Review of the thesis by Petr Macek

The thesis is composed of six papers, a short introduction and a summary; the candidate is the first author of five of them. Four of them are either published in international journals or in press. All papers but one deal with wetlands in Belize and range from an ecophysiological approach (chapter 2) through population biology to mineral cycling and ecosystem processes.

Chapter 2

This is an essentially ecophysiological study of response of one plant species to gradients in flooding. A certain weakness of the paper is the lack of comparative approach as only one species is studied.

- Description of the statistical analysis is not satisfactory: was really 2-way ANOVA used? The data seem to be better suited for repeated measures ANOVA. Since the d.f. are not provided, it is impossible to check the correctness of the residual d.f. (This is a common omission throughout the thesis.)
- I understand that in the second experiment, shoots submerged for longer time produced more shoot. Isn't this a result of simple effect of more time available for growth? I assume that the null hypothesis here should not be "equal number of shoots of all treatments" but "equal rate of shoot formation over time" and the statistical tests should be changed accordingly.

Chapter 3

This is a rather rare study on environmental response of clonal growth parameters based on a detailed field study.

- Why author's own data and the data from the Czech National Phytosociological Database were analysed separately, and results from both analyses compared only informally? The author thus missed a chance to see the position of his data in the whole ecological range of *Potentilla palustris*.
- In the stepwise regressions, the author performed stepwise variable selections based on three non-overlapping sets of environmental variables. In the next step variables selected from these three sets were combined and put into a further analysis. This approach may does not optimize the global set of predictors; in contrast, in each set it may choose variables closely correlated with variables from some other set. The high values of set intercorrelations (p. 47) may then be due to this selection procedure and need not say much about the total data set. Could the author comment on this?
- The author does not mention the possibility that genetic makeup of individual populations could underlie differences in clonal growth morphology. For obvious reasons, an observational study cannot provide an answer, but there may be indirect clues available from other data sets (e.g. on gene flow among populations). Comments?

Chapter 4

This is an experimental mesocosm study of the response of three wetland species from Belize to addition of phosphorus and nitrogen, performed under three salinity levels. It nicely shows the dominant effect of phosphorus on the ecology of the wetland system.

- Why N and P levels were coded as 1-2-3? This is not fully linear, but not logarithmic either, and thus introduces artificial pattern to the data.
- Take logarithms of response variables changes assumption of additive variation to the assumption of multiplicative variation, not vice versa (p. 68).
- The multivariate analyses are confounded by the fact that mathematically dependent variables (such as Biomass, P-content of biomass and PUE) are used as predictors. Such variables are not linearly related (i.e. are likely not to be identified when linear dependence of predictors is checked by the program), but use of all three simultaneously does not introduce meaningful variation to the analyses. (The same problem is found also in paper 5.)
- The experiment was designed to test different response to nutrient under different salinity levels. Was there any *a priori* reason to assume that this would differ across species?
- As there were no replicates of individual nutrient/salinity treatments, the replicates of ANOVAs

Chapter 5

This is a paper based on an extensive fertilization experiment in the field, addressing both species responses and overall nutrient cycling in the system.

- Do you have an idea why CBM did not respond to P concentration (p. 99)?
- In the multivariate analyses, it is not fully clear which environmental variables were passively projected into the ordination space. I would assume that correct approach would be to use only treatment variables as predictors, and all soil variables as passive variables.
- It is not clear to me how phosphorus budget was calculated. This is an extremely interesting part of the paper, but without sufficient scientific rigour. I assume it was done by a series of indirect calculations, and therefore should be accompanied by some kind of error analysis. (It is also surprising to read that nitrogen recovery was [exactly] zero.)
- In discussion, the authors suggest that by phosphorus addition the system becomes more nitrogen limited. This sounds intuitively correct, but how would you then explain the low difference between P and NP treatments?

Chapter 6

This is a paper based on the same experiment as the previous chapter, but addresses population biology of individuals of *Typha domingensis* implanted into individual fertilization/salinity treatments.

- how many cells were used to assess effects for larger neighbour distances? Only the four cells with exact distances, or a range of distances within a certain distance class (e.g. 6 – 7 m)?
- p. 126: last lines in the first para seem to imply that analysis of the *Typha* spreading was done in fertilized plots only. Is this true?
- description of the model is unsatisfactory (namely of the processes occurring in already colonized cells). Formulas for all possible types of transition probabilities

should be given. I also assume (correctly?) that the remaining parameters other than Cp were used for the model parameterization, and both fertilized and non-fertilized parameter values were used to run different model scenaria.

Chapter 7

This is an elegant study of patch generation and maintenance due to interactions between plants, animals, and nutrient cycling. It also uses a wide array of methodological approaches (vegetation mapping, behavioural data on birds, isotope analysis).

- Comparison of shell density between patches and open marsh depend on the assumption that they can be located with the same likelihood in both habitats. Is this true?
- Do you have any behavioural data on the correlation of defecation with their other activities? The assumption here is that the droppings are produced when the birds spend their time in the patches, but I was not able to find any direct evidence for this.

