**Ocean Acidification: Predator-Prey Interactions of Intertidal Snails and Sea Stars**

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Virginia Commonwealth University Spring 2022

*Lesson Duration: ~ 30-40 minutes*

The purpose of this lesson is to explore the environmental issue of ocean acidification utilizing a study focusing on predator-prey interactions and lowered seawater pH. Students will explore data from a study conducted by Jellison et al. (2016) in order to understand generalized linear models, their uses, and how to interpret their output using the R programming language. At the completion of this lesson, students will have a better understanding of this quantitative skill and a better understanding of how lowered pH due to ocean acidification may alter trophic interactions in marine ecosystems.

**Learning Objectives:** Students should be focused on understanding the following topics. At the completion of the lesson, students should be able to answer the following questions.

**Environmental Studies**

*Conservation Component*

*Section Duration: ~ 15-20 minutes*

**Students should have:**

* Rudimentary background in ecology or environmental studies

**Understand the environmental and conservation issue of ocean acidification, its causes and dangers, and how a lowered pH may alter predator-prey interactions**

* What is ocean acidification? What are its causes, and what dangers can it pose on marine ecosystems?
* What is the predator-prey interaction focused on in the focal paper? How might interactions like this affect marine ecosystems?

**Quantitative Skills**

*Statistical Component*

*Section Duration: ~ 15-20 minutes*

**Students should have:**

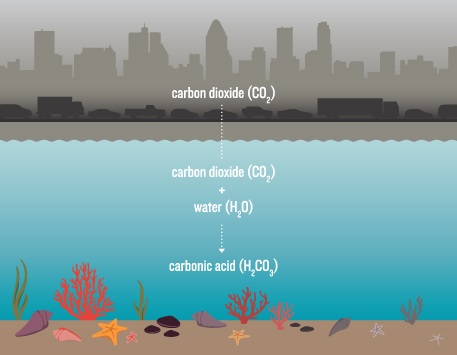
* R and R studio previously installed
* Basic understanding of R programming language for statistical computing and graphics

**Understand generalized linear models and related components using the R programming language by utilizing data from the focal paper**

* Are the data provided normally distributed, and what does this have to do with the utilization of a generalized linear model?
* What is the difference between a linear model and a generalized linear model?
* What are the components of generalized linear models?
* How can we interpret the generalized linear model output from R, and how does this compare to the focal paper’s findings

**Environmental Studies**

Encourage students to read through and understand each section as there will be assessment questions at the end of the lesson. Highlighted text provides hints for information that will help with assessment questions.

**Ocean Acidification**

Approximately one third of the carbon dioxide (CO2) emitted through anthropogenic activities enters the ocean, driving ocean acidification. Ocean acidification is characterized by an increase in aqueous CO2 and results in reduced seawater pH. Ocean acidification is just one aspect of global climate change, which is not surprising as CO2 is largely to blame for most contemporary climate change-related issues.

Acidification occurs when CO2 is absorbed into the water at a high rate, reacting with H20 molecules (water) to form carbonic acid (H2CO3). Once this compound breaks down, hydrogen ions (H+) and bicarbonate (HCO3-) are released. The hydrogen ions are the molecules that cause a decrease in the ocean’s pH (Climate Interpreter). With the increase of carbon dioxide (CO2) in the atmosphere, the pH of ocean waters has decreased by 0.1 pH units. This decrease marks an approximately 30% increase in acidity, making the ocean’s average pH now 8.1 (NOAA). Though 8.1 is still considered basic according to the pH scale, the ocean will continue to absorb more CO2 with continuing human-driven increased levels of CO2 in the atmosphere.

Two major sources that play large roles in the increasing CO2 being pumped into our atmosphere include fossil fuel emissions and deforestation (Climate Interpreter). The most commonly known sources of fossil fuel emissions include automobiles, airplanes, and factories that actively burn fossil fuels (i.e., coal, oil, gas). Similar to burning fossil fuels in factories, deforestation also emits large amounts of CO2 into the atmosphere. What’s more, forests are responsible for the intake of carbon dioxide through the process of photosynthesis. Deforestation is an activity that creates increased CO2 while preventing CO2 intake, further contributing to the rapid influx of CO2 into our atmosphere driving ocean acidification.

**Want to Learn More?**

For more information on climate change and what is being done to address its significant impacts, please refer to the United Nations’ page on [Climate Action: Tackling Climate Change](https://www.un.org/sustainabledevelopment/climate-action/).

