Toruń, Poland, 10 March 2022

Prof. dr hab. Jarosław Kobak
Nicolaus Copernicus University in Toruń
Faculty of Biological and Veterinarian Sciences
Institute of Biology
Department of Invertebrate Zoology and Parasitology
Lwowska 1, 87-100 Toruń, Poland
jkob73@umk.pl

Review of the PhD thesis by Pavel Soukup (PhD Studies in Hydrobiology, Faculty of Science, University of South Bohemia in České Budějovice, Czechia): "Role of habitat complexity and predation in the structuring of aquatic communities" (supervisor: Assoc. Prof. David Boukal, Ph.D.)

The thesis consists of 3 chapters, two of which have been published in international scientific journals, while the third one is a manuscript prepared to submission:

Chapter 1. Soukup P.R., Näslund J., Höjesjö J., Boukal D.S. (2022) From individuals to communities: habitat complexity affects all levels of organization in aquatic environments. WIREs Water, DOI: 10.1002/wat2.1575 (IF=6.1)

Chapter 2. Mocq J., Soukup P.R., Näslund J., Boukal D.S. (2021) Disentangling the nonlinear effects of habitat complexity on functional responses. Journal of Animal Ecology, DOI: 10.1111/1365-2656.13473 (IF=5.09)

Chapter 3. Soukup P.R., Kolář V., Boukal D.S. Invasive crayfish predation risk and habitat complexity effects on community assembly in small standing waters. (Manuscript)

The candidate described his contributions to the studies conducted within each chapter, declaring his leading role in the research. Chapters 1 and 2 have already been published in highly renowned ecological journals (Q1 in the Web of Science rankings, Impact Factor >5.0), and Chapter 3, judging on its content, is likely to follow successfully the same path. The candidate is the first (though not corresponding) author of all the chapters. In Chapter 2, he is listed as second, but with a note indicating the equal contribution of the first two authors to the study. The thesis also includes a declaration of the supervisor, prof. David Boukal, confirming the leading role of the candidate in all the papers, and a short CV of the candidate. The chapters are accompanied by a 26-page introductory overview containing the theoretical background, aims and scope of the thesis.

The thesis deals with an important topic of habitat complexity in the aquatic environment, with particular emphasis on its effect on predator-prey interactions. Both these phenomena are crucial factors shaping the functioning and composition of communities. Habitat complexity is also important in quality assessment, protection and restoration of natural aquatic habitats. Both habitat complexity and predator-prey interactions have been studied extensively, but important gaps still exist in our knowledge of these phenomena, particularly concerning their interactions with each other and with other ecosystem processes. Definitely, the candidate did a good job identifying these gaps and attempting to fill them with the results of his research.

Chapter 1 is a review paper showing the state of the art of the knowledge of habitat complexity in aquatic environments. This review constitutes a good introduction to the two following experimental parts, describing comprehensively all known aspects of factors affecting and affected by habitat complexity, known and potential feedbacks acting in the system, measurement methods and, what is the most important in such work, crucial gaps in our current understanding of the topic. In particular, the authors emphasize the need for focusing further research on:

- Using quantitative measures of habitat complexity and unifying these measures in various studies to produce comparable results;
- Using the higher resolution of experimental habitat complexity levels: having a range of complexity levels instead of just a binary approach (high vs. low complexity), as well as testing different levels of temporal and spatial resolution;
- Paying attention to non-linear effects of habitat complexity (e.g. threshold responses);
- Identifying feedbacks between higher and lower levels of biological organization and habitat complexity.

Certainly, knowledge accumulated in this review and recommendations provided by the authors are highly valuable for readers interested in habitat complexity and, more generally, ecosystem functioning. These recommendations have been followed by the authors in the experimental studies described below.

