha$ , turbidity, DIN, TOC, and percent plant cover all had a negative effect on fish log(biomass), whereas depth, DO, salinity, NO<sub>3</sub>, TN, TP, SRP, and depth had an overall positive effect (Table 7).





**Figure 21.** Coefficient plot of scaled coefficient estimates for the “full” and “subset” models for hypothesis 2. Response variable = % periphyton cover; Full model = Model 1; Subset model = Model 2 (missing DO, TP, SRP, and % plant cover).

**Table 7.** Mixed effects model summary for hypothesis 2 (AIC = 1027.026, BIC = 1069.792, logLik = -497.513, response variable = % periphyton cover).

<i>Fixed Effects</i>						
Model Parameter	Standardized Coefficients		t	Sig.	95% Confidence Interval for Beta	
	Beta	Std. Error			Lower Bound	Upper Bound
(Intercept)	43.166	4.343	9.940	0.000	34.334	51.913
Temperature	0.874	2.158	0.405	0.687	-3.149	4.884
DO	2.954	2.064	1.431	0.156	-0.899	6.788
Salinity	5.516	3.579	1.541	0.127	-1.219	12.171
NO <sub>3</sub>	1.252	2.730	0.459	0.127	-3.831	6.328
Chl- $\alpha$	-3.401	2.321	-1.465	0.648	-7.714	0.916
Turbidity	-4.264	2.340	-1.822	0.071	-8.624	0.083
TN	4.076	2.429	1.678	0.097	-0.484	8.626
DIN	-2.762	2.573	-1.074	0.286	-7.553	2.019
TP	1.468	2.046	0.717	0.475	-2.382	5.271
SRP	3.711	2.027	1.831	0.070	-0.054	7.504
TOC	-0.447	3.365	-0.133	0.895	-6.800	5.812
Depth	0.532	2.629	0.202	0.840	-4.368	5.418
% Plant Cover	-2.835	1.937	-1.464	0.146	-6.448	0.847
<i>Random Effects</i>						
Groups		Name			Variance	
Year		(Intercept)			161.600	
Residuals					307.200	

In the subset model, which excluded DO, TP, SRP, and percent plant cover, we observed this same effect for each of the remaining variables. When comparing the two models using an ANOVA, there was a marginal significant difference between the two models, indicating that

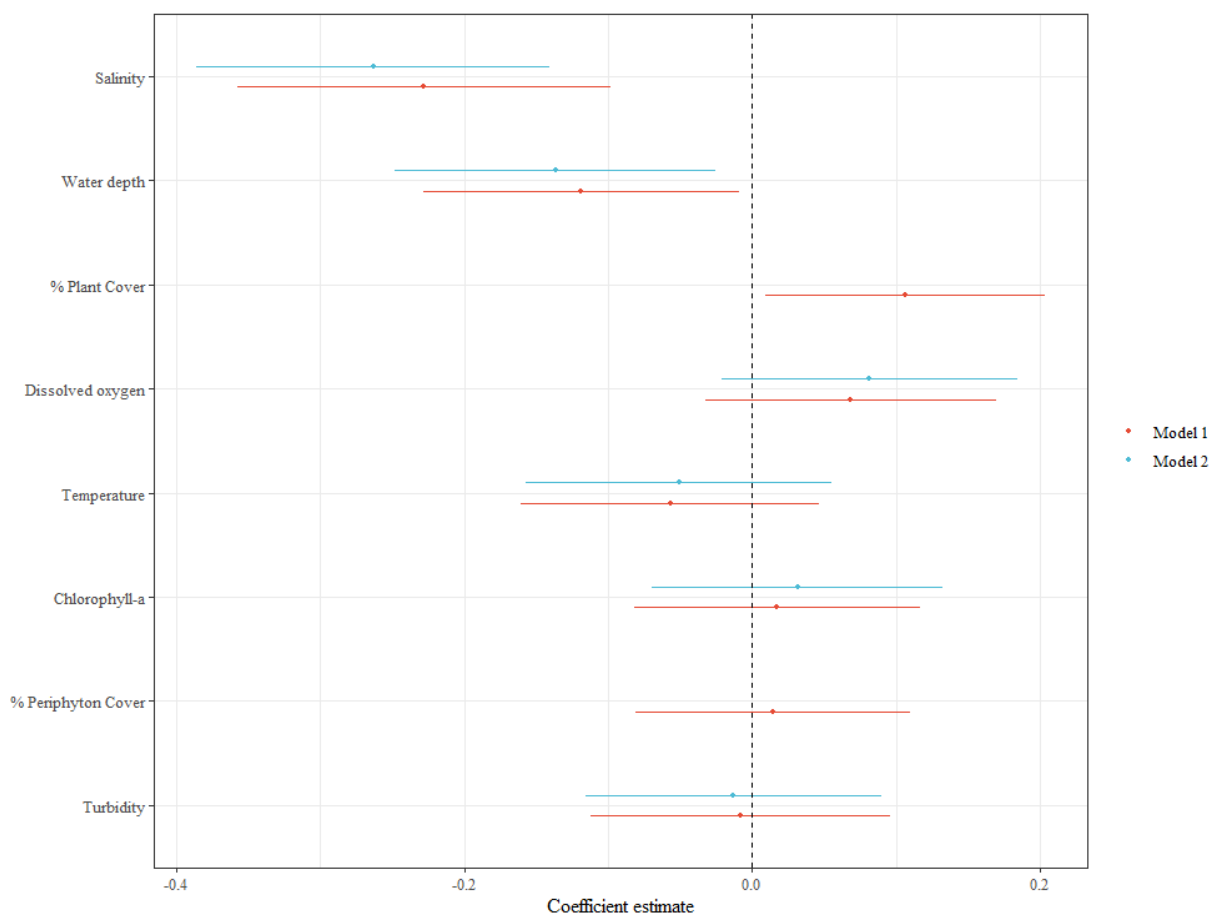
these four variables may account for some variation in percent periphyton cover, however other parameters are still necessary to better predict its growth in the Florida Everglades (Table 8;  $\chi^2 = 8.593$ ,  $p = 0.072$ ).