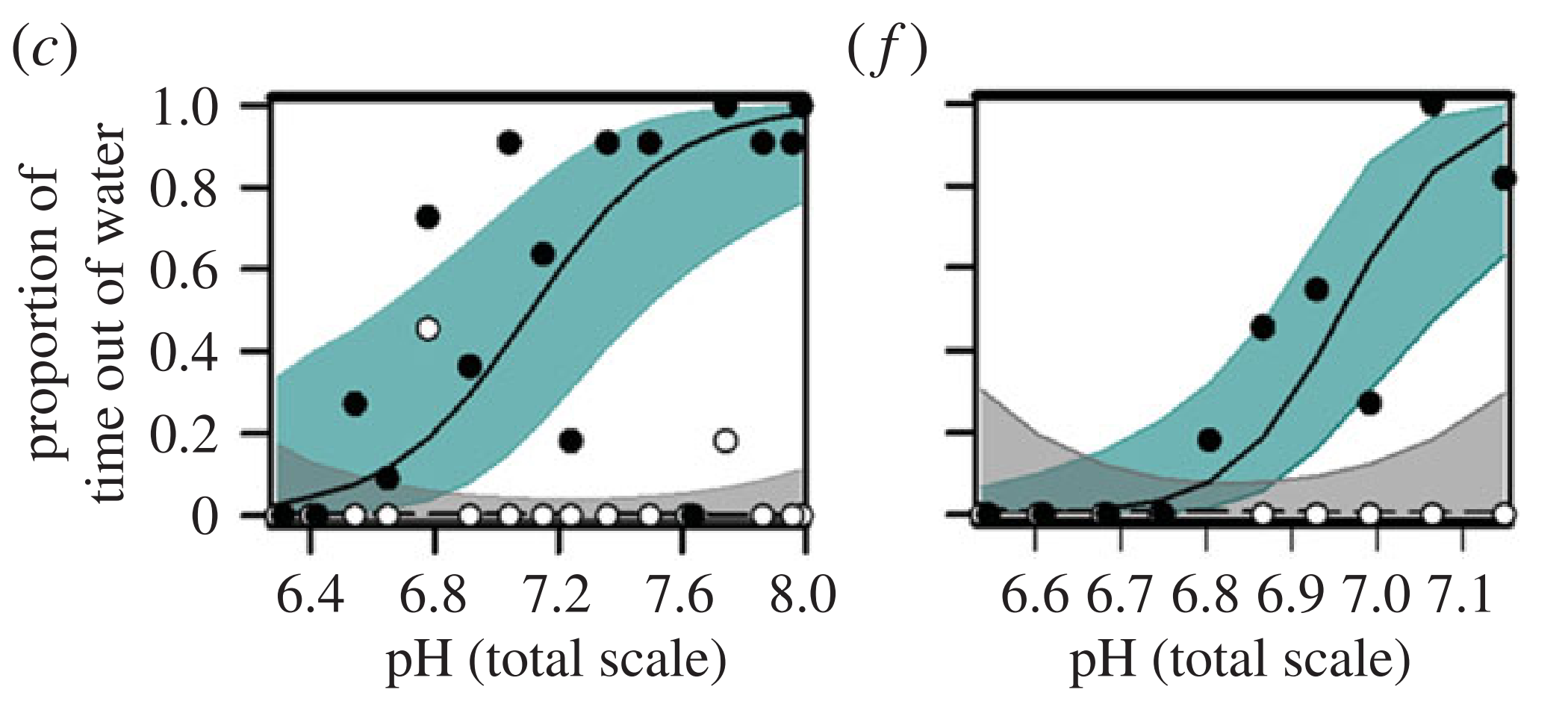
What can be done about to combat ocean acidification? As ocean acidification is an aspect of global climate change, any conservation efforts done to mitigate climate change will benefit the ocean as a whole. As the National Oceanic and Atmospheric Administration (NOAA) states, sustained efforts to monitor ocean acidification are only beginning as we look to understand the effects of the changing ocean chemistry. As more specific effects are studied and discovered, conservation efforts can become more specialized to tailor to specific effects. What we can do now, according to the United Nations is combat global climate change through general conservation efforts: investments to accelerate decarbonization and investing in sustainable solutions (i.e., decrease fossil fuel subsidies and increase investment in clean energy with the promotion of responsible consumption and production).

**Ecological Effects: Predator-Prey Interactions**

Previous studies have shown that reduced seawater pH results in physiological and demographic responses in marine organisms, contributing to the potential for profound ecological shifts. Of these responses, a distinct ecological effect includes the ability of ocean acidification to alter interactions between predators and their prey, predator-prey interactions. Consequently, disturbance of these interactions could create detrimental consequences for trophic interactions within food web systems in aquatic ecosystems.

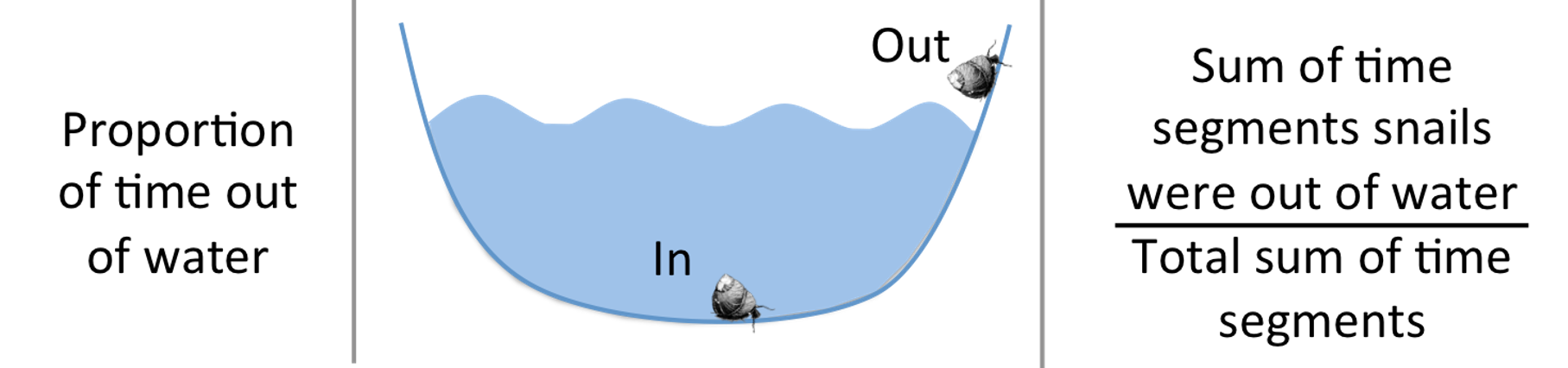
The importance of trophic interactions in marine ecosystems will become clearer in the next section of this lesson to discuss our focal paper, “Ocean Acidification Alters the Response of Intertidal Snails to a Key Sea Star Predator” by Jellison et al.

**Focal Paper**



“Ocean Acidification Alters the Response of Intertidal Snails to a Key Sea Star Predator” by Jellison et al. (2016), explores the relevance of ocean acidification effects on coastal invertebrate predator-prey relationships. The authors of this study wanted to observe how ocean acidification can affect individual marine organisms by examining how the responses of the black turban snails to predator cues were affected when met with a decreased pH, mimicking the effect of ocean acidification. In this way, the researchers studied a specific predator-prey interaction to better understand how ocean acidification could negatively affect trophic interactions within aquatic ecosystems. By observing anti-predator behaviors of *T. funebralis* (black turban snails) in varying levels of pH, the authors found that behavioral responses in these snails were impaired when individuals experienced reductions in seawater pH (seen in figure 4c from the focal paper).

