

## Homework 7 – Contrasts

Here we will analyze experimental data from Leibold et al. (2017), in which they used mesocosm manipulations to test whether grazers promote phytoplankton diversity in ponds. They factorially implemented a number of experimental treatments: N:P ratio, light, nutrient level, presence of grazers, and presence of a predator of the grazers. For the purposes of this assignment we will only use the balanced N:P treatment, and we will include both light levels but will not analyze the effect of light (the authors found it has no effect).

The column NutrientLevel has two levels (L = low and H = high), and the column FoodWeb has three levels (A = algae, G = algae + grazers, P = algae + grazers + predator). The treatments were randomly assigned to mesocosms within four blocks (column Block). The response variable we will analyze is the column Phyto\_Chao1, which is the Chao-1 estimator of taxonomic richness.

Create a linear model that tests whether richness is explained by nutrient level and/or food web treatment, and whether the effect of food web treatment differs between nutrient levels, while including block as a covariate. Why is including block as a covariate important? Use residual plots to decide whether it is better to model Chao-1 richness as normally or lognormally distributed. Report marginal F-tests on the model terms, and plot the fitted effects. What is your interpretation of these results?

Now we will use contrasts to test some hypotheses, and we will imagine that we formulated these hypotheses *a priori* (as opposed to formulating them after we looked at the data). We would like to know specifically whether the grazer treatment (G) has greater richness than the algae treatment (A), and whether the effect of grazers differs between high and low nutrient levels (we think the effect of grazers on coexistence may be greater at high nutrient loading).

Use emmeans to calculate the estimated marginal means of each combination of nutrient level and food web treatment (i.e., H + A, H + G, H + P, L + A, L + G, L + P). Now define contrasts to test (1) whether G is different from A in the H treatment, (2) whether G is different from A in the L treatment, and (3) whether G is different from A, averaging over the L and H treatments. There are multiple ways you can achieve this with emmeans, because it has many functions for specifying contrasts (see the vignettes here: <https://cran.r-project.org/web/packages/emmeans/index.html>), but it may be easiest to define your own custom contrasts using the function contrast(). An example was given in the lecture slides, and there is also a well-explained example on this blog: (<https://aosmith.rbind.io/2019/04/15/custom-contrasts-emmeans/>). Finally, (4) define an *interaction contrast* that tests whether the difference between G and A is itself different between L and H treatments. I.e., is  $(G - A)$  for the high nutrient treatments greater or smaller than  $(G - A)$  for the low nutrient treatments? Hint: for the interaction contrast you are essentially taking contrast #2 and subtracting it from contrast #1, in order to test whether the two contrasts are the same, which means that the difference between them is zero.

How do you interpret these results?

Now, repeat the same set of 4 contrasts, but this time ask whether the P treatment is different from the G treatment. We are interested in these contrasts *a priori* because we think that adding a predator of the grazers may mean that grazers have a weaker effect on phytoplankton diversity,

and that this trophic cascade may be more important under high nutrient supply. How do you interpret the results?