**Table 8.** ANOVA comparing hypothesis 2 “full” and “subset” models. Subset model is missing four key variables of interest (DO, TP, SRP, and % plant cover).

Model	# of parameters	AIC	BIC	logLik	Deviance	Chi-sq	Df	<i>p</i>
Full Model	16	1073.300	1118.000	-520.660	1041.300			
Subset Model	14	1073.900	1107.500	-524.960	1049.900	8.593	4	0.072

### Hypothesis 3

The coefficient plot of the full and subset models for hypothesis 3 (Fig. 22) demonstrated that salinity had the largest negative effect on fish log(biomass) and percent plant cover had the largest positive effect. In general, it was observed that turbidity, salinity, turbidity, temperature, and depth all had a negative effect on fish log(biomass), whereas DO, chlorophyll- $\alpha$ , percent periphyton cover, and percent plant cover had an overall positive effect (Table 9).



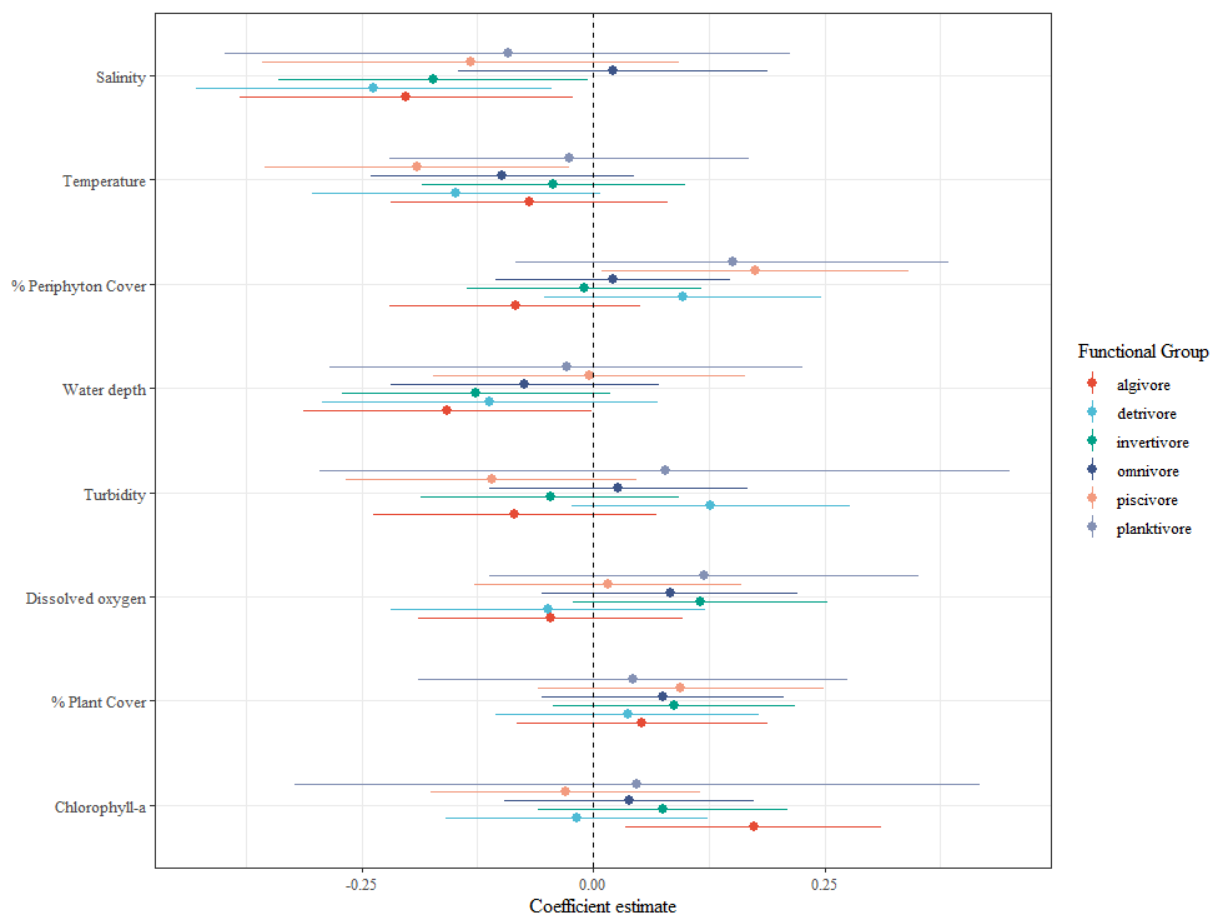
**Figure 22.** Coefficient plot of scaled coefficient estimates for the “full” and “subset” models for hypothesis 3. Response variable = log(biomass); Full model = Model 1; Subset model = Model 2 (missing % plant and periphyton cover).

**Table 9.** All fish model summary for hypothesis 3 ( $R^2 = 0.373$ ,  $F = 5.422$ ,  $p < 0.0001$ , response variable =  $\log(\text{biomass})$ ).

Model Parameter	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Intercept)	0.748	0.558	0.111	1.342	0.184	-0.363	1.860
Temperature	-0.016	0.015	-0.057	-1.092	0.278	-0.046	0.013
DO	0.057	0.042	0.069	1.351	0.181	-0.027	0.140
