



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Iryna Kuklina, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Pavel Kozák, Ph.D.
Title of PhD thesis: Crayfish as bioindicators of water quality	

REVIEWER:

Surname: Reynolds	Institution: 115 Weirview Drive Stillorgan Co. Dublin IRELAND
Name: Julian	E-mail: jrynolds@tcd.ie
Titles: Dr.	
Please describe your professional relationship to the PhD student: She attended my seminar in Vodnany	Please describe your field of expertise: Ecology and conservation of Freshwater Crayfish

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

Crayfish are relatively large and robust freshwater invertebrates, and their biology has received much study, some of it in their putative roles as bioindicators of water quality. This Thesis by Iryna Kuklina brings together knowledge in the well-worked fields of crayfish ethology and physiology to develop a less-studied area, the use of behavioural and physiological reactions for environmental monitoring. A centre of excellence for such work is the laboratory of Fetodov and Sladkova in St Petersburg, visited by the student. The thesis presents a novel non-invasive development using electronic information (patents applied for) which is ahead of the field, and whose applications in municipal water quality monitoring appear potentially useful. Iryna Kuklina shows familiarity with the field and has developed advanced techniques and a prototype system for its study. This makes the thesis internationally competitive.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):

This thesis is, at first glance, relatively short (just over 100 pages of text), but well elaborated, as over half of the total (70 pages) is taken up by published papers in their printed format. The thesis is structured around a General Introduction to crayfish as bioindicators of water quality (Chapter 1), including bioaccumulation of harmful substances. This is followed by five published and peer-reviewed papers covering the field of crayfish in water quality monitoring (Chapter 2), with Kuklina as first author in three of them, including their accumulation of heavy metals and an investigation of the impact of a pesticide and disinfectant on crayfish cardiac activity. These publications are evaluated below. The next two chapters describe development of the system for biological monitoring, and experimental trials of its ability to record crayfish reactions to a series of different odours as representative of the stimuli which may be encountered in nature. There is a short General Discussion (Chapter 5), and an English and Czech Summary. The order of the chapters is logical. The approaches used are original, as indicated by the application for a patent. Overall, the results presented reflect well the stated objectives of the thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Overall, this thesis shows the standard necessary for a PhD. The English language needs some improvement, starting at the Contents page.

Chapter 1. General introduction. (10p.)

This is a useful short summary of current understanding of biomonitoring and biomonitorers; crayfish as essential components of freshwater habitats; the bioaccumulation of harmful substances in tissues and its use in toxicological studies; and ethophysiological responses in water quality evaluation. Further introductory detail is provided in the papers in Chapter 2.

Chapter 2. Investigation of crayfish as water quality possible bioindicator. (70p.)

This chapter consists of five papers, mostly published, in Journals with good impact factors, with Kuklina as first author in three and junior author in two.

2.1 Real-time monitoring of water quality using fish and crayfish as bioindicators: a review. Kuklina, Kouba and Kozák 2013. Environ Monit Assess 185: 5043-5053



This paper presents a comprehensive review of bioindicators, and methodology used to date to monitor physiological (cardiac and respiratory activity) and behavioural responses in fish and crayfish to various stimuli.

2.2 Crayfish as tools in water quality monitoring. Kozák & Kuklina 2014. (23p.) (No Journal attribution given).

This detailed paper takes on from the previous, concentrating solely on the use of crayfish in biomonitoring. The writing style is more colloquial and the English less perfect. Fourteen crayfish species from Europe, North America and Australia are listed as having been used as biosensors. Measuring crayfish behaviour is first discussed, using cameras to track locomotion and fine movements of appendages and also optical gate technology and acoustic monitoring and telemetry. Invasive and non-invasive methods of monitoring behavioural and physiological changes are described. There is a table of chemical and physical stressors investigated in crayfish, and over 80 references.

2.3 Accumulation of heavy metals in crayfish and fish from selected Czech reservoirs. Kuklina, Kouba, Buric, Herka, Dureis & Kozák, 2014. Biomed research International, Article ID 306103 (9p.).

