Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

The review of the Mgr. thesis by Bc. Pavla Heinclová

The effect of direct predation by fish on cercarial populations of selected trematode species (Digenea)

The predation of cercariae by fish is an interesting topic that has not yet been studied in detail. Pavla confirmed that cercariae appear to be one of diet components of fish. She used four fish species and cercariae of three trematode species to study predation of cercarie with regard to specific life-history traits of both predators and parasites. Unfortunately, the laboratory conditions were not suitable for long-term survival of rainbow trout, roach and white bream. Therefore only stone moroko could be used in all experiments testing all possible fish-trematode combinations.

Despite these difficulties, number of priority findings has been achieved in carefully prepared experiments. The results of the experiments were evaluated using sophisticated statistical methods. Although I am not able to judge statistical analyses in details, I have one objection. I am not convinced that it is right to exclude replication with a distinctly different result (e.g. fthe first replication of combinationof small stone moroko and *T. szidati*). Such an operation is warranted if the cause of the large difference is revealed (or at least estimated). However, the results obtained from the "selected" groups appear to be reliable.

In the discussion, the obtained results are compared with the results of other authors and are related to the properties of cercaria and fish species. I highly appreciate this part he diploma thesis. At the end of the discussion, 6 issues are proposed to address further research. Among them, I miss a proposal to evaluate of the role of cercariae in fish food in terms of the flow of energy in the food chain.

Without biomass measurement, dry matter determination and calculations of nutritional values, the conclusion no. 8 is not justified: "The results also confirm that trematode cercariae play an important ecological role in the energy flow in food-webs of aquatic ecosystems."

Meanwhile, the methodology of biomass determination of larval stages of the trematode has already been developed and has been repeatedly used; e,g.:

Lambden J and Johnson PTJ: Quantifying the biomass of parasites to understand their role in aquatic communities. Ecology and Evolution 2013; 3: 2310–2321

Yurlova NI.: Productivity and Biomass of Trematode (Digenea) Parasites in Lake Ecosystems. Doklady Akademii Nauk, 2016, 466, 500–503.

Lagrue, C. and Poulin, R. The scaling of parasite biomass with host biomass in lake ecosystems: are parasites limited by host resources? Ecography 2016 39: 507–514

Rosenkranz M, Lagrue C, Poulin R,Selbach C. Small snails, high productivity? Larval output of parasites from an abundant host. Freshwater Biol. 2018; 63: 1602–1609.

In my opinion, these works should at least be quoted.

I fully agree with all the other conclusions. Finally, a few questions:

1. Compared with other planktonic organisms, cercariae contain cathepsins and other enzymes. It does not matter in the fish intestine but can it not affect the prey preference of predators?
2. Some fish were excluded from the experiments, because learning behavior during predation could not be excluded (which was correct). However, was such a behavior observed in your experiments? Could it play important role in cercariae predation?
3. Regarding conclusion 6: Do you think that fish (or other predators of cercariae) could be used for biological control of some trematode species, e.g. causative agents of cercarial dermatitis?
4. You do not consider the stone moroco a species suitable for biological control because it is invasive. What about common minnow, whose reintroduction to many localities would be desirable?

The diploma thesis under consideration undoubtedly meets the criteria of the Faculty of Science. I recommend it for the defnse..

České Budějovice January 18. 2019

Doc. RNDr. Oleg Ditrich, CSc

Reviewer report on master thesis

Opponent: RNDr. Jana Bulantová, Ph.D.

Student: Bc. Pavla Heinclová

Thesis: **The effect of direct predation by fish on cercarial populations of selected trematode species (Digenea)**

Theme of the diploma thesis

Diploma thesis of Pavla Heinclová is focused on the possible effect of various fish species to the population of free living cercariae. It confirms that cercariae can serve i.a. as a source of energy for predators including fish.

Introduction

Introduction summarizes the role of parasites on food webs and their important contribution to the energy flow in ecosystems not only as possible pathogens but also as a pray. Impact of predators to the parasites can results in decrease of final numbers of free-living stages of parasites able to infect their suitable hosts and can affect final distribution/density of trematode parasites in environment.

Aims

Aims are well defined

Methods

Chapter materials and methods contains extensive description of used organisms, their main morphological characteristics, biology and behaviour. Text is supplemented with many explanatory pictures illustrating individual steps of experimental workflow. Two main experimental designs are described here together with asked questions and supposed hypotheses. For higher clarity, both experimental set-ups are summarized also in a form of tables. Also procedures leading to statistical analysis of results are mentioned in close details.

Results

Due to frequent death of experimental fish, not all planned experiments were performed. Obtained results are presented separately based on appropriate experimental design and followed by numerous tables and graphs summarizing data mentioned in text and/or their evaluation by statistical analysis.

Discussion

In discussion, student compares her results with previous studies and critically evaluates possible weaknesses of some hypotheses. She also presents new ideas, which should be solved for verification and better understanding of complex relationships between cercariae and their possible fish predators.

Conclusions

Results of presented study are briefly concluded.

Formal and language level of thesis

Text contains several common types of mistakes arising probably from inattention or lack of time during finalization of thesis (typing errors, duplication of whole paragraph, confusion in numbering of pictures in text vs. figure table, usage of unexplained abbreviation). Unfortunately, there can be found also some insufficiencies in explanation of some terminology, formulation inaccuracies or inconsistencies in used ordering of studied organisms in text or in graphs/tables; making orientation in text more difficult.