**From Paper: Figure 4c. Effects of seawater pH during constant pH experiment on proportion of time out of water in the presence and absence of predator cue**

****To understand the authors’ findings, we will explore the chemical ecology of predator-prey interactions. Specifically discussed in the paper are flight responses, namely, a cue-mediated behavior observed in the studied species. Responses to chemical alarm cues have been shown to have survival benefits for the prey, such as antipredator responses like area avoidance (Ferrari et al., 2010). The organisms studied in the focal paper were *T. funebralis* (black turban snails) and *P. ochraceus* (purple sea star). In their relationship, the black turban snail is the prey, and the purple sea star is the predator. Purple sea stars emit a chemical cue as predators. In response to the presence of this cue, black turban snails typically partake in area avoidance by seeking refuge (i.e., exiting the water). These snails typically crawl up and out of pools to avoid and escape from their sea star predators. Researchers focused on this specific predator-prey interaction while under 16 different levels of seawater pH ranging from present levels to those expected for rocky intertidal pools by the year 2100, 6.42-7.99 pH units. The authors found that more acidic waters led to an impairment in the black turban snails’ escape response, shown through a marked decline in proportion of time out of water when predator cue was present, an escape response in which the snails would seek refuge. When pH levels fell to 7.1 and below, the snails failed to implement their escape response.

**Source:** [**Young Investigators Review**](https://upload.wikimedia.org/wikipedia/commons/a/ab/Pisaster_ochraceus_%28purple_sea_star_or_ochre_sea_star%29_%282132256087%29.jpg)

**Source:** [**Central Coast Biodiversity**](https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.centralcoastbiodiversity.org%2Fblack-turban-snail-bull-tegula-funebralis.html&psig=AOvVaw2axHHouSvZNhRH4TJNs7yD&ust=1646289332454000&source=images&cd=vfe&ved=0CAsQjRxqFwoTCLCV9-7npvYCFQAAAAAdAAAAABAD)

**Data Introduction**

We will focus on the data for proportion of time out of water for this lesson as these data operate as an index of a fully realized avoidance response, distinguishing between flight behaviors exhibited by the black turban snails that led to refuge-seeking or lack thereof (Jellison et al., 2016). These data were gathered by observing pictures captured every two minutes for a total of 28 minutes per individual within their laboratory arenas. The proportion of time out of water data were quantified by noting the fraction of the two-minute assessment intervals that a snail was above the water. As previously stated, the authors found that low pH strongly modified the ability of the snails to respond to predation risk, shown through a decline in the proportion of time out of water when predator cue was present at these lower levels of pH.

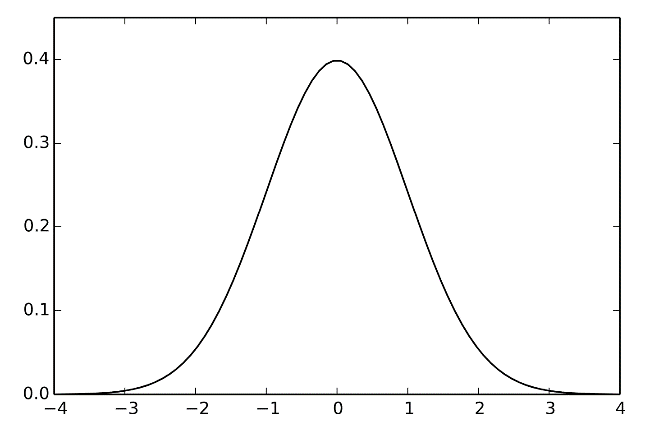
**From Paper: Figure S1. Proportion of time out of water metric**

**Quantitative Skills**

See the provided R Markdown document with accompanying R code for this portion of the lesson. The beginning code should contain the addition of packages and the inputting of our required data. Assist students as needed to ensure that everyone is able to follow the code.

In this lesson, you will receive an introduction to generalized linear models (GLMs) for data with non-normal distributions using data from the study conducted by Jellison et al. (2016) available through Dryad. We will cover why you might use a GLM, the components of a GLM, and go through a tutorial with data from the focal paper in order to understand output from a GLM using the R programming language. We will then conclude with a tutorial on how to produce visualization of the data used in the GLM modelling.

**Example of Gaussian (normal) distribution**

**Generalized Linear Models**

Some data follow a normal distribution, but not all data. If you want to model data, you can do so with a linear model (LM) if your data follow a Gaussian distribution (normal distribution), or if you are able to transform your data to reflect a normal distribution. However, you may not be able to transform your data to create a normal distribution, or it may be easier to utilize a different modelling method. If your data are not normally distributed or cannot be transformed to follow a normal distribution, GLMs come in handy. Non-normal distributions include, but are not limited to, Poisson, binomial, and exponential.

The first component of GLMs we will discuss is the family; the family of a GLM is the distribution of the response variable. The family is also known as the random component. The response variable is the variable that changes or “responds” as a result of an applied explanatory variable, the variable that is changed and “explains” the resulting response. In an LM, a linear regression model, the family is Gaussian as the response variable in an LM exhibits a normal distribution. As previously stated, non-normal distributions, common families, that GLMs are useful for modelling include Poisson, binomial, and exponential. The second component in our discussion is the linear predictor, also referred to as the systematic component, and this describes the linear function of the regressors in which the regressors are functions of the explanatory variable or variables. Lastly, the link function is the “link” or relation between the first and second components of the GLM, the response variable and the linear predictor.

**Components of GLMs:**

1. Family
2. Linear Predictor
3. Link Function

****It may be easier to see and understand these components by going through the following tutorial in R using data from the focal paper.