Chapter 2 is a short-term experimental study on the effect of habitat complexity on the functional response of predatory dragonflies hunting on phantom midge (*Chaoborus* sp.) larvae. I particularly enjoyed reading this paper due to its elegant experimental design and multi-sided approach to the problem: the authors looked at foraging parameters from the community perspective, calculating them from the functional response equations, and simultaneously identified them through direct behavioural observations in individuals to obtain reliable and robust results. The authors demonstrated that habitat complexity does not affect the shape of the functional response curve, but can change prey encounter rate and handling time. Furthermore, they showed the presence of nonlinear relationships between habitat complexity and community processes, suggesting that a more detailed approach and higher resolution should be used in future studies. The study also contains a short review of functional responses in various predator-prey systems under different habitat complexity conditions, indicating the high variability of outcomes depending on a range of additional factors.

Chapter 3 is a longer-term outdoor experimental study on the effect of habitat complexity on consumptive and non-consumptive predator effects of an invasive crayfish on early invertebrate colonizers in a pond community. Here, the authors had to face the nightmare of an experimental biologist: negative results. This means that, although there were clear effects of habitat complexity and, to a lesser extent, predator presence alone on various community parameters, the effect of the interaction of these two factors (which would be potentially the most interesting outcome) was rather negligible, meaning that habitat complexity did not have any profound impact on predator effects (with a single exception of the size spectrum of chironomid larvae). Anyway, the authors efficiently handled this problem, drawing noteworthy conclusions and being able to show the importance of their results. Among the strong sides of this part, I would like to point out the following:

- The whole community approach in a long-term outdoor experiment, which is a valuable addition to simple laboratory experiments dealing with single elements of the ecosystem. Although such approach can generate results that are more difficult to interpret due to multiple factors enhancing or attenuating one another, it presents the final outcome produced by combined interactions among all single phenomena observed in simple experiments dealing with one factor and taxon a time.
- Careful handling of potential sources of error in the experimental design, a good example of which is the approach to correct the potential error in animal size measurements.
- Good discussion of results, with many possible explanations shown as more or less plausible on the basis of various experimental outcomes and earlier literature.

A reviewer's duty, though not pleasure, is also to point out weaknesses of the thesis being reviewed. These are the issues which, in my opinion, need further attention, discussion and better explanation or justification by the candidate, or perhaps just more effort and better understanding on the reader's side:

Chapter 1:

1. The appeal for unifying complexity measures seems to me a bit unrealistic. Researchers have their specific goals, research questions and hypotheses, which they try to resolve in an optimum way, adjusted to their particular case. It is difficult to demand that they would use metrics which are not optimal for their purposes just to be consistent with other studies on habitat complexity. I mean, it would be nice if they did, but I'm afraid they probably just won't... So, perhaps a better idea would be to have raw data freely available, so everybody would be able to recalculate them as needed.
2. Also, as to complexity measures, I miss a comprehensive list of available quantitative measures of habitat complexity, for instance as an appendix table. That would be really helpful in a paper like this, especially give the recommendation to use such metrics more widely and consistently. Several metrics are mentioned in the text, but only as examples taken from a presumably longer list.
3. I would be grateful for the justification of time limits in the literature search (only papers published since 2015). What if a particular aspect of the topic was comprehensively covered earlier, so no cutting edge articles appeared after this date? Just hypothetically, could it be possible that it would be falsely considered as a gap in knowledge?
4. I'm not sure how to understand the term "species interactions", used several times in the text (e.g. in the *caption of Fig. 1*). Interactions, such as predation, competition or parasitism, take place among individuals, which can belong to the same or different species.
5. In my opinion, the topic of oviposition deserves more attention in *section 2 (effects on individuals)* than just a single sentence at the end.
6. Similarly, in *section 3 (effects on interactions)*, I would expect some more attention paid to competition (indicated in *Fig. 1* as one of the most important issues affected by habitat complexity). Please note that the competition of individuals for an optimum (here: more complex) habitat is not the same as the effect of complexity on competition for other resources.
7. Another aspect needed to show the really full picture of the phenomenon is the effect of habitat complexity on parasitism. For instance, habitat complexity could change transmission rates, survival of free-living life stages of parasites and their encounter rates

with potential predators and hosts, contacts between infected and non-infected individuals of the host species, etc. See for instance:

- Sousa & Grosholz 1991. The influence of habitat structure on the transmission of parasites. In: Bell S.S., McCoy E.D., Mushinsky H.R. (eds). Habitat Structure. Population and Community Biology Series, vol 8. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-3076-9_15
- Brothers & Blakeslee 2021. Alien vs predator play hide and seek: How habitat complexity alters parasite mediated host survival, J. Exp. Mar. Biol. Ecol. 535: 151488, https://doi.org/10.1016/j.jembe.2020.151488.

8. In my opinion, the topic of abiotic factors (temperature, light, water flow, etc.) could be covered in a separate section to show what is currently known on their relationships with habitat complexity, rather than only in the to-do list in the summary.

Chapter 2:

1. Please be careful with the term "substrate preference", as you cannot easily determine real animal choices made in your setup. They occupied plant models more often when more of them were present in the environment, which is not the same. Preference means active selection of some habitats or objects over other, equally available alternatives. So, to talk about preferences, it should be demonstrated that plants were occupied more often than assumed on the basis of the random distribution of animals among available surface types.
2. You defined the probability of successful prey capture as a ratio between the number of successful and failed attacks (*Methods, page 4, Experiment 2*). Was it really so? I would say a ratio of successful vs. all attacks would be a more intuitive measure, and this is also suggested by *Fig. 4b*, where the attack success is always <1. According to your definition given in the *Methods*, this would have meant that there were always more failed attacks than successful ones...
3. What were the attacks with zero handling times in Experiment 2 (*Methods, page 4*)? How was it possible?
4. In the *Methods*, you mentioned that in Experiment 2, each predator individual was subject to two prey densities in randomized order. But later, in the description of the statistical model, this fact seems to be somehow lost. You reported using individual as a random factor, but what about the fact that some individuals got the low prey density first and the others started with the high prey density? I think a kind of a cross-over design would be suitable here.
5. How was it possible to apply a binomial model to microhabitat use data? It doesn't seem to be a binary measure, like the number of independent events assuming one of two possible states. Habitat use was measured as percentage time spent in a given habitat, thus it was a continuous measure.

Chapter 3:

1. A technical issue: please note that the crayfish from your experiment currently belongs to the genus *Faxonius* instead of *Orconectes*
2. I'm not sure whether this is a perfect model to study the effects of an invasive predator on the local community, which is emphasized as one of the main goals and outcomes of the study. Please note that, looking from the prey perspective, it is not important whether a predator is native or alien, instead, it matters whether it is known or unknown to the prey

(i.e. whether the danger can be properly recognized and handled). The North American spiny cheek crayfish (*Faxonius limosus*) in an "old invader", present in European habitats for much more than 100 years. Given the fast rate of evolution of anti-predator defences in prey populations, it is likely that they can respond to such a danger just as they do in the case of native predators. The difference which invasive predators make in such systems is that they are not known to their prey, which cannot adequately respond to their presence. I wonder whether a similar study including additionally another, really novel crayfish species, e.g. the marbled crayfish, wouldn't be better to test this aspect of the phenomenon. Nevertheless, I must emphasize that the study is still strong in all its other aspects, i.e. tests of the effect of habitat complexity, predator presence and their interaction on invertebrate colonization, community composition, growth and survival, all irrespective of the predator invasiveness.