Salinity	-0.025	0.007	-0.228	-3.507	0.001	-0.038	-0.011
Chl- $\alpha$	0.011	0.033	0.017	0.347	0.730	-0.054	0.076
Turbidity	-0.002	0.013	-0.008	-0.154	0.878	-0.028	0.024
Depth	-0.008	0.004	-0.119	-2.157	0.034	-0.015	-0.001
% Periphyton Cover	0.001	0.003	0.015	0.306	0.761	-0.004	0.006
% Plant Cover	0.012	0.006	0.107	2.188	0.032	0.001	0.023

In the subset model, which excluded percent periphyton and plant cover, we observed this same effect for each of the remaining variables. When comparing the two models using an ANOVA, there was a slight significant difference between the two models, indicating that percent periphyton and plant cover may account for a significant impact on fish biomass ( $F = 3.094$ ,  $p = 0.051$ ).

The coefficient plot for the functional group level model (Fig. 23) demonstrated that salinity, temperature, and percent periphyton cover have the largest effect on fish  $\log(\text{biomass})$ . Intergroup differences in effect size of a given variable were also observed, with the sign of the relationship being different in many cases. That being said, taken into consideration the 95% confidence intervals of the coefficient estimates, group differences significantly overlap (Fig. 23; Table 10). This overlap was supported by the PCA plot output, which showed nearly 100% overlap in cluster ellipses of functional groups (Fig. 24). Variation in these ellipses (stretch by the second PCA axis) appears to be driven by salinity and depth.