**linear predictors**

**link function**

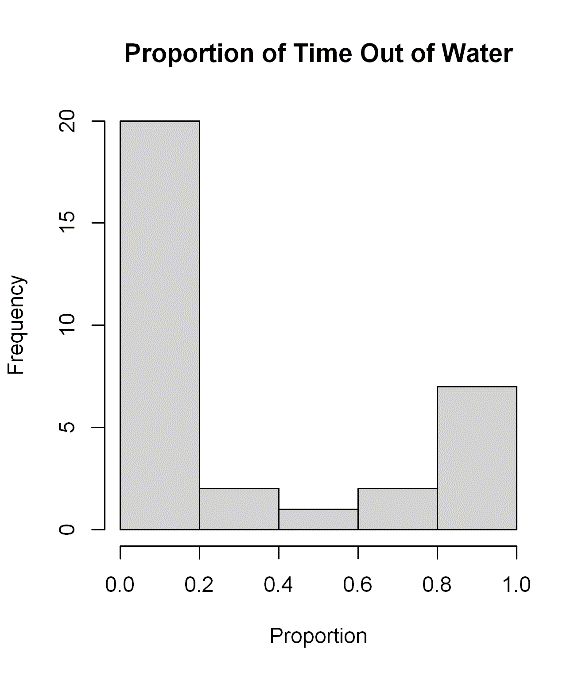
**family**

**Modelling Data from the Focal Paper**

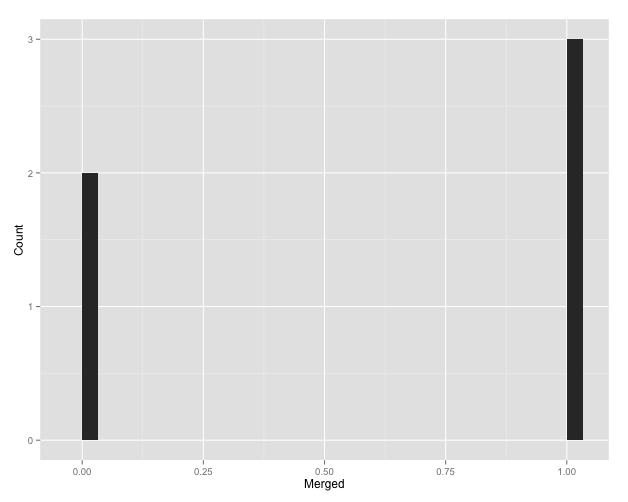
**Mixed Models:**

Random effects are grouping variables, always categorical. These variables typically cause variation in the system and are used to account for noise and grouping effects.

In “Ocean Acidification Alters the Response of Intertidal Snails to a Key Sea Star Predator” by Jellison et al. (2016), the researchers utilized a generalized linear mixed-effects model (GLMM) with a binomial distribution, logit link function, with snail included as a random effect to account for repeated measures and over-dispersion. For our purposes, because the data obtained from Dryad does not include repeated measures, we will repeat this assessment with a GLM as there are no random effects without these repeated measures.

You may choose to follow along using the R markdown document provided to accompany this lesson.