As both crayfish and fish are valuable food sources, this paper is a survey of heavy metal concentrations in crayfish *Astacus astacus* from 3 drinking water reservoirs and a contaminated reservoir (Darkovské), and relating them to comparable data on coarse fish (bream, rudd, roach, tench and pikeperch) from these same sites. Crayfish hepatopancreas showed high rates of accumulation compared to other tissues. Results for metals differed between reservoirs, with Darkovské crayfish generally showing lower levels of most metals than seen from drinking water reservoirs. However, Zn in muscle from Darkovské was double that in drinking waters. Carnivorous fish show biomagnifications of Hg, indicating their relative unsuitability for biomonitoring.

2.4 Investigation of chloramine-T impact on crayfish *Astacus leptodactylus* (Esch., 1823) cardiac activity. Kuklina, Sladkova, Kouba, Kholodkevich and Kozák, 2014. Environ Sci Pollut Research (8p.).

This paper goes further, by investigating a specific chemical, the biocide chloramine-T with a different species of crayfish. Crayfish heart rate responded rapidly to presence of the biocide. Long-term (24 hr) exposure led to loss of energy for the affected crayfish when subsequently stress-tested, in contrast to short-term (1 hr) exposure.

2.5 Stress reaction in crayfish: chlorides help to withstand stress in high nitrite concentration conditions – preliminary study. Kozák, Policar, Fedotov, Kuznetsova, Buric, Kouba, Kuklina and Kholodkevich, 2011. KMAE 401, 05 (12 p.).

This paper is in many ways a fore-runner of the last, and would be better placed before it than after it. Using the same crayfish species, heart rate changes and stress reaction to suspension above the floor was measured under different chemical conditions.

Chapter 3. Development of the system for biological monitoring. (6p.)

A non-invasive sensor attached to the carapace emits infrared radiation and measures radiation reflected from the haemolymph contained in the heart muscle and changing during each beat



contraction. The benefits are that it is noninvasive, fast, easily attached and detached, allows continuous real-time recording, and animals are unrestrained. The biomonitoring system consists of 12 aquaria monitored by two cameras.

A standard IP video camera, observing from below and monitored through internet, allows detection of even slight limb movement. There is simultaneous recording and analysis of movement to eliminate its effects on heart rate. Its potential application includes use in municipal industrial facilities for drinking and waste water treatment and supply monitoring

An Appendix refers to applications for 2 patents – monitoring methodology for crayfish/molluscs, and non-invasive sensor for measurement of crayfish heart rate.

This chapter lacks references. References would help to link the developments here with the current literature and practice, including Chapter 2.

Chapter 4. First experimental trials with biomonitoring system. 9p.

This chapter is written in paper format, and the English is mostly good.

Crayfish can sense and discriminate between chemicals and odours, showing behavioural and physiological adaptations, including changes in locomotion, cardiac rate and ventilation.

Twelve large wild-trapped signal crayfish of both sexes were used in controlled trials including tests of food odour (milled chironomid larvae), odour of predator *Perca*; odour of conspecifics, male and female; odour of injured conspecifics; and odour of the disinfectant chloramine-T at 10 mg.L

Crayfish were monitored for 30 mins before odours were introduced, and for 30 mins after, to test primary reactions. Heart rate at first slows and this period should be excluded from tests. Heart rate did not show a significant increase with conspecifics and predators, and there were no difference between sexes outside the reproductive period. The presence of food, injured crayfish and the chemical all led to increases in heart rate, although the chemical concentration was very low. Wave-like and stable high heart-beats are described – these should be depicted as in Figs 1 below.

The conclusions in point form need further teasing out, including specific reference to Kuklina et al. 2014 (Chap 2.4).

4.2 Peculiarities of the heartbeat p. 95

This section deals with heartbeat changes in relation to visual cues (food), eating, etc., and relating to myograms of scaphognathites etc. It should perhaps be better integrated with the rest of the chapter.

Chapter 5. General discussion. 8p.