**Figure 23.** Coefficient plot of scaled coefficient estimates for hypothesis 3, broken down by functional group. Response variable = log(biomass).

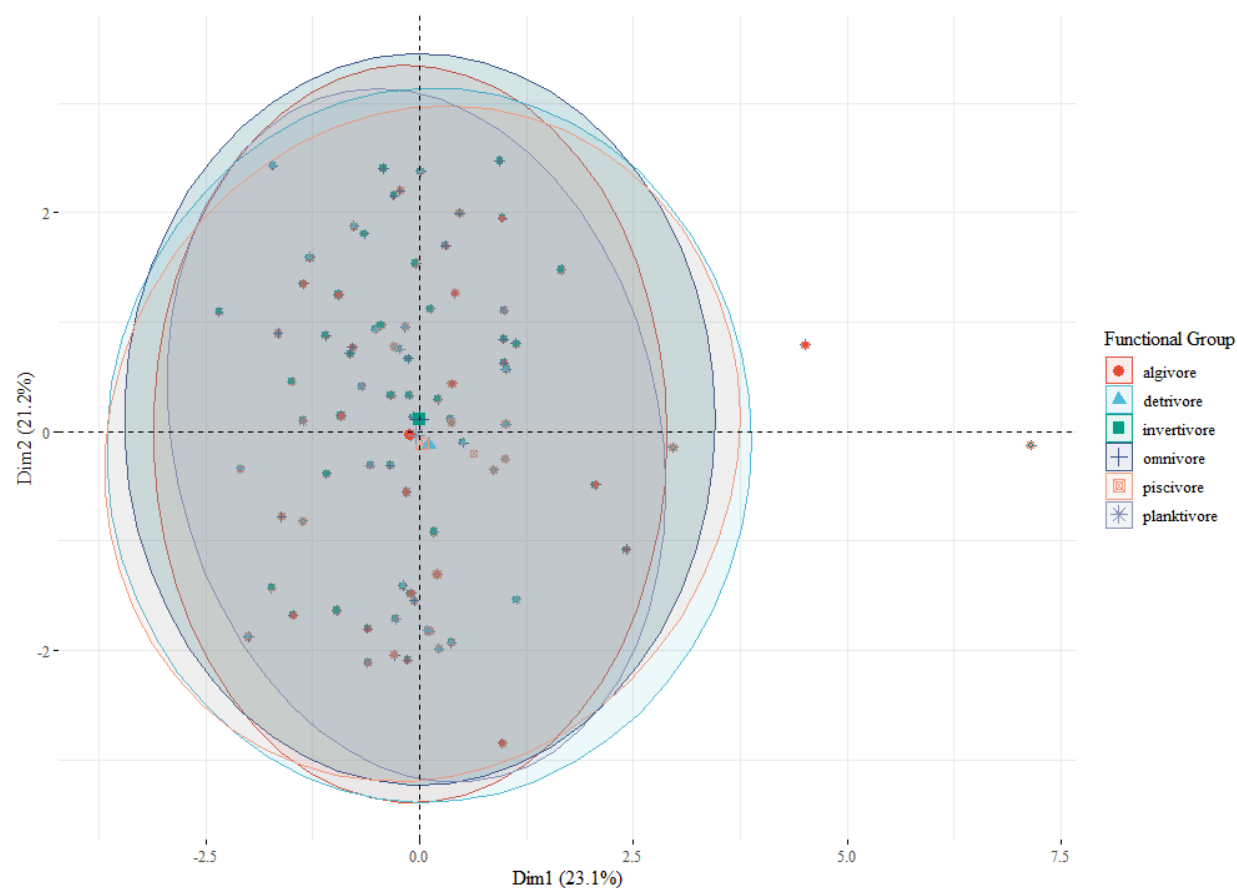
**Table 10.** Functional group level model summary ( $R^2 = 0.82$ ,  $F = 28.85$ ,  $p < 0.0001$ , response variable =  $\log(\text{biomass})$ ).