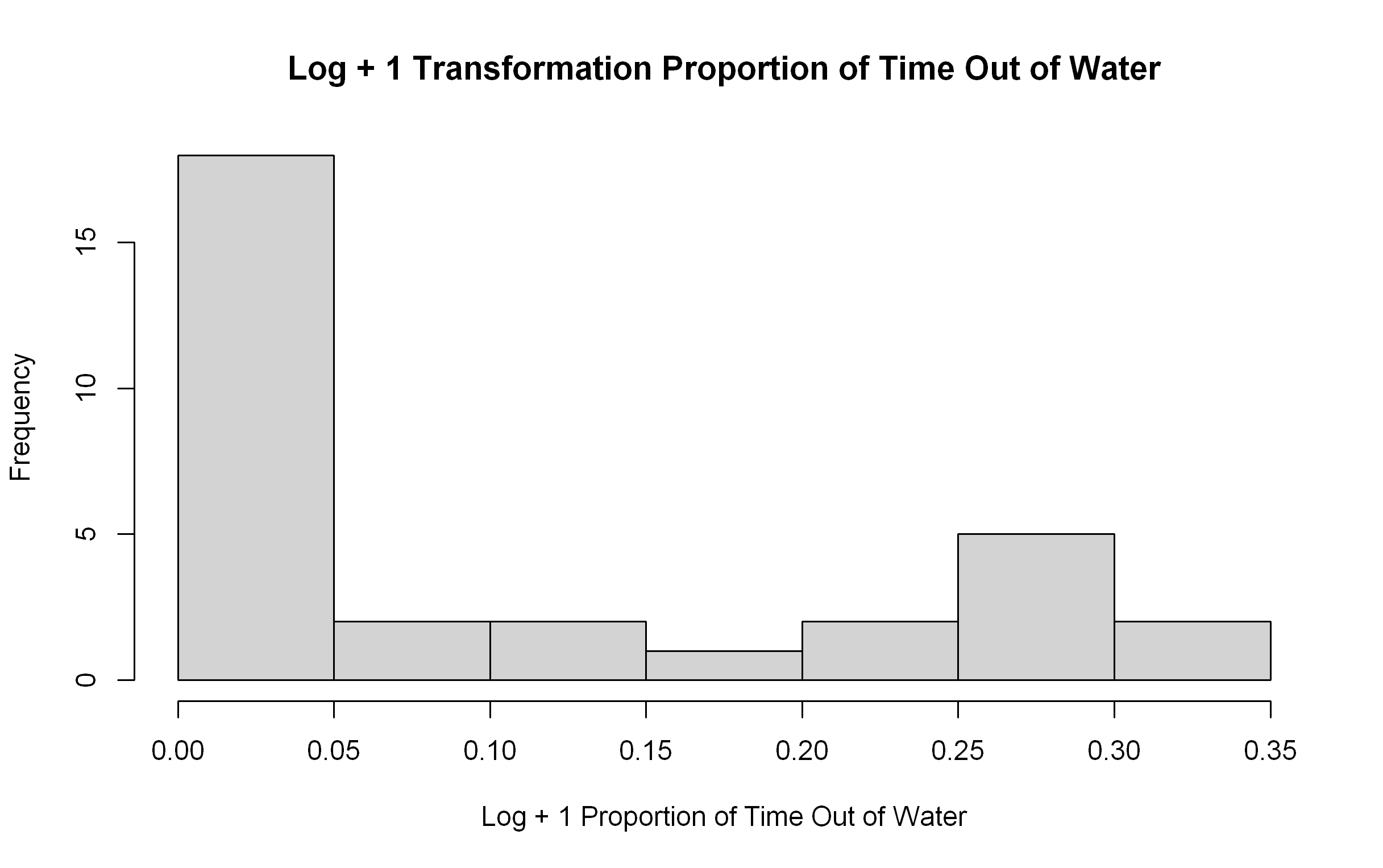
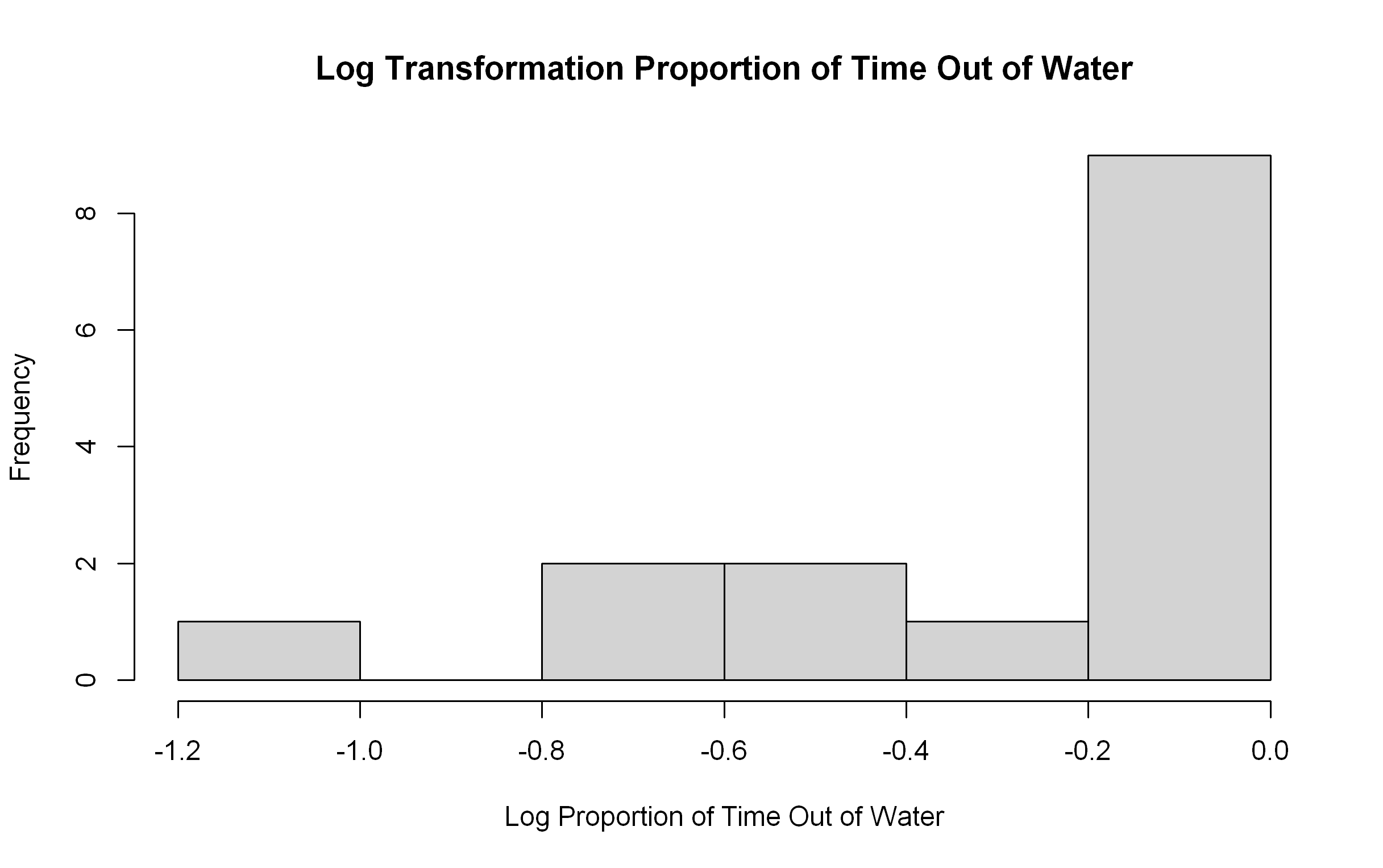
**Histogram produced from R code**



**Example of histogram of binary data**

First, taking a look at the histogram of the proportion data, we can see that they are not normally distributed; it does not follow a Gaussian distribution. These data look visually similar to a distribution that would be produced from binary data.

Before deciding that we must utilize a GLM to model our data, we can attempt to transform these data. Log or log + 1 transformations can sometimes improve normality of data when looking to visualize linear relationships. If you are able to transform your data to reflect a normal distribution in this way, you may be better suited to use an LM as opposed to a GLM for modeling your data. After conducting these transformations, we can visually see that normality is not improved. To be sure, we can conduct Shapiro-Wilkes Normality tests. A significant p-value, below 0.05, shows us that we can conclude that, at a 5% significance, our data is non-normal. From this, we can deduce that a GLM may be the way to go about modelling our data.