This chapter starts with a useful discussion of species of crayfish and their use in monitoring (Table 1), with 31 references. This is followed by a brief paragraph on ‘Conclusions and future perspectives’, with no text references. This would be an opportunity to review or refer to the author’s own publications and those of the team, rounding off the thesis. There is an impressive List of Publications and abstracts or Conference Proceedings on p.108

Provided attention is paid to the points raised above, I believe that this Thesis can go forward for defence.



Fakulta rybnářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

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

FINAL RECOMMENDATION

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

Dublin, 9 June 2014
Date and place

Julian Reynolds
Name and signature
JULIAN D REYNOLDS



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Iryna Kuklina, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Pavel Kozák, Ph.D.
Title of PhD thesis: Crayfish as bioindicators of water quality	
REVIEWER:	
Surname: Tricarico	Institution: Department of Biology University of Florence Via Romana 17 50125 Florence, Italy
Name: Elena	E-mail: elena.tricarico@unifi.it
Titles: Dr.	
Please describe your professional relationship to the PhD student: I hosted her for a training period in my lab	Please describe your field of expertise: Crustacean, crayfish, inland waters, alien species, behavioural ecology

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

Biomonitoring is a quite interesting and crucial issue, considering the currently increasing pollution rate, particularly in inland waters, and crayfish represent optimal model organisms to investigate this topic. Moreover, there is a growing demand to find the best non-invasive methods to apply on bioindicators to obtain reliable results without altering too much the behaviour of test animals. The PhD thesis focuses on the use of crayfish as bioindicators, coupling physiological and ethological aspects, and testing non-invasive methods for biomonitoring. It is thus very interesting and timely for the scientific pertinent field.

Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):



The thesis has four main aims, well elucidated at the beginning of the volume, for whose achievement the candidate conducted several laboratory experiments. Overall, the thesis is a very good work, well and clearly structured, and the English results almost fluent and well written. I also appreciated the deep and interesting review of the literature relating to her PhD subject; particularly useful are the Chapter 2.1. and 2.2., summarising the known used biomonitoring techniques also in crayfish. The methods are appropriate, clearly illustrated and explained, even if a higher number of test animals would have been better for some analyses and the study on odours should be deepened in the future (see specific comments). The statistical analyses result almost always well applied (see specific comments). All results are well illustrated and discussed with a good critical ability, and accomplished the thesis objectives. The candidate also developed non-invasive techniques for biomonitoring and widely disseminated her results through conferences and papers. The thesis confirms the possibility to use crayfish as bioindicator, even if the studies should be extended to more species and more in depth investigations are needed for the cardiac activity heartbeat. In conclusion, the thesis is very good, gives a relevant contribute to the field of biomonitoring in crayfish, and offers important scientific relapses.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Specific comments

Chapter 1

1.1, 2nd paragraph: also presence/absence of a certain species is an indicator of water quality

1.1, 3rd paragraph: I would say “sedentary life/low dispersal rate”

1.2, 2nd paragraph: please mention crayfish as k-stone species. Moreover, also the red swamp crayfish is not a good bioindicator being a highly plastic and adaptable species.

1.3, 4th line: “ but it is also a question of their...”. What about accumulation of toxins in crayfish? Please discuss also this issue.

1.3.1, 13th line: astaciculture

1.3.1, 16th line: “ other detrimental “habits” (high migration ability, etc.; Holdich et al., 2009; Jackson et al., 2014).

1.4, first sentence: please better rephrase it and delete the comma after be.

1.4., 2nd paragraph: “behaviour is usually observed directly or through video registration..”

1.4., 3rd paragraph: not all the crayfish are nocturnal. So, most are nocturnal. Moreover: “ circadian cycle, whereas stabilization...etc (please delete the comma after the parenthesis)”.

1.4., 4th paragraph: “, while Bini and Chelazzi (2006) have shown the heart and ventilatory rate to be correlated and decelerate under toxic impact of copper.” Moreover, “In sum, investigation of more than one characteristics of crayfish state (look into the combination and interaction of several parameters) is thus crucial.”



Chapter 2

2.1: I would say that Ethovision is just a technique not another method to study animals behaviour.

