



Kouba

University of South Bohemia in České Budějovice
Faculty of Fisheries and Protection of Waters
Institute of Aquaculture

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Confidential

Review of USB FFPW PhD Thesis

Surname of the PhD student: Ing. Antonín Kouba		Name of supervisor: doc. Ing. Pavel Kozák, Ph.D.	
Title of PhD thesis: Intensification of juvenile crayfish culture			
REVIEWER:			
Surname: Reynolds		Institution: University of Dublin, Dept. Zoology, Ireland	
Name: Julian			
Titles: Prof.		E-mail: jreynolds@tcd.ie	
Please describe your professional relationship to the PhD student: I met him at a conference in Czech Republic and assisted with the English in some chapters.		Please describe your field of expertise: Ecology and culture of freshwater crayfish	

QUESTIONNAIRE

Originality, scientific importance, prospects of the PhD thesis and benefits for basic or applied research

Evaluate its competitiveness in the international context and compare its level with the current state of the art in the field:

This thesis relates essentially to applied research. With 7 published papers, there are no doubts as to its originality, and the author and his team now form a recognised group in Central Europe.

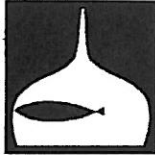
This thesis takes the form of 7 multi-authored papers published between 2009 and 2011, or accepted for publication. The candidate A. Kouba is senior author in 4 and a junior author in 3. However, I have not previously seen a thesis chapter represented by a paper with a junior authorship. I would therefore prefer to see the precise role of A. Kouba elaborated in papers where his is not the leading author, as this also has future implications for other junior authors in these studies.

Preparation of the PhD thesis, targets of the work and deliverables

Evaluate the overall level of preparation of the PhD thesis and the originality of the selected approaches; evaluate publications and whether the targets set in the PhD thesis correspond with the declared purpose of the thesis:

The thesis is well prepared, both in the published papers and in the introduction and discussion chapters. The chapters form a unit, as described in the objectives (Introduction). The publications have been peer reviewed and are of an international standard.

OVERALL COMMENTARY ON THE PhD THESIS



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Please write comments:

The Introduction (Chapter 1) and Discussion are the main parts uniting the thesis.

Chapter 1: Introduction:

1.1 Crayfish Biology: note the following minor comments: p.8: crayfish plague is not lethal to all astacids, e.g. *P. Leniusculus*. p.9: *Astacopsis gouldi* is not in the same situation as the others mentioned, as there are no alien crayfish in Tasmania.

1.2 Aquaculture, 1.3 Key aspects of culture. These sections should refer to the Moneycarragh hatchery, which forms the background for chapters 2, 4 and 6, with *A. Pallipes* as test animal.

There are over 120 references, apparently correctly cited. As a minor point, the author should decide whether the initial R. for Gonzalez precedes or goes after the surname.

Overall, this is a coherent and concise chapter, with clear objectives leading into the chapter structure to follow. It would be helpful to explain why some chapters dealt with *Astacus astacus* and others with *Austropotamobius pallipes*.

Chapter 2: The effect of different cold period during maternal incubation on incubation efficiency and hatching term in *Austropotamobius pallipes*. AUTHORS: Policar, Smyth, Flanigan, Kozak, Kouba. KMAE (2009).

This study was carried out in Moneycarragh, N. Ireland, where the lead author worked. The precise role of A. Kouba should be indicated. This experiment appears well devised and executed, but the chapter writing is not very clear-cut, either in providing detail of experimental layout or in conclusions.

Chapter 3: The effect of water temperature on the number of moults and growth of juvenile noble crayfish, *Astacus astacus* (Linnaeus). AUTHORS: Kouba, Kanta, Buric, Policar, Kozak. FC 17 (2010).

Growth in crayfish remains a complex topic. The findings in this well-structured chapter include that growth rate decreases over time, and that survival is best at low temperatures, because there are fewer hazardous moults to survive.

Chapter 4: Optimum water temperature for intensive production of *Austropotamobius pallipes* (Lereboullet) juveniles. AUTHORS: Policar, Smyth, Flanigan, Kozak, Kouba. FC 17 (2010).

Again carried out at Moneycarragh, this study showed that in intensive culture, survival at 20.3 C (higher than usually encountered in Irish conditions) was better than at 18.3 C, while a natural river showed maximum temperatures of under 15 C. Rearing at ambient temperatures is therefore not recommended. This is a well-structured chapter.