General

Overall, this is a good thesis. Its core is the set of six self-contained experimental papers that are either published or publishable. They form a coherent group (some of them are based on different data sets collected in the same field experiment) and the author was therefore able to use information collected in one study to interpret or extend results of some other study. This is a really strong point in the thesis. (In this context, I do not fully understand why the Chapter 3 was included – the thesis would have been strong enough without this paper, and much more focussed. But there is nothing wrong with that in the strict sense.)

The papers are based on well-designed data collection and generally use appropriate techniques for data analysis. In most papers however, the data analysis parts are rather sketchy and important details on the exact procedures used (covariates, standardization, randomization type) are sometimes missing. They often use multivariate statistics; the analyses seem to be correct and informative, although, again, not always well described. In the univariate statistics, almost never any information on residual degrees of freedom is given. This is a serious omission as the reader/reviewer is left in doubts whether appropriate ANOVA type was used. (Also quite often no info on the software used for the univariate analysis is given.)

I also miss a statement on the candidate's contribution to the multiauthored papers; there is a statement by Eliška Rejmánková on the chapter five (i.e. paper four), but no information on the other papers.

The thesis has demonstrated that the candidate is able to do research of his own, and process the results to produce good and publishable papers. Therefore I recommend the thesis for defence. Still I reserve further questions concerning this work for the defense.

Tomáš Herben
4th January, 2009

Evaluation of the PhD thesis of Petr Macek: The role of clonal plants in wetlands.

The thesis presents original plant ecological research on the processes that permit plant species to survive stressful environmental conditions in wetlands mainly by varying to different degrees clonal plant traits resulting in the apparent vegetation structure that is typical for wetlands wherever they occur.

The thesis is an impressive large scale investigation, comparing wetland characteristics and corresponding clonal plant traits both in temperate and tropical ecosystems. In this way the thesis allows conclusions to be drawn at the general level of the relation between ecosystem properties and plant characteristics. In this respect the thesis far exceeds the local aspects of specific plant species responding to specific local conditions. Moreover a variety of techniques have been applied ranging from ecophysiological experiment via mesocosm manipulation to the level of full blown field experiments. This makes the thesis very worth while for the broader scientific community and this is reflected by the fact that 4 out of the 6 research chapters have been published in international peer reviewed journals of high scientific standards. It is clear that an important contribution to plant ecology has been produced by the author and therefore this thesis certainly must be admitted to be defended in public in order for the author to obtain his doctor's degree.

However, notwithstanding the quality of the actual separate chapters, the overall conclusions remain somewhat vague. The fact that species respond differently to different environmental drivers is hardly surprising as it constitutes the core of evolutionary ecology. Also the fact that nutrient availability drives the species responses and thereby controls their interaction and resulting community structure, is not entirely new. What is relatively new is assigning a crucial role to two clonal plant traits: internode length and number of internodes/branching frequency in modulating the effects of nutrient availability. It is in this respect that the thesis formulates general principles of escaping or tolerating stress and also where questions can be asked whether this really holds.

First of all it is assumed that clonal structures are costly to a different degree: internode elongation is less costly then increasing internode number. This raises the question of the currency of these costs. Most of the clonal structures are carbon sinks but in open ecosystems like wetlands, light is not a limiting factor and extensive carbon sinks can be maintained at low costs (see the excavated picture of Potentilla palustris at the beginning of ch3: few leaves support a gigantic stolon system). Even more, they allow extensive foraging for the real limiting factor: nutrients. This leads to two contrasting explanatory theories that pervade this thesis: a cost-benefit approach based on escaping stress and a competitive foraging approach quickly pre-empting and occupying space both explained by longer internodes and reduced branching. This ambiguity is also apparent in the final discussion.

Another paradox is in the fact that regeneration through seedling establishment is seemingly important. This is understandable given the fact that all individuals eventually die. However, seedlings and young individuals are extremely sensitive to flooding and therefore regeneration is mostly confined to the higher less flooded parts of the wetland. If this is true it is hard to understand the dynamics that will eventually result in clear separation of species along flooding gradient. It would be interesting to discuss possible clonal mechanisms that would allow a collective common start and a subsequent sorting along a flooding gradient in addition to understanding the current interactions driven by nutrients between different clonal strategies.

Lastly, it is assumed that clonal growth forms are the ultimate answer to the stressful environment of a wetland. But looked at it from a different angle: aquatic systems are less stressful then dry ecosystems and harbor the older monocotyledonous life forms. With no need for expensive secondary growth structures these species are perfectly adapted to their ‘stress’, whereas dicots conquered the land surface only to return to the aquatic system secondarily. Given the important ontogenetic differences that result from a monocotyledonous life form compared to the dicotyledonous one, I would have expected some discussion in this direction, certainly when comparisons are made between species in their clonal strategy across these two tribes as is the case in this thesis (Potentilla versus Eleocharis+Cladium+Typha).

However, these are typically points for a further discussion and do not in any way distract from the main conclusion: excellent plant ecological research that is well published internationally and tries to achieve a more general understanding exceeding the local, by comparing across continents, across species and applying a spectrum of relevant experimental techniques.