**Log transformed histogram produced from R code**

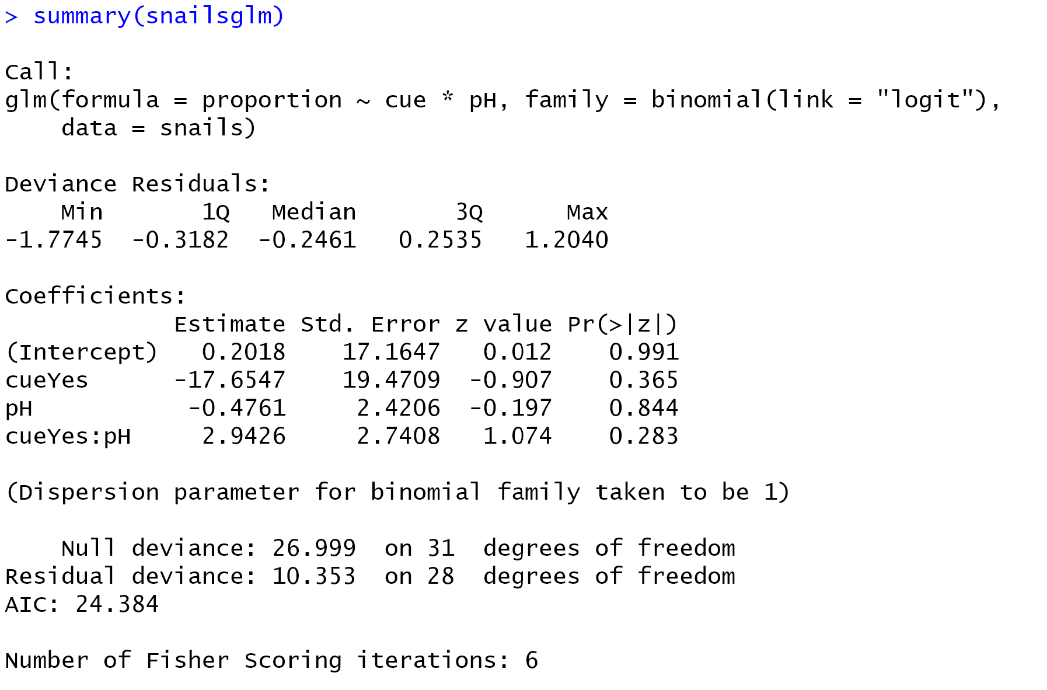
**Log + 1 transformed histogram produced from R code**

As previously discussed, the distribution of the original non-transformed data looks somewhat similar to the distribution of binary data. For binary data, we would look to use a binomial distribution for modelling. With a binomial family, the logistic link is canonical. So, we can use logistic regression for our modelling. Logistic regression can be used to describe data to explain the relationship between one dependent variable and one or more independent variables. In our case, the snails’ proportion of time out of water is our dependent variable. Both pH and presence of cue are our independent variables; these are also our linear predictors.

****If you are following along with the R markdown document, you can see that we fit the model with the glm () function in R. We utilize the “\*” to express an interaction between cue and pH; we want to determine if there is an effect of this interaction on proportion of time out of water.

Once we fit the model and label it with “snailsglm,” we can use the summary () function in R to observe and analyze the output.

**Understanding the Output**

Following with the R markdown document, the output of our model using the summary() function is shown here.

Paying attention to the “Coefficients” part of the summary, we can look to the last column, showing our p-values. None of these values indicate significance with an alpha value of 0.05 (below 0.05). We found that our predictors (cue, pH, as well as the cue and pH interaction term) did not have a statistically significant effect on proportion of time out of water.

**From paper supplementary material: Table S4. Results generated for GLMM comparing anti-predator behavior for snails exposed to no cue or cue water at different pH levels**

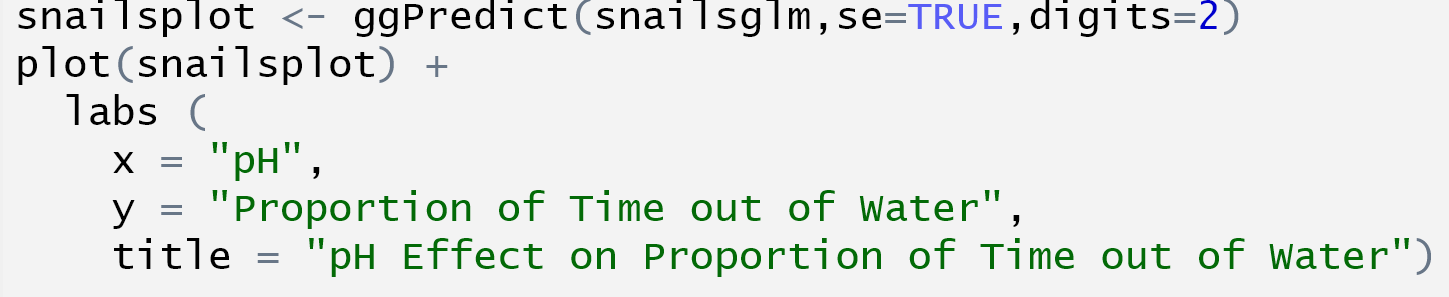
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Experiment** | **Response Variable** | **Model Type** | **Predictor** | **Estimate** | **±SE** | **t value/ Wald Z** | **P-value** |
| Constant | Proportion of time out of water | GLMM | **Intercept** | **-3.61** | **1.47** | **-2.45** | **0.01** |
| Cue | -1.86 | 2.28 | -0.82 | 0.42 |
| **pH** | **4.40** | **1.46** | **3.02** | **0.002** |
| **Cue x pH** | **-4.44** | **2.21** | **-2.01** | **0.04** |

Unlike our results, through the GLMM results, the authors were able to determine statistically significant effects for the predictors of intercept, pH, and the cue and pH interaction term. Specifically, this interaction term reflects a difference in the effect of pH on snail refuge-seeking between the cue and non-cue treatments, where reduced pH only decreased the likelihood for snails to exit the water when low pH was accompanied by predator cue (Jellison et al., 2016).

Remember, the authors utilized a GLMM to account for random effects within their full data. Since we were not provided with their full set of data, we were unable to recreate this modelling and found different results with our more simplified model.

Despite the findings from the output summary of our logistic regression model, data visualization shown in the next part of this lesson shows us similarities with our produced figure and figure 4c from the focal paper.