Chapter 5: Artificial incubation of noble crayfish (*Astacus astacus*) eggs in a partially recirculating system using formaldehyde as an antifungal treatment. AUTHORS: Kouba, Carral, Buric, Mraz, Policar, Kozak. AQ RES (2010)

This chapter describes work carried out in Czech Republic, developing ideas initiated by the team of JM Carral et al. in Spain to minimize egg infections by fungi in culture. Good survival was obtained using formaldehyde.

Chapter 6: Sodium chloride as effective antifungal treatment for artificial egg incubation in *Austropotamobius pallipes*. AUTHORS: Policar, Smyth, Flanigan, Kouba, Kozak. KMAE (2011)

An alternative to the previous chapter is described in this clear chapter, comparing the use of formaldehyde with the use of salt as an anti-fungal agent for *Austropotamobius pallipes* eggs in Moneycarragh hatchery. Formaldehyde resulted in good egg survival to hatching, as did high concentrations of salt, but lower levels of salt were less effective. Removal of dead eggs is recommended in systems without antifungals.

Chapter 7: Use of three forms of decapsulated *Artemia* cysts as food for juvenile noble crayfish (*Astacus astacus*).

AUTHORS: Kouba, Hamackova, Buric, Policar, Kozak. CZECH J AN SCI (2011)

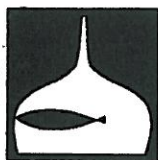
This chapter investigates recommended foods for initiation of feeding, and concludes that freshly decapsulated *Artemia* cysts give best results in growth and survival. Such studies are essential in determining the conditions under which intensive culture of crayfish may be economic.

Chapter 8: Evaluation of body appendage injuries to juvenile signal crayfish (*Paifastacus leniusculus*): relationships and consequences. AUTHORS: Kouba, Buric, Policar, Kozak. KMAE (2011)

This interesting study shows that aggressive contacts during intensive rearing result in injuries to antennae, chelae and, to a lesser extent, walking legs, and such injuries may affect future survival. Cannibalism of injured crayfish is suggested. This chapter contributes to our fundamental understanding of crayfish social behaviour.

Chapter 9: Discussion. 8 pages, 73 references (including 5 with A. Kouba as 1st author)

This chapter acts to integrate the various papers into a coherent study of ways to improve the efficiency of crayfish egg hatching and juvenile rearing. It starts by noting that aquaculture can be useful both for food production and also to provide stocks for increasingly necessary conservation activities. It makes a persuasive case for the set of papers put together in this thesis.



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FINAL RECOMMENDATION

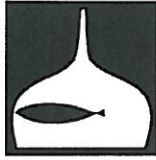
- can be recommended for defence of PhD Thesis**
 can be recommended with reservations for defence of PhD Thesis
 can not be recommended for defence of PhD Thesis

.....Dublin, 29 May 2011.....

Date and place

Surname and signature

REYNOLDS



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Title of PhD thesis: Intensification of juvenile crayfish culture	

REVIEWER:

Surname: Souty-Grosset	Institution: Université de Poitiers, laboratoire Ecologie, Evolution, Symbiose , France
Name: Catherine	
Titles: Ph.D.	E-mail: catherine.souty@univ-poitiers.fr
Please describe your professional relationship to the PhD student: I am researcher in astacology working on conservation of native European crayfish species	Please describe your field of expertise: Europe, Crayfish, native, invasive, Ecology, genetics, conservation biology Past coordinator of the European programme CRAYNET

QUESTIONNAIRE

Originality, scientific importance, prospects of the PhD thesis and benefits for basic or applied research

Evaluate its competitiveness in the international context and compare its level with the current state of the art in the field:

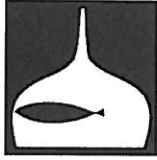
This manuscript is of important value for astacologists in Europe with the problem of conserving and restoring populations of native crayfish species. Here the main species studied are the noble crayfish *Astacus astacus* and the white-clawed crayfish *Austropotamobius pallipes* Up to date only a few studies concerned artificial incubation of eggs, compared with the ones conducted on the invasive crayfish *Pacifastacus leniusculus* in Spain.