2.2.: should the already cited species reported with abbreviation in all the text?

2.2., 2, 4th paragraph: I would also add the reproductive status for females.

2.2., 2, 5th paragraph: please be aware about alien invasive crayfish.

2.2., 3.1, 3rd line: " Overall, behaviour demonstrates.."

2.2., 3.1.1., camera based, 2nd paragraph: please better rephrase this part.

2.2., 3.1.1., movement patterns: please again remember that not all crayfish are nocturnal.

2.2., 3.1.4, radio telemetry: "...30 to 300 MHz; Cook et al. 2013).

2.2., 3.1.4., acoustic telemetry: what about the time spent in refuge by crayfish? Is this a problem for acoustic monitoring? And what about vibrations?

2.2., 3.1.4., PIT, 2nd paragraph: "But if one thinks this idea out (is this correct?)" . Moreover, movement patterns can be influenced also by reproductive state or by need of food.

2.2., 3.2., 1st paragraph: "...of selected bioindicators, while bioindicators are..."

2.2., 3.2., 2nd paragraph: "...carried out exactly for that purpose..."

2.2., 3.2.1, IPG, 2nd paragraph: " Suggesting the IPG as very useful (...".

2.2., 3.2.1, PPG, 1st paragraph: 1984 in parenthesis, as 1990 for Depledge and Andersen below.

2.2.: What about the use of oxygen consumption monitoring among the cited method?

2.2., Table 1, carbon dioxide: please ventilatory system on the line above.

2.2., Table 1, prey-predator behaviour: please specify "injured conspecific odour".

2.2., Table 2: Bini and Chelazzi (2006) studied *P. clarkii*, not *cubensis*.

2.3, Methods: why did the authors not analyze also the exoskeleton? Why was the nickel of Darvoske samples not measured?

2.3, Discussion: It would have been noteworthy to cite also the paper by Gherardi et al. (2002). A comparison of trace metal accumulation in indigenous and alien freshwater macro-decapods. *Marine and Freshwater Behaviour and Physiology* 35: 179-188. What about the concentration of these metals in the water of the studied reservoirs?

2.3, Conclusion: About low migration rates: I would be more careful about this statement. For sure, crayfish are less mobile than fish, but some crayfish can disperse quite a lot.

2.4., Methods: Which is the sex of analyzed crayfish? Moreover, the n is low, as evidenced also by the recorded variability in the test crayfish (see for example S.D. of Fig. 3 or Fig. 5). And this can influence results and discussion. Why did the authors not use an ANOVA?

2.4., Results: Where are the statistical outputs?

2.5., Methods: again, which is the sex of the analyzed crayfish?

Chapter 3

3.1: Are the crayfish able to use the refuge without problems?

3.2.: Are the cameras able to record also the maxilliped movements (important to detect in the presence of some odours)?

Chapter 4



4.1., Introduction: please uniform the font. Moreover, nearly the end: “crayfish species-signal crayfish, *Pacifastacus leniusculus*”.

4.1., Methods: a control group (plain water before and after) is missing. Each crayfish was subject to each treatment. Was the order random?

4.1., Results and discussion: where are the statistical comparisons among treatments? It is better not to use the shelter in this experiment to add a further variable. The reaction towards the odour of injured conspecific can be explained considering that this odour is primarily an alarm odour for crayfish, signalling danger situations.

4.2, 2nd paragraph, 9th line: please write a comma after activity instead of full stop.

Chapter 5

General discussion, 3rd paragraph: “with the exception of *P. clarkii*” seems a nonsense, being the species invasive. Please delete or better explain it. Moreover, the wider use of this species can be explained also by its plasticity, flexibility, tolerance and easiness to be maintained in the lab.

Page 100, 1st paragraph: “.of potentially unsafe compounds, while tolerant crayfish (..”.

Page 100, 2nd paragraph: please consider also the reproductive status.

Table 1: again Bini and Chelazzi (2006) studied *P. clarkii*, not *cubensis*.

FINAL RECOMMENDATION

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

Florence, 2014 June 23rd

Date and place

Elena Tricarico

Name and signature