Model Parameter	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Algivore	0.189	0.782	-1.362	0.242	0.809	-1.348	1.726
Detrivore	0.958	0.946	-0.736	1.012	0.312	-0.904	2.819
Invertivore	0.075	0.757	-0.174	0.099	0.921	-1.414	1.565
Omnivore	-0.868	0.757	-1.025	-1.146	0.253	-2.358	0.622
Piscivore	0.208	0.877	-0.927	0.237	0.813	-1.516	1.932
Planktivore	-2.257	1.391	-1.540	-1.622	0.106	-4.993	0.480
Algivore:Depth	-0.011	0.005	-0.157	-1.980	0.049	-0.021	0.000
Detrivore:Depth	-0.008	0.006	-0.112	-1.211	0.227	-0.020	0.005
Invertivore:Depth	-0.009	0.005	-0.126	-1.720	0.086	-0.019	0.001
Omnivore:Depth	-0.005	0.005	-0.074	-1.002	0.317	-0.015	0.005
Piscivore:Depth	0.000	0.006	-0.004	-0.051	0.960	-0.012	0.011
Planktivore:Depth	-0.002	0.009	-0.029	-0.222	0.825	-0.019	0.016
Algivore:Chl- $\alpha$	0.114	0.046	0.173	2.472	0.014	0.023	0.205
Detrivore:Chl- $\alpha$	-0.012	0.047	-0.018	-0.245	0.807	-0.105	0.082
Invertivore:Chl- $\alpha$	0.050	0.045	0.075	1.097	0.273	-0.039	0.138
Omnivore:Chl- $\alpha$	0.026	0.045	0.039	0.575	0.566	-0.063	0.115
Piscivore:Chl- $\alpha$	-0.020	0.049	-0.030	-0.402	0.688	-0.115	0.076
Planktivore:Chl- $\alpha$	0.031	0.124	0.048	0.253	0.800	-0.213	0.275
Algivore:Salinity	-0.023	0.010	-0.202	-2.209	0.028	-0.043	-0.002
Detrivore:Salinity	-0.026	0.011	-0.237	-2.428	0.016	-0.048	-0.005
Invertivore:Salinity	-0.019	0.009	-0.172	-2.024	0.044	-0.038	-0.001
Omnivore:Salinity	0.002	0.009	0.021	0.248	0.804	-0.016	0.021
Piscivore:Salinity	-0.015	0.013	-0.132	-1.150	0.251	-0.040	0.010
Planktivore:Salinity	-0.010	0.017	-0.092	-0.592	0.554	-0.044	0.024

Model Parameter	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Algivore:% Plant Cover	0.006	0.008	0.053	0.763	0.446	-0.010	0.022
Detrivore:% Plant Cover	0.004	0.009	0.037	0.514	0.607	-0.012	0.021
Invertivore:% Plant Cover	0.010	0.008	0.087	1.313	0.190	-0.005	0.026
Omnivore:% Plant Cover	0.009	0.008	0.075	1.130	0.259	-0.007	0.024
Piscivore:% Plant Cover	0.011	0.009	0.095	1.205	0.229	-0.007	0.029
Planktivore:% Plant Cover	0.005	0.014	0.043	0.369	0.713	-0.022	0.033
Algivore:% Periphyton Cover	-0.004	0.004	-0.084	-1.225	0.221	-0.012	0.003
Detrivore:% Periphyton Cover	0.005	0.004	0.097	1.272	0.204	-0.003	0.013
Invertivore:% Periphyton Cover	0.000	0.003	-0.009	-0.141	0.888	-0.007	0.006
Omnivore:% Periphyton Cover	0.001	0.003	0.021	0.333	0.740	-0.006	0.008
Piscivore:% Periphyton Cover	0.009	0.005	0.176	2.083	0.038	0.001	0.018
Planktivore:% Periphyton Cover	0.008	0.006	0.150	1.261	0.208	-0.004	0.021
Algivore:Temperature	-0.019	0.021	-0.069	-0.904	0.367	-0.061	0.023
Detrivore:Temperature	-0.041	0.022	-0.148	-1.863	0.063	-0.085	0.002
Invertivore:Temperature	-0.012	0.020	-0.043	-0.593	0.554	-0.052	0.028
Omnivore:Temperature	-0.027	0.020	-0.098	-1.354	0.177	-0.067	0.012
Piscivore:Temperature	-0.053	0.023	-0.190	-2.279	0.023	-0.100	-0.007
Planktivore:Temperature	-0.007	0.028	-0.025	-0.256	0.798	-0.062	0.047