Preparation of the PhD thesis, targets of the work and deliverables

Evaluate the overall level of preparation of the PhD thesis and the originality of the selected approaches; evaluate publications and whether the targets set in the PhD thesis correspond with the declared purpose of the thesis:

The manuscript in an association of seven papers placed chronologically from 2009 to 2011, published or in press. The candidate is first author about 4 papers ; all the papers are well related to the declared purpose of the thesis

OVERALL COMMENTARY ON THE PhD THESIS



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Please write comments:

Introduction of 4 pages is very rich in references (more than hundred); Crayfish Biology: as the work deals mainly with native European crayfish, it would be interesting to develop more the aspects of restoring populations (see last paragraph before 1.2 aquaculture): explain more the distribution of *A. Astacus* and habitat and *A. pallipes* in streams; Aquaculture: it is important to consider that even if many studies were performed on *P. leniusculus* (non native and invasive species) we could hypothesize that the native European crayfish are more fragile and particularly *A. pallipes* and the aim of such a MS is well to consider rather papers gathered per species and no more chronologically, ie a section on *A. pallipes* (3 chapters) with an introduction of the state of knowledge on *A. pallipes* and the very few astaciculture present in western Europe and still important problem of urgently restoring populations; a section 2 gathering the two chapters about *Astacus astacus*, certainly more documented with Scandinavia but still to be improved about aquaculture; the last chapter dealing with *P. leniusculus* is to be replaced among the huge amount of Spanish papers.

Ricciardi is OK in the text but in references Ricciardi Key aspects of culture.

As the problems are very correlated with fungi, its could be more developed on the nature of these fungi (many papers for very short piece of sentence)

The final sentence, The overall aim.....juvenile crayfish must be completed by conservation implication of the species by restocking

Chapter 2: Effect of different cold period during maternal incubation on incubation efficiency and hatching term in *Austropotamobius pallipes*. Author 4°/4.

I don't know if it is usual to place the paper without any comments; in France, usually we recommend to introduce briefly the paper and this would be very useful if papers dealing with the same species are joined as explained above.

Chapter 3: Effect of water temperature on the number of moults and growth of juvenile noble crayfish, *Astacus astacus*. Author 1°/4, the paper is good and very well written.

Chapter 4: Optimum water temperature for intensive production of *Austropotamobius pallipes* juveniles. Author 4°/4 as for chapter 2. As the candidate did not go to Ireland (see foreign stays during PhD study), what was the part of the work (only help during writing of the papers?)

Chapter 5: Artificial incubation of noble crayfish (*Astacus astacus*) eggs in a partially recirculating system using formaldehyde as an antifungal treatment. Author 1°/4 and impact factor 1.099; the paper is good and very well written.

Chapter 6: Sodium chloride as effective antifungal treatment for artificial egg incubation in *Austropotamobius pallipes*. Author 4°/5. Again the same remark about the location of the experiment.

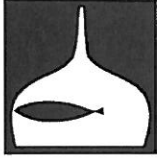
Chapter 7: Use of 3 forms of decapsulated *Artemia* cysts as food for juvenile noble crayfish (*Astacus astacus*). Author 1°/5 impact factor 1.08. Good paper concluding that freshly decapsulated *Artemia* cysts allowed best results in growth and survival. Is it planned to compare with *A. pallipes*?

Chapter 8: Evaluation of body appendage injuries to juvenile signal crayfish (*Paifastacus leniusculus*) Author 1°/4; the papers deals with the problem of intensive culture leading to aggressivity of young crayfish and cannibalism.

Chapter 9: This is the final discussion dealing with both production and conservation purposes (but too late for me). I don't agree with the term concurrent about research from Carral's team as they work on *P. leniusculus* (see comments about introduction); the sensitivity to fungi is certainly different according to the species (native or non native) and if research would be conducted about *P. clarkii*, results will also changed according to the degree of tolerance of the species.

Perez et al is cited 1998 in the text and 1998b in references

Finally, If I am not wrong I think that the PHD duration was from 2008 to 2010 (based on PHD courses); during the first period a certain number of publications dealt with O. Limosus as coauthors of Buric's works. In 2010 it is interesting to point out the publication written as Author 1°/3 in Water, air and soil pollution (impact factor 1.676) showing that the candidate is capable to write a very good synthetic paper dealing with the state of knowledge about bioaccumulation in crayfish and indeed to access to Journals of higher impact factor.



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To sum up, the MS could be recommended for defence of the thesis; I would be glad if the candidate would have time to organize sections per species, or at least this exercise could be planned for the defence with a final and pedagogical comparative analysis of results obtained in two native species (mainly his MS) and a non native species (mainly from the literature).

FINAL RECOMMENDATION

- can be recommended for defence of PhD Thesis
 can be recommended with reservations for defence of PhD Thesis
 can not be recommended for defence of PhD Thesis

.....
Date and place

24 th June 2011

.....
Surname and signature

Catherine SOUTY-GROSSET