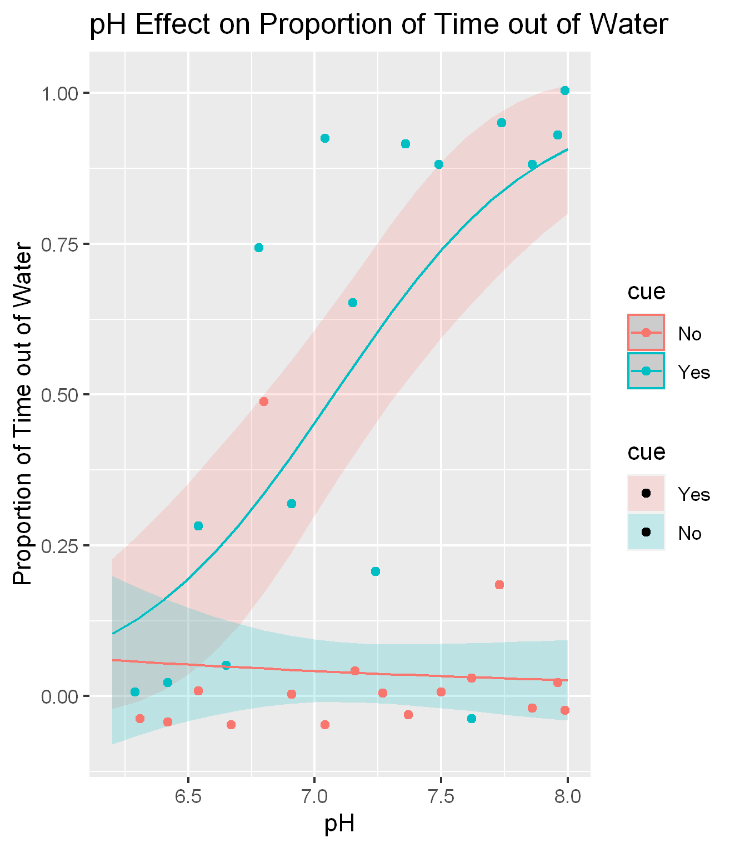
**Data Visualization**

There are many ways to visualize data using the R programming language. For this tutorial, we will be using the ggPredict() function within the R package ggiraphExtra. Instructions have been provided in the R markdown document accompanying this lesson.

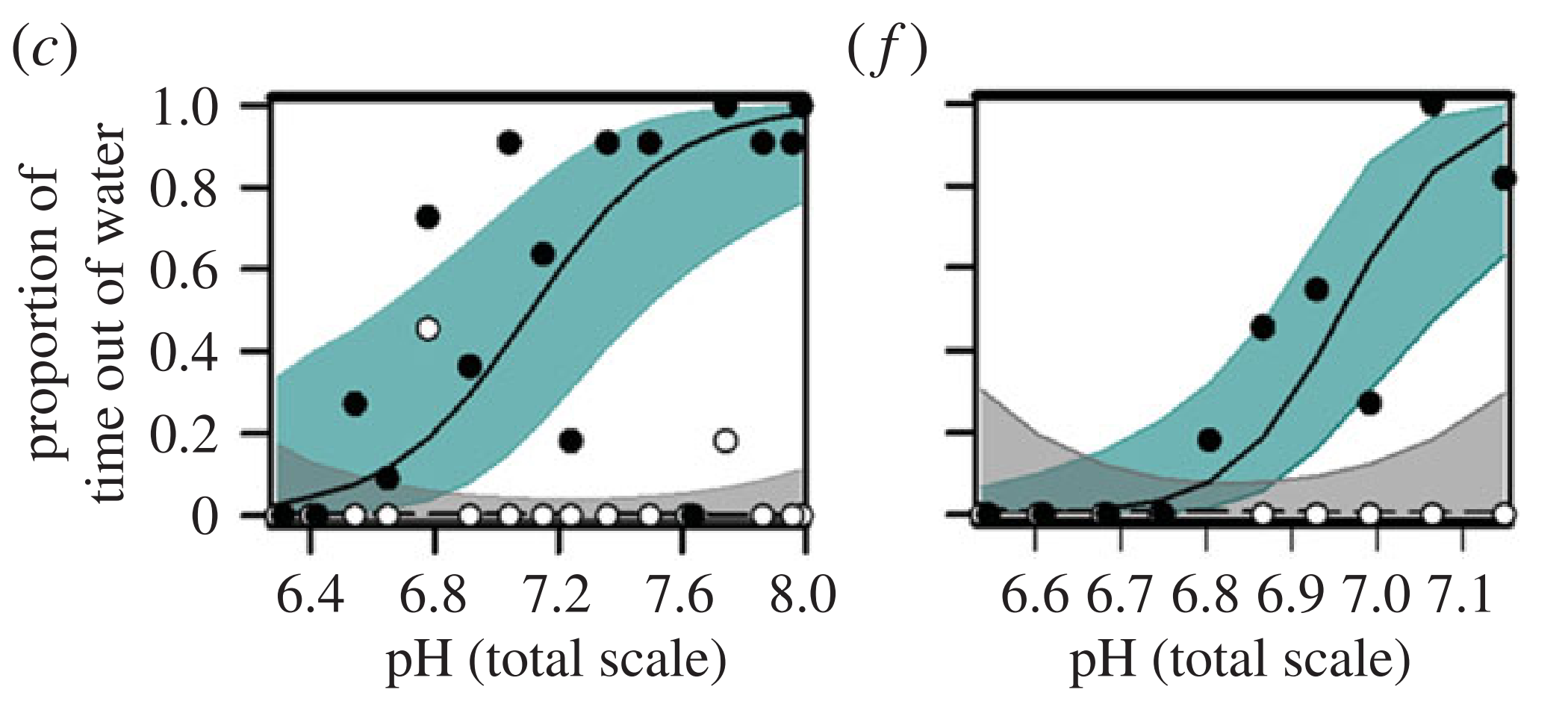
Using ggPredict (), the arguments we utilize are “fit,” “se,” and “digits.” For “fit,” we use the model label from the previous section “snailsglm.” For “se,” we use “TRUE” as we can to show standard error on our plot. For “digits,” we use “2” as we want two decimal places to be shown. We label our plot “snailsplot” to be able to edit axis labels and the plot title.

**Want to Learn More?**

Follow this [link](https://cran.r-project.org/web/packages/ggiraphExtra/vignettes/ggPredict.html) to a tutorial on using the ggPredict () function to visualize multiple regression models.



**Plot produced in R**



As we can see from our produced plot on the left and figure 4c from the focal paper on the right, there are similarities in these visualizations. Because we used logistic regression in our modelling of the data, we were able to achieve a similar S-curve achieved by the authors from their GLMM model. Despite our contrasting modelling results, we are still able to visually see a reduction in proportion of time out of water for these snails in the presence of predator cue at lower levels of seawater pH. However, given the differences in our results, we are able to recognize the effect of different modelling methods given available data.

