22nd March 2022

Dear Prof Vrba,

Thank you for the invitation to review Pavel Soukup's PhD thesis from the University of South Bohemia, České Budějovice in Czechia. I enclose here my thoughts on the introduction, three main data chapters, and summary. This is an enjoyable thesis, underpinned by quality research, and written to a high standard. My overall recommendation is for the PhD to be awarded.

Introduction:

Generally very well written and a solid introduction to the major themes that will be addressed in the thesis, and the tools that will be used to explore them (laboratory microcosm and field mesocosm experiments). I have a couple of minor recommendations.

C0.1. I found the statements about the lack of herbivorous and detritivorous interactions in freshwaters a little surprising. Sure, predation is important, but it is still the minority portion of most food webs (*i.e.* the classic Elton pyramid of numbers). I'm thinking about snails grazing biofilms, blackfly larvae filtering fine particulate organic matter (FPOM) from the water, midgefly larvae collecting and gathering diatoms and FPOM from the benthos, plus the many shredders and scavengers of CPOM and dead organic matter. It would be good to see a more balanced appraisal of the various interactions that make up freshwater food webs.

C0.2. A brief overview of the different types of functional response would increase understanding of this concept. The explanation of changes in functional responses due to temperature and habitat complexity are also quite vague and require better explanation.

Chapter 1:

A very nice review of habitat complexity impacts across individuals, interactions, and communities. Figure 1 provides a really great road-map to the paper and the various sections adequately summarise the key studies at each level of biological organisation. The identification of three major gaps in the field is a strong point of the review, and it is nice to see some links here to research attempting to fill those gaps later in the thesis (*i.e.* the gradient approach to habitat complexity in Chapter 2 experiments). Could the quantification of habitat complexity and ecological feedbacks also have been addressed in the thesis? Some additional comments below.

C1.1. Don't oversell the importance of habitat complexity in aquatic environments…it is also a major player in terrestrial environments, e.g. trees and vegetation.

C1.2. Why limit the literature review to 2015-2020 and not prior studies? Simply to avoid having too many papers to wade through, or some other logic?

C1.3 Figure 1 is great, but shouldn't populations be a separate level of organisation between individuals and communities?

C1.4 Figure 3 could be improved with some real or hypothetical examples of ecological scenarios that lead to each type of response.

C1.5. Section 5 on ecological feedbacks seems surprisingly short. Even if there is insufficient material to review here, I would expect some commentary on the lack of studies and the directions in the field that are now needed to address that major gap.

Colchester Campus
Wivenhoe Park
Colchester CO4 3SQ
United Kingdom

School of Life Sciences
T 01206 873333
F 01206 872592

Chapter 2:

This is a very nice chapter, examining changes in the functional response of dragonfly nymph predators over a gradient of habitat complexity. The comparison of results from functional response and video-tracking behavioural experiments is a real highlight, adding nuance to the understanding of the mechanisms underpinning the observed changes with habitat complexity. I appreciated the integrity of the information theoretic approach and the transparency in presenting multiple possible model outputs in the results section. The quantitative comparison of experimental results to previous literature on the topic in the discussion is really neat, particularly the exploration of how habitat complexity has different consequences for foraging parameters if the interactions are 2- or 3-dimensional. I have some minor comments and questions for consideration.

C2.1. Functional response experiments are typically run for 24 hours, but the experiments here are just 8 hours in duration. Unless they were all started at the same time of day, could this have introduced some bias, *e.g.* if predators have more active feeding periods during the day?

C2.2. I understand that the analysis of background prey mortality was not included in the main paper, but it would be cool to see this and an associated figure in supporting information for the thesis. In fact, it would be good to include the supporting information from the paper in the thesis as well – we are currently missing a lot of figures and tables referenced in the chapter.

C2.3. Why reuse individuals in the behavioural experiment? Does that introduce any potential biases and how was this accounted for, *e.g.* randomising the order in which habitat complexity or prey densities were examined for the same predator individuals?

C2.4. Calculating the duration of foraging as the time until the end of the trial would seem to introduce a bias, *e.g.* if the last event began seconds before the end of the trial, it would be forced to have a very short duration which does not reflect the actual duration of the event or time until the next one. Worth excluding the end-of-trial data to avoid this "edge effect".

C2.5. It is unclear what P0, P1, P2, and P3 mean in Equation 1.

C2.6. Is the lack of a prey density effect in Experiment 2 just down to the limited spread of prey densities in the experiment (6 and 24)?

C2.7. Did the video-tracking also allow activity of the prey to be quantified? The increased attack rates with habitat complexity may be driven by greater prey activity, and it would be neat to test this if the data are available.

C2.8. Is it problematic that experimental duration determines the attack rate? Are there any studies that try to identify the optimum duration for more realistic attack rates?

Chapter 3:

This chapter presents the results of a mesocosm experiment, which must have taken a considerable amount of time and management to establish, sample, process, and analyse. The endeavour to explore the interactive effects of habitat complexity and presence/absence of predators (and their cues) is impressive. The multitrophic mesocosms also provide a more realistic setting for exploring effects of habitat complexity in the thesis. There are surprisingly few treatment effects, with little change in the abundance or biomass of most taxonomic groups. Some interesting effects of habitat complexity were noted on the biomass of snails and the size spectrum of midgefly larvae, but I wonder if some further exploration may be needed to really nail the story of this experiment, particularly considering the role of fluctuations in temperature among replicates of each treatment. I have some further suggestions below.

C3.1. The experiment only examines presence/absence of habitat complexity, and not a gradient, which was advised against in the earlier chapters. Is that problematic?

C3.2. Was there just one individual crayfish per mesocosm and if so, does it matter that this excludes potential effects of intraspecific competition?

C3.3. Were the temperatures consistent across each mesocosm? This could have a huge effect on the results if not, given the strong temperature dependence of so many different biological rates. Temperature could be a key co-variate to include in the analyses to account for this.

C3.4. Why was temperature not included in the RDA when it varied so much over the course of the experiment?

C3.5. I'm not convinced by the technique for avoiding gaps in the size spectra. Is there a precedent for it? Empty size bins seem totally plausible, *e.g.* inverts often jump a step in size when they move between instars, which could leave gaps in the size spectrum, or some treatments may simply be missing larger individuals. Artificially filling those gaps with nominally low abundances is potentially dangerous and leads me to question the results and associated interpretation of Figure 2. I would suggest removing these "almost zero" points and reanalysing the data.

C3.6. Consider examining how the overall size spectra or species averaged mass-abundance relationship changes as a function of the treatments. That would say more about how the entire community is responding, rather than individual groups.

C3.7. Consider formally calculating and examining treatment effects on the consumptive and non-consumptive effects on various taxonomic groups by comparing abundances in the treatments with and without crayfish and their predatory cue. There is a rich literature on how to do this, *e.g.* search TMII / non-consumptive effects / dynamic index.

Summary:

Perhaps this is the typical length for the summary in the Czech system, but it felt too short to me, with quite a limited and narrow set of future directions. I would expect to see a more articulate integration of the take-home messages from each chapter and how they link together, and a more exciting take on what we need to be considering next in this field, *e.g.* what to tackle in a post-doc or a major research grant on this topic, or how the results inform conservation and applied management of systems.

In summary, I would be happy to recommend Pavel Soukup for the award of a PhD based this excellent thesis.

Yours sincerely,

Dr Eoin O'Gorman
Lecturer in Ecology
University of Essex
Phone: +44 (0)1206 876389
Email: e.ogorman@essex.ac.uk