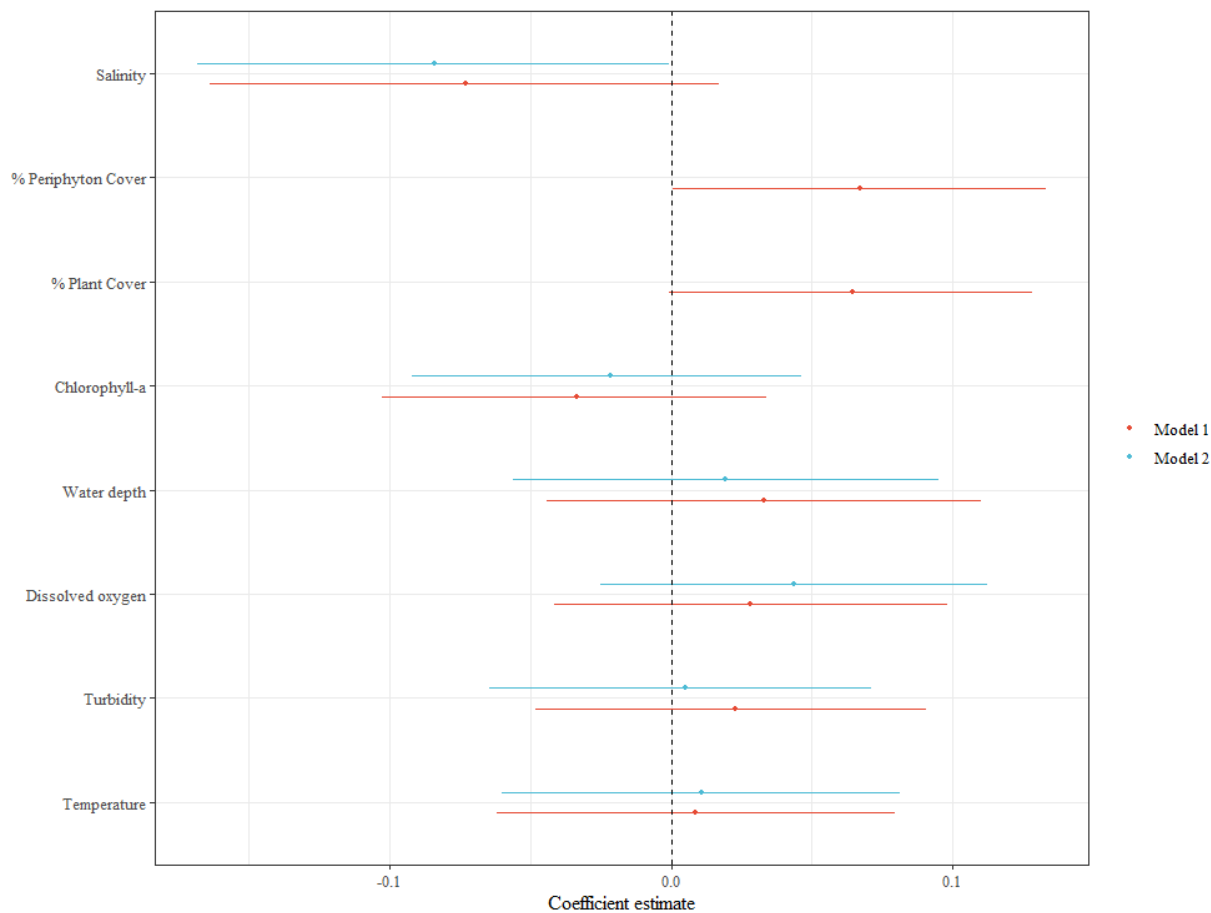


Model Parameter	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Algivore:DO	-0.038	0.061	-0.046	-0.628	0.530	-0.158	0.081
Detrivore:DO	-0.040	0.073	-0.048	-0.558	0.577	-0.183	0.102
Invertivore:DO	0.097	0.059	0.116	1.657	0.099	-0.018	0.213
Omnivore:DO	0.070	0.059	0.083	1.185	0.237	-0.046	0.185
Piscivore:DO	0.013	0.062	0.016	0.217	0.828	-0.108	0.134
Planktivore:DO	0.100	0.099	0.119	1.013	0.312	-0.094	0.295
Algivore:Turbidity	-0.021	0.019	-0.085	-1.091	0.276	-0.059	0.017
Detrivore:Turbidity	0.032	0.019	0.127	1.672	0.095	-0.006	0.069
Invertivore:Turbidity	-0.012	0.018	-0.046	-0.651	0.516	-0.046	0.023
Omnivore:Turbidity	0.007	0.018	0.027	0.385	0.701	-0.028	0.042
Piscivore:Turbidity	-0.027	0.020	-0.110	-1.372	0.171	-0.067	0.012
Planktivore:Turbidity	0.019	0.047	0.078	0.411	0.682	-0.074	0.112



