Bc. Mónica Patricia Romero Cueva: Responses of Blue Tits (Cyanistes caeruleus) and Great Tits (Parus major) to con- and hetero-specifics alarm calls. MSc. thesis review by Pavel Linhart

It was my pleasure to review the Master thesis of Mónica Romero. The thesis investigates the response of blue tits and great tits on the feeder to conspecific and heterospecific alarm calls. There is a good introduction summarizing why it is interesting to study alarm calls, what can be encoded in alarm calls, and, how and why conspecific and heterospecific species can respond to different alarm calls. It fails a bit to make a good connection for the Aim 3 (which develops findings of previous studies that revealed heterospecific response to black-capped chickadee alarm calls). The amount of experimental work is not excessive and in my opinion suits well to demands of master thesis. The experiments and analyses are simple, but mostly appropriate. Observations are not strictly independent though (specific individuals can drive the response at the feeder) so using tests assuming independent samples may be seen as oversimplification and rationale for the tests should be maybe better explained in the methods. Discussion could be longer and should probably include more thorough assessment of limitations (and maybe benefits?) of the used study design (single feeder - response affected by specific individuals and, therefore, non-independent observations; responses affected by the other species) in respect to other similar studies. It remains unclear to me why both European tits should respond to black capped chickadee alarm calls and not to very similar calls of sympatric willow tit. I would appreciate better conclusions summarizing how the results fit the proposed hypotheses.

The thesis is written in good and very well understandable English. References are relevant and up to date, also citing older works when appropriate. I appreciate citation style with links to papers. There are some minor issues with appropriate use of citations (inappropriate references and copying parts of reference text) but they are not systematic. Consistent and appropriate names of species should be used throughout the paper – American X Black-Capped Chickadee; Titmice X Tits X Poecile?

I think this is a good thesis which has a good potential to be published and I classify the thesis to be between 1 (great) and 2 (good) with the final classification depending on the thesis defense.

Questions

- 1) Compare the limitations and benefits of your experimental design to few selected relevant studies (e.g. those of Doutour et al, or Randler, study on a feeder) and propose changes that could be made to make the study stronger but still doable.
- 2) Which species drives the response at the feeder? Why the two tested species respond differently? Responses of one species should affect the other species on the feeder, no? This is actually an interesting finding, isn't it?
- 3) 1 minute after playback seems like a lot of time. Intuitively, with little actual knowledge of the test conditions, I would expect that the situation immediately before and after playback is crucial. How the actual response looked like? Immediate reaction was to fly away and then return or other (just some birds flew away)? Same in all cases? What would happen if you scored 10s before and 10s after playback?
- 4) Why you calculate proportions as after / (before + after) and not after / before? Could this weaken your results (if there is fewer birds after the playback your proportions will range 0-0.5 instead 0-1)?

Pavel Linhart, Ceske Budejovice, 14. 5. 2021

Specific comments

Introduction

- Alarm calls in titmice Poecile species?
- Aims Far East Tit (is not a name), Varied Tit
- Aims 1. Blue Tits respond only **TO** conspecific alarm calls.
- Aims 3. Blue Tits as well as Great Tits respond to all alarms of titmice species Poecile clade?, because they are structurally similar?

Methods

- To test for the difference in the number of visits to the feeder before and after the playback we ran a PAIRED? t-test
- Experimental design observations are not independent, 5 variants of playbacks for each species = each variant was played 3 times? Why not 15 different variants (probably available on Xeno Canto and avoids pseudoreplication completely)
- better description of distribution of experiments and treatments over time and dates random but were there any clusters? Five stimuli each day? All Five treatments played back before moving into next replicate?

Results

- and American Black-capped? Chickadee alarm call was presented (t = 2.23, p = 0.03, df = 27.9)
- The proportion of the Great Tits visiting the feeder after the playback was not affected by the playback presented (LM, F = 1.52, p = 0.2) and the post-hoc comparison between the conditions was not significant in any case (p > 0.05) (Figure 4). Should mirror the t-test results? Should be close to 0.5? (e.g. in the case of great tit reaction to a blue tit song same numbers of great tits before and after playback? Probably, Y axis is a proportion of birds already after arcsine transformation?
- Figure 4. The effect of particular playbacks on the proportion of Great Tits visiting the feeder after the playback, out of all Blue Tits Great tits? visiting the feeder before and after the playback. The solid line refers to median value, box refers to 75% quartile, whiskers refer to non-outlier range and dots refer to outliers.

Discussion

- Blue Tits responded to conspecific alarm call by decreasing their feeding rates. Feeding rates were not measured.
- It is therefore surprising that in our experiments, the effect of Blue Tit alarm was so weak... In our experiment, the playback represented a single bird, which might be too weak stimulus for the Great Tits.March – heterospecific alarms are no more relevant; different foraging behavior so they do not listen to heterospecific alarms – More on why? Explanation would apply for other species as well, no? Your and others' methods differ? (feeders, one minute after playback?)

Lack of discussion of study limitations. – Experimental design.

Conclusion

 We may conclude that Blue Tits and Great Tits do not respond to any alarm of any titmice species. - ???

Details on Reference issues

Using the **same formulations** as the cited papers without enclosing the formulations into quotation marks, but this does not seem to be a rule as my repeated random investigations did not reveal more such examples. Further, the text flows well and the quality of the English does not deteriorate when moving from Intro to other parts of manuscript.

- Hollen and Radford 2009 Alarm call behaviour, as with all vocal communication, comprises
 three aspects: the delivery of calls with a specific set of acoustic features (call production); the
 use of calls in particular contexts (call usage); and the response to calls produced by others (call
 responses).
- Thesis: Alarm call behaviour involves three aspects: the emission of calls with a specific set of acoustic features (call production); the use of calls in specific contexts (call use); and the response to calls produced by others (call responses) (Hollén & Radford, 2009).

Non-fitting references (just a few cases found, does not seem to be a rule):

To scape immediate predation risk birds can gain information individually observing a predator, or socially using other's signals and cues (Santema et al., 2019). – Santema is about manipulation of predation risk through individual onbservations of predators (broadcasting predator calls) – no mentions of social effects in Santema

Further we presented alarm of one unfamiliar North American Tit, American Chickadee (Poecile atricapillus), and one Far East Tit, Varied Tit (Sittiparus varius). These species were shown to share some similarities in their alarm-calling system (Dutour et al., 2016) - **Doutour et al 2016 does not address Black-capped Chickadee or Varied Tit**