**From Paper: Figure 4c. Effects of seawater pH during constant pH experiment on proportion of time out of water in the presence (black dots and blue shading) and absence of predator cue (white dots and grey shading)**

**Takeaways**

In this lesson, you have studied the environmental issue of ocean acidification, its causes and dangers, and how a lowered pH may alter predator-prey interactions. You have also received an introduction to GLMs and one method of data visualization using data from a relevant study focused on this issue of ocean acidification as it pertains to trophic interactions in the marine food web.

The authors of “Ocean Acidification Alters the Response of Intertidal Snails to a Key Sea Star Predator,” Jellison et al. (2016), emphasize that more research is needed to understand why the black turban snails failed to fully implement their escape response within more acidic conditions and to deduce if these organisms may even adapt to these conditions in the future. As previously stated by NOAA, sustained efforts to monitor ocean acidification are only beginning as we look to understand the effects of the changing ocean chemistry. Through this lesson, we have found that this changing ocean chemistry due to ocean acidification may have a negative effect on the marine food web through effects on trophic interactions. Global climate change has been shown to be a significant threat to our environment, as well as all living things existing within our environment. As we continue to learn about the specifics of this threat and what it may mean for humans, plants, and other living organisms, it is important to understand that all ecosystems are unique and complex, requiring ongoing research to fully appreciate how deeply calamity runs. Harm to marine food webs is just one issue that must be combatted to solve the issue of global climate change.

**Lesson Assessment**

**Environmental Studies**

1. What is ocean acidification? What are its causes?

Ocean acidification is one aspect of global climate change, characterized by an increase in aqueous CO2 and results in reduced seawater pH. Two major sources that play large roles in the increasing CO2 being pumped into our atmosphere include fossil fuel emissions and deforestation (Climate Interpreter).

1. What is the predator-prey interaction focused on in the focal paper?

Specifically discussed in the paper are flight responses, specifically a cue-mediated behavior observed between the black turban snail and the purple sea star. In their relationship, the black turban snail is the prey, and the purple sea star is the predator. Purple sea stars emit a chemical cue as predators. In response to the presence of this cue, black turban snails typically partake in area avoidance by seeking refuge (i.e., exiting the water).

1. What dangers does ocean acidification pose in relation to the predator-prey interaction between the black turban snail and the purple sea star and other trophic interactions within the ecosystem?

Ocean acidification has the ability to alter interactions between predators and their prey; disturbance of these interactions could create detrimental consequences for trophic interactions within food web systems in aquatic ecosystems, affecting the functioning of these ecosystems. In this way, ocean acidification may have a negative effect on the marine food web through effects on trophic interactions.

**Quantitative Skills**

1. Are the data provided normally distributed, and what does this have to do with the utilization of a generalized linear model?

By taking a look at the histogram displaying our data, we can see that they are not normally distributed. From this, we can be able to deduce that a GLM may be the way to go about modelling our data. We were also able to see that the visual distribution of the original non-transformed data looks somewhat similar to the distribution of binary data. For binary data, we would look to use a binomial distribution for modelling. With a binomial family, the logistic link is canonical. So, we can use logistic regression, a GLM, for our modelling.

1. What is the difference between a linear model and a generalized linear model?

If you want to model data, you can do so with a linear model (LM) if your data follow a Gaussian distribution (normally distribution), or if you are able to transform your data to reflect a normal distribution. However, you may not be able to transform your data to create a normal distribution, or it may be easier to utilize a different modelling method. If your data are not normally distributed or cannot be transformed to follow a normal distribution, GLMs come in handy. Non-normal distributions, common families, that GLMs are useful for modelling include Poisson, binomial, and exponential.

1. What are the three main components of generalized linear models?

The three main components of GLMs are the family, linear predictor, and link function.

1. How can we interpret the generalized linear model output from R, and how does this compare to the focal paper’s findings?

We found that our predictors (cue, pH, as well as the cue and pH interaction term) did not have a statistically significant effect on proportion of time out of water.

Unlike our results, through the GLMM results, the authors were able to determine statistically significant effects for the predictors of intercept, pH, and the cue and pH interaction term. Specifically, this interaction term reflects a difference in the effect of pH on snail refuge-seeking between the cue and non-cue treatments, where reduced pH only decreased the likelihood for snails to exit the water when low pH was accompanied by predator cue (Jellison et al., 2016).

**References**

Climate Interpreter. (n.d.) *Ocean Acidification.* How are humans causing ocean acidification? Retrieved April 2, 2022, from <https://climateinterpreter.org/content/how-are-humans-causing-ocean-acidification>

Fox, J. (2015). Generalized Linear Models, *Applied Regression & Generalized Linear Models* (373-423). SAGE Publications.

Ferrari, M. C., Wisenden, B. D., & Chivers, D. P. (2010). Chemical ecology of predator-prey interaction in aquatic ecosystems: a review and prospectus. *Can. J. Zool.* 88: 698-724. doi:10.1139/Z10-029.

Hector, A. (2015). Generalized Linear Models for Data with Non-Normal Distributions, *New Statistics with R* (121-140)*. Ox*ford University Press.

Jellison, B. M., Ninokawa, A. T., Hill, T. M., Sanford, E., & Gaylord, B. (2016). Ocean acidification alters the response of intertidal snails to a key sea star predator. *Proc. R. Soc. B* 283: 20160890. <http://dx.doi.org/10.1098/rspb.2016.0890>

Jellison, Brittany M. et al. (2016), Data from: Ocean acidification alters the response of intertidal snails to a key sea star predator, Dryad, Dataset, <https://doi.org/10.5061/dryad.38m23>

National Oceanic and Atmospheric Administration. (n.d.). *Ocean acidification*. Ocean acidification | National Oceanic and Atmospheric Administration. Retrieved March 2, 2022, from https://www.noaa.gov/education/resource-collections/ocean-coasts/ocean-acidification