Namely e.g.:

Text p. 6 – „andpredators", p. 17 "Live cercariae" probably instead of "Living cercariae"

Text from p. 11 – description of fish species – maximal value should be only one number, not range; size of fish species should be described constantly for all four species, e.g. in *Blicca bjoerkna* is range of length and maximum weight, but not maximal length like in other species.

Text p. 16 – formulation inaccurancy - „As other echinostome cercariae, those of *E. aconiatum* are typical by continuous swimming and the speed of swimming depends predominately on the size of the body and temperature. Larger echinostome cercariae swim much faster than smaller species (see review of Haas 2000)." It seems like *E. aconiatum* could have variable size.

Text p. 17 – there is not clear how did you sampled stone morocco (*Pseudorasbora parva*)? With nets or by electrofishing?

Text p. 19 – "functional response Type III" is mentioned without closer explanation in methods, but this term is firstly explained on page 27, I would supposed that types of functional responses should be explained within introductory part.

Text p. 20 – *Pseudorasbora parva* has not 23-44 cm, but measurements are obviously in mm

Text p. 20-23 – whole paragraph is doubled

Text p. 23 – Table 1 – there is used abbreviation FR without any comment what does it mean

Text p. 33-34 – Usual order of studied organisms is *Plagiorchis, Echinoparyphium, Trichobilharzia*, nevertheless, data in graphs are ordered in different order what is confusing.

- Text of chapter 3.3.1 (from p. 24) and 4 (Results; from p. 29) overlap in some aspects and thanks to extensive description of statistical methods, it is not easy to keep attention.

Fortunately, well-arranged tables for better orientation in experimental designs or results are included, what was very useful.

Figures and tables

Figures are usually in good quality. I very appreciate especially fig. 5 and 6 (methods, p. 21, 22). They are very important and usable for better understanding of all experiments mentioned in the text. Nevertheless, I have some minute remarks to figures.

Namely e.g.:

- When author of the text is the same as the author of the picture, the name is usually not mentioned in figure caption, what is not respected in this thesis.

- Fig. 1 (p. 3) – I very appreciate effort of student to make hers own diagram which should illustrate relationships of trematodes and other organisms (hosts, predators), but used organization of pictures in figure is more confusing than clarifying (too much graphical styles in one figure – photographs, drawings, line arts; inconsistency – representatives of intermediate hosts are depicted but not definitive hosts, arrows with or without description, etc.

- Fig. 3 (p. 15) – pictures in figure table are in really good quality and nicely illustrates typical characteristics of cercariae mentioned in the text, but unfortunately, overall impression is ruined by various format and position of scale bars; numbers are sometimes small, thus illegible.

- Table 7, 8, 9 – I would appreciate dividing sections with low, medium and high density e.g. with lines, it could help to be more clear.

Overall conclusion

The theme of thesis is well and clearly defined. It contains field part with collection and determination of snails and cercariae, gaining and maintenance of snails and fishes in laboratory conditions. Student was able to plan and realize extensive experiments with various fish species and adapt following procedures to unexpected circumstances like death of experimental animals. She gained experiences with statistics and learned how to work with data from experiments. In discussion, she is able to critically evaluate results and statistical data in context of possible usage of gained knowledge in field practice.

Despite of my comments above, I recommend thesis for the defence with tentative classification 2. Final classification will depend on course of defence and on student's answers to questions below.

Questions:

1. How did you sample stone morocco (*Pseudorasbora parva*)? With nets or by electrofishing?

2. How long did you use individual snails as a source of cercariae for experiments? Did you recognize any changes in cercarial species diversity in captured snails during their laboratory maintenance?

3. On p. 46, you mentioned: "it is possible that the use of different water volumes in experimental containers resulted in higher consumption rates (Kaplan et al. 2009, Orlofske et al. 2013, 2015).". So, what is usual volume of water during this type of experiments and if it differs from your experimental design – why did you choose this small volume?

4. You mentioned that different fish species prefer various time optima for feeding during the day in natural conditions. Did you considered this fact to your experimental design, or you made experiments anytime regardless of the day time?

5. Small particles in water column (including cercariae) are filtered on fish gills, so can we count fish among filtrators? How can be distinguished active consumption from passive accidental consumption in filtrators/fish?

6. How small particles can be filtered and used by fish as a source of food? How it vary between fish individuals of different age/body length? Can it be somehow standardized?

7. What is the reason for decreased number of cercariae "eaten" by fish in cases where highest cercarial density was present in experimental container? Could it be also because of stressfull presence of high numbers of cercariae in small water volume all around the fish?

8. In text (p. 4-5) you mentioned: „However, because they are affected by various biotic and abiotic environmental factors, only a small fraction of cercarial population reaches its target hosts (e.g. Pietrock and Marcogliese 2003, Thieltges et al. 2008a, Morley 2012). Part of remaining cercarial population play their secondary ecological role within aquatic ecosystems as an important source of energy flow through food webs by predation (Fig. 1J) (Thieltges et al. 2008 a, b, Johnson et al. 2010, Goedknegt et al. 2012, Morley 2012)."

Are there known any more specific numbers or proportions between cercariae reaching their target hosts vs. cercariae serving as a food source for predators vs. dead cercariae decomposed by other organisms at the bottom?

18.1.2019 in Prague

RNDr. Jana Bulantová, Ph.D.

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