Jan van Groenendael
Head of Department of Aquatic Ecology and Environmental Biology
Faculty of Science
Radboud University Nijmegen
The Netherlands

Review of the Ph.D. Thesis "The Role of Clonal Plants in Wetlands"
By Mgr. Petr Macek

This thesis consists of eight chapters of which the first and last ones are the General Introduction and General Discussion, respectively. The remaining six chapters comprise six original papers of which P. Macek is the author or co-author. Out of these papers, three have been published in respectable journals, one has been accepted and is in press (also in a respectable journal) and two have been submitted for publications. To which journals? In any case, however, the submitted thesis more than fulfils the conditions set by the Faculty of Science of the University of South Bohemia for the acceptance of Ph.D. theses.

The subject of the thesis is of great interest not only to plant ecology, but also to general ecology and ecosystem management. The publication of the papers is timely in view of the present encroachment of anthropogenic influences on wetland ecosystems, in this case mainly tropical ones. Clonal emergent macrophytes (helophytes) form the structural matrix of these ecosystems. The description and analysis of the effects of eutrophication on the dominant helophytes in the marshes of Belize therefore represents a starting point for developing effective measures for the control of the eutrophication of these marshes. The analysis of the clonal behaviour of a temperate wetland plant, namely *Potentilla palustris*, provides a useful parallel with the other studies conducted on dominants of the Belize marshes, especially *Eleocharis cellulosa, Cladium jamaicense* and *Typha domingensis.*

The General Introduction (Chapter I) points to the importance of clonal vascular plants in wetlands and presents a brief outline of the problems to be studied. At the end, there is a list of four main questions the thesis should find answers to. **I would welcome if the candidate would explain in more detail the reasons for which he was asking these questions**. I suspect that he sometimes makes a shortcut between submergence and anoxia, but these two factors need not necessarily be coupled with each other.

The arrangement of the chapters II to VII of the thesis follows a logical progression from the individual plant level to the ecosystem level, which facilitates the readers´ orientation in the thesis. The graphical setup of the thesis is ingenious and excellent. Since the first five chapters have already passed though the peer-review process, there is only little to criticise in them. At the same time, however, the methods used and results presented in these five chapters induce some questions that the candidate might consider.

Chapter II is a paper by P. Macek et al. (2006) on the effects of submergence on *Eleocharis cellulosa.* This helophyte apparently dominates the Belize marshes if they are oligo- to mesotrophic. The results of the experiments carried out within this study are convincing. In my opinion, the storage of non-structural carbohydrates in belowground organs of this plant is among the most important physiological factors affecting its survival. The paper seems to be somewhat short of a full appreciation of its importance. **Since the carbohydrate storage was not studied in this paper, I assume that sufficient support to its importance not only in temperate helophytes, but also tropical ones, can be found in the literature. Is it so?** Quite a few normally emergent macrophytes, e.g, *Scirpus lacustris* or *Phalaris arundinacea*, can relatively well adapt to life in submergence by adopting the structure and physiology of submerged macrophytes. The main adaptation concerns the plants´ photosynthesis, which relies on the uptake of either CO_2 or HCO_3^- from the water. **Is there really evidence that *E. cellulosa* is not capable of taking up bicarbonate,** which evidently is the by far prevailing form of carbon available in the hardwater Belize marshes**?** The statement about the investment of the plants into shoot elongation in the last paragraph on p.11 sounds somewhat anthropomorphic. In submerged plants, stem elongation normally depends on the presence of ethylene in the tissues. **How fast do the plants, emerged after long-term submergence, recover from the post-anoxic injury?**

Chapter III is a paper by P. Macek and J. Lepš (2008) on the growth traits of *Potentilla palustris,* a plant typical of temperate meadows and somewhat acidic fens. In these habitats, it often becomes dominant in patches of quacking fen, being freed of the competition with most of the other fen species. Consequently, *P. palustris* can be considered as a weak competitor whose clonal growth involving the formation of stolons is part of its evasive life strategy. The study presents a number of both abiotic and biotic factors explaining the behaviour of *P. palustris* plants in different habitats. I must confess my having certain difficulties in accepting the anticipated equivalence of the abiotic and biotic factors, and more so the greater importance ascribed to the latter factors in this paper. In my opinion, the influence of the surrounding vegetation, presented as a directly acting biotic factor, can mostly be explained in terms of the modification of various elementary abiotic factors by the vegetation. **Could the candidate express his opinion about this question?** It might be helpful to consider the life and growth forms (*sensu* Hejný et al. 1998) of the plants interacting with *P. palustris* when trying to brake down their complex influence into individual elementary environmental factors. On pages 41 (bottom) and 42 (top), a list is given of four vegetation types containing *P. palustris*. **Can these types be also expressed in terms of phytocoenological units** that could perhaps be correlated with the DCA results given in Fig. 1**?** I would find it useful for a broader generalisation of this finding.

Chapter IV presents a paper by P. Macek and E. Rejmánková (2007), describing an experimental study of the effects of nutrient (N and P) supply and differentiated salinity on three dominants