3. *The first paragraph of Introduction.* This seems to be a mistake: as far as I know, out of the two species mentioned here, *Orconectes rusticus* is invasive and *O. propinquus* is native, not the other way round.
4. *Introduction, paragraph 4.* I'm not sure about the statement that "...indirect non-consumptive effects (...) should (...) manifest regardless of habitat complexity..." I would say it is quite likely that organisms living in a simpler habitat (thus being more exposed to the danger) could respond more strongly to the presence of predators, thus showing stronger non-consumptive effects. See for instance:
    - Catano et al. 2016. Reefscapes of fear: predation risk and reef heterogeneity interact to shape herbivore foraging behaviour. J. Anim. Ecol. 85: 146-156, https://doi.org/10.1111/1365-2656.12440.
5. *Methods, Experimental protocol, paragraph 1.* Given your recommendation formulated in the previous work, postulating the higher number of habitat complexity levels in studies on its community effects, I wonder why here you used just two levels (complex vs. simple) of habitat complexity.
6. *Methods, Experimental protocol, paragraph 3.* Here I've noticed a potential problem in the study design. If the crayfish in your experiment were not additionally fed, then the free-ranging animals consumed much more food than the caged individuals. It is important to note that predation cues, triggering non-consumptive predator effects, include not only predator kairomones (acting predominantly in the caged predator treatment), but also alarm substances released by injured prey and dietary cues, released in faeces of predators feeding on particular types of food (these were additionally present in the free-ranging predator treatment). If so, the treatments differed not only in consumptive effects, but also in the quality and strength of non-consumptive effects. Organisms can respond more strongly or even differently to such a mixture of cues compared to their reactions to pure kairomones. This is a minor problem given the actual results of the experiment (no differences between the caged and free-ranging predator treatments), but if such differences did occur, it would have been difficult to tell their real causes (consumptive vs. stronger non-consumptive effects of free-ranging predators).
7. *Methods, Statistical analyses, paragraph 3.* I must admit I had a problem with understanding the details of the regression analysis of size spectra (i.e. what was the dependent and independent variable in the analysis) on the basis of this description. This became clear later, when I looked at the results and graphs, but perhaps could be made easier to understand here. Also, what kind of a post-hoc test (mentioned and reported in the *Results* and *Table S7*), did you apply to the regression slopes?

8. *Results, Effect of habitat complexity and predation risk on macroinvertebrate size spectra.* Here, you mentioned a comparison between the predator-free complex habitat and the simple habitat with free-ranging predators (also in T*able S7*). But this seems to make no sense, as they differ in two traits simultaneously. I think only comparisons between various habitat complexities within a given level of the predator presence, and between various predator treatments within a given habitat complexity are valid (this would also make the correction for multiple comparisons less conservative).
9. *Discussion, Effect of predation risk, paragraph 2.* What do you mean by "positive effects of predation risk" and how should they be manifested in you results if present?
10. *Discussion, Effect of predation risk, paragraph 5.* What do you mean by the statement: "a direct evidence for gastropod behavioural responses to predation risk by invasive crayfish is lacking"? Perhaps this is true specifically for *Gyraulus albus*, but definitely not for gastropods in general. See for instance:
    - Orr et al. 2007. Predator detection in *Lymnaea stagnalis*. J. Exp. Biol. 210: 4150-4158, https://doi.org/10.1242/jeb.010173.

I must emphasize that the above remarks mostly concern just a few minor details of the studies, having no profound impact on the quality of the main results and conclusions following from the thesis, which are reliable and well proven by experimental evidence. Therefore, my general opinion on the thesis and the candidate is highly positive.

To summarize, I have found the thesis by Pavel Soukup as a valuable contribution to science, adding considerable content to the contemporary knowledge on the role of habitat complexity in ecosystem functioning, particularly in predator-prey interactions. The candidate proved his ability to extract necessary information from scientific literature, to apply diverse experimental methods to study ecological phenomena, to design elegant, carefully planned ecological experiments, to use sophisticated statistical methods, as well as to properly interpret and discuss obtained results. Briefly, he proved to be an independent, resourceful scientist capable of conducting the high quality research. Thus, I am fully convinced that the thesis submitted by Pavel Soukup clearly exceeds the requirements of a proper PhD thesis and I recommend it to be admitted to the next stage of the PhD procedure and to be defended at the Faculty of Science of the University of South Bohemia in České Budějovice.

Jarosław Kobak