**Figure 24.** Principal component analysis plot for hypothesis 3, broken down by functional group. Response variable =  $\log(\text{biomass})$ . Colour of points and ellipses indicates functional group.

The coefficient plot of the full and subset models for hypothesis 3 (Fig. 25) demonstrated that salinity had the largest negative effect on species richness, whereas percent periphyton and plant cover had the largest positive effect. In general, it was observed that salinity, and chlorophyll- $\alpha$  had a negative effect on species richness, whereas temperature, DO, percent periphyton cover, percent plant cover, water depth, and turbidity had an overall positive effect (Table 11).



**Figure 25.** Coefficient plot of scaled coefficient estimates for the “full” and “subset” models for hypothesis 3. Response variable = species richness; Full model = Model 1; Subset model = Model 2 (missing % plant and periphyton cover).

**Table 11.** Species richness generalized linear model summary for hypothesis 3 (McFadden's pseudo- $R^2 = 0.260$ , AIC = 447, response variable = species richness, distribution = Poisson, link function = logistic).

Model Parameter	Unstandardized Coefficients		Standardized Coefficients	z	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Intercept)	2.239	0.395	2.628	5.671	0.000	1.464	3.011
Temperature	0.003	0.010	0.009	0.246	0.806	-0.018	0.023
DO	0.023	0.029	0.028	0.791	0.429	-0.034	0.081
Salinity	-0.008	0.005	-0.073	-1.585	0.113	-0.018	0.002
Chl- $\alpha$	-0.022	0.023	-0.033	-0.959	0.337	-0.067	0.022
Turbidity	0.006	0.009	0.023	0.645	0.519	-0.012	0.023
Depth	0.002	0.003	0.033	0.834	0.404	-0.003	0.007
% Periphyton Cover	0.004	0.002	0.067	1.983	0.047	0.000	0.007
% Plant Cover	0.007	0.004	0.064	1.955	0.051	0.000	0.015

In the subset model, which excluded percent periphyton and plant cover, we observed this same effect for each of the remaining variables. When comparing the two models using an ANOVA, there was a deviance of -12.66 ( $p = 0.002$ ), meaning periphyton and plant cover significantly affects species richness positively, even when other explanatory variables are accounted for.

## Discussion

### Interpretation of results

#### Hypothesis 1

The results of the analysis of hypothesis 1 demonstrate that depth had the largest effect on fish biomass. This relationship demonstrated that as depth increases, so does fish biomass. The variable with the next largest impact on fish biomass was ammonia, which had a significant negative relationship with fish biomass. The coefficients of each variable demonstrate that the effect size between variables differs. In support of our hypothesis, temperature and DO had a significant impact on fish biomass. Temperature and DO demonstrated a slight negative and

positive relationship with fish biomass, respectively. When comparing this full model to the subset model which excluded temperature and DO, we confirmed that these variables in combination significantly impacted fish biomass (ANOVA:  $F = 4.20$ ,  $p = 0.02$ ). The negative relationship between temperature and fish biomass, combined with the positive relationship between DO and fish biomass could be attributed to an increase in temperature due to climate change, which reduces DO and limits the carrying capacity of an aquatic environment to support a larger fish community (Farwell et al., 2007). Water depth can be an important characteristic of aquatic ecosystems as well. Spatial variation can influence habitat use by prey fish and their predators, which results in an ecosystem that can support many niches (Gelwick et al., 1997).

Interguild differences in variable effect size were also observed, though none of these differences were significant. The lack of significant differences between guilds is likely due to the fact that though these thermal guilds are the optimal temperature for each species, these species reside in the same study area and are experiencing more or less similar environmental conditions. Overall, the results of the models created to test hypothesis 1 provide support for the suggested hypothesis.

