Homework 6 – Effects of temperature on predation pressure in belowground foodwebs

This assignment will analyze data from Thakur et al. (2018) [attached], to practice modeling zero-inflated count data. The authors performed an experiment using two species of Collembola (springtails) and two species of mites they prey upon the springtails. Springtails are common hexapods in leaf litter and top soil layers that consume fungus and detritus. The authors were interested in how temperature may alter a variety of food web processes: suppression of prey by predators, trophic cascades, key traits such as body size and lipid:protein ratio.

For this assignment we will focus on springtail abundance and how these are affected by temperature and mite predation. Excerpts from the paper:

> Our experimental community consisted of two predatory mite species as predators and two Collembola species as their prey. The two congeneric predatory species were *Hypoaspis aculeifer* and *Hypoaspis miles*. The two prey species were *Folsomia candida* and *Proisotoma minuta,* both belonging to the Isotomidae family. These Collembola species were cultured in the laboratory facility of Leipzig University (cultured with dry yeast at a temperature of 14°C), whereas predatory mites were commercially obtained from *Schneckenprofi* in Germany. These organisms occur mostly in the litter and top layers of the soil. Collembola are generally fungal grazers, but also ingest litter material in their diet, and thus are important litter detritivore species (Chahartaghi, Langel, Scheu, & Ruess, 2005). The Collembola species *F. candida* is larger in body size (body length ranging from 1,500 to 3,000 µm) than *P. minuta*(body length ranging from 600 to 1,100 µm) (Thakur, Künne, Griffin, & Eisenhauer,). The two predatory species range from 700 to 800 µm in their body sizes (Jess & Bingham, 2004), but despite their smaller body size, they both are known as voracious predators of Collembola species (Heckmann, Ruf, Nienstedt, & Krogh, 2007), as confirmed by our own trials. However, their foraging success may vary with temperature (Thakur, Künne, et al.,).
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> We performed a microcosm experiment with the above-described experimental community along a temperature gradient. We used temperature treatments with a day and night cycle of 16 hr and 8 hr, respectively, but with no light to keep dark conditions for animals. The dark conditions for litter and soil animals resemble more to their natural habitat conditions. The experiment ran in three different temperature regimes: 12–15°C (12°C for 8 hr night and 15°C for 16 hr day; representing ambient conditions that the two Collembola species had experienced for several generations; Thakur, Künne, et al.,), 17–20°C (17°C for 8 hr night and 20°C for 16 hr day), and 22–25°C (22°C for 8 hr night and 25°C for 16 hr day). The warmer temperature regimes (+5 and +10°C) were to mimic moderate to extreme warming scenarios for the next 100 years as per the predictions of the IPCC for several regions (Buckley & Huey, 2016; IPCC, 2014). Our previous trials with the monocultures of the model species showed that all these species survived in these temperature regimes (Thakur, Künne, et al.,).
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> In total, we established four communities (two prey species, two prey species + predator 1, two prey species + predator 2, and two prey species + predator 1 + predator 2) across three

> temperature regimes (12–15°C, 17–20°C, and 22–25°C), each replicated five times. At the start of the experiment, we added 10 individuals of each Collembola species. These individuals were carefully sorted from the laboratory cultures to be identical in their body size. Immediately after the addition of Collembola individuals (within hours), we added predatory mites in the following combination: predator monocultures received six individuals, whereas predator polycuture treatments received three individuals of each predator species. By keeping the total predator density constant, we established a substitutive design.

In the attached dataset we will use the columns total.prey (total Collembola count), temp.factor (the temperature treatment), and predators (the predator treatment).

1. First use an appropriate 'standard' model for counts (i.e., not zero-inflated) to ask whether the abundance of Collembola is affected by temperature, by the presence of one or more predators, and whether the effect of predators depends on temperature. Use the function glmmTMB() to fit the model, because later we will compare this model to others fit with this function.

Plot fitted effects and perform likelihood ratio tests on the relevant terms. To perform marginal tests you will want to *compare pairs of models* using the function anova(). Previously we used the function Anova() (the capitalized and uncapitalized functions are different) to automate this process, but this function returns a less accurate test for glmmTMB (a Wald test). So, *for each term you want to test*, compare a model *with* this term to a model *without* this term.

How do you interpret the results at this stage?

2. A large proportion of the data are zeros, and it may be the case that processes controlling abundance are different from processes controlling 'extra' zeros – if, in fact, there are extra zeros. Use glmmTMB to fit zero-inflated count model(s). You should decide how many to fit, and which kind of probability distribution(s) to use.

Use AIC to do model selection, to determine whether incorporating zero inflation improves model fit.

Using the best model (i.e., the lowest AIC), perform marginal likelihood ratio tests on the predictors, again using anova() to compare pairs of models. How have the results changed from #1?

3. If your results look the way mine do it seems like the three treatments with predators (HA, HM, HA+HM) may not be very different in their effect on the prey. Let's test the effect of predators, but treating all treatments with predators as the same. Create a new column that recodes the predator treatment so that it only has two levels: predators or no predators. Fit a model using the new predator predictor instead of the old one, and plot fitted effects and perform likelihood ratio tests as before (you only need to use the 'best' probability distribution model determined in #2). How do these results compare to what

you saw previously? Why do you think the results have changed? How do you interpret these patterns?

4. Finally, we have not considered that 'extra' zeros could themselves vary across the experimental treatments. Use an appropriate zero-inflated model and allow the extra zeros to vary with temperature, predators, and the interaction between these treatments. Use likelihood ratio tests to test these terms. The various packages for plotting fitted effects do not (to my knowledge) have a convenient way to plot zero-inflated effects (although this could be done 'manually' by extracting model predictions). Instead, look at the model coefficients returned by summary() to interpret what's going on with the extra zeros. It may be easier to interpret the coefficients if you use a model that removes the non-significant terms. What is the pattern in the extra zeros according to this model? How does this pattern differ from the patterns in the count model?