## Hypothesis 2

The results of the analysis of hypothesis 2 demonstrate that salinity had the largest effect on percent periphyton cover. This relationship demonstrated that as salinity increases, so does periphyton cover. This was the opposite effect from what we predicted. While we cannot confirm why this is the case, we can theorize it may be due to the species of periphytic algae which were growing in the study area. Mazzei et al. (2018) found that freshwater periphyton species exposed to 1 ppt increase in salinity, were negatively affected with reduced productivity and nutrient sequestration into their tissues. Whereas, brackish water species were much more tolerant of such salinity changes, and that marine periphyton species actually produce at faster rates in certain salinities. The variable with the next largest impact on periphyton was turbidity, which had a marginally significant negative relationship with percent periphyton cover. This relationship was to be expected, since as turbidity increases, light penetration into the water column decreases, reducing photosynthetic rates of primary producers, such as periphyton.

The coefficients of each variable demonstrate that the effect size between variables differs. In support of our hypothesis, SRP, and to a lesser degree, TP, had a slight positive impact on periphyton cover. Contrary to our predictions however, increasing DO increased periphyton cover, whereas increasing plant cover decreased periphyton cover. While the effects of nutrients such as phosphorus are well understood in the literature, due to their utilization by primary producers for growth, DO and plant cover's effects on periphyton are much more contested. In the case of plant cover in particular, there are many positive (mutualistic) and negative (competitive) factors which play out when periphyton interacts with macrophytes (i.e., attaches to their leaf or stem surfaces) (Wetzel, 1983). When comparing this full model to the subset model however, it was found that these four variables only provided marginally significant

information on periphyton cover on their own ( $\chi^2 = 8.593$ ,  $p = 0.072$ ). Further exploration of the literature shows that this is usually the case with the effect of water quality parameters on periphyton and/or plant growth. Rather than one single variable having a large effect size, many variables appear to have marginal effects, which fluctuate in importance over temporal and spatial scales (Hillebrand and Kahlert, 2001). Overall, the results of the models created to test hypothesis 2 provide some support for our suggested hypothesis, however single abiotic characteristics were not sufficient to explain relative importance to overall periphyton growth.

### Hypothesis 3

The results of the analysis of hypothesis 3 demonstrate that as periphyton and plant cover increases, so too does fish biomass and richness. Such a relationship is to be expected, given the “pyramid” structure of almost all ecosystem food webs. Periphyton and plants are primary producers, who form the base of the Everglades food web. The energy they produce through photosynthesis, ultimately cascades up the food web to the higher trophic levels, which includes fish. The more primary producers that are available, the more energy there is for fish to reproduce and co-exist with other species. When comparing this full model to the subset model which excluded percent periphyton and plant cover, we confirmed that these variables, in combination with one another, significantly impacted fish biomass in particular (ANOVA:  $F = 3.094$ ,  $p = 0.051$ ).

Functional group differences in variable effect size were also observed and highly variable (shifting sign from one group to another), though none of these differences were significant (Table 10; Fig. 25). The lack of significant differences between functional groups is likely due to the fact that these groups were generated based on the main food source of a given species. However, in reality, a vast majority of the species within the Everglades are generalist feeders, and therefore feed “across” functional groups, which dampened differences across groups. Overall, the results of the models created to test hypothesis 3 provide support for the suggested hypothesis.

### Significance

Understanding the abiotic factors which influence fish biomass and diversity is critical knowledge for ecosystem management practices to be most effective at sustaining target fish populations. Assessing past and current effects of variables such as temperature is also critical to predict potential future effects of climate change and their subsequent effect on fish populations. Additionally, evaluating factors which impact periphyton and other primary producers is also critical to understanding the stability of the aquatic system being studied. Finally, Everglades National Park includes a variety of ecosystems and in turn, provides an important habitat for wildlife. Long-term investigation of ecological trends is imperative for the conservation of one of America’s largest National parks.

## Future Steps

We recommend greater communication between research project leaders to allow greater data integration the ability to draw more powerful and ecologically relevant conclusions. This would include the designation of standard site IDs and sampling procedures, as well as sampling abiotic and biotic factors concurrently with one another to improve consistency and ecological relevance of the data. Additionally, continued data collection would make for greater temporal and spatial resolution specific to the Florida Everglades over time. Finally, though this data was not collected by us in conjunction with our research projects, the analysis we completed in this report will allow each of us to utilize the learned skills and methods which can be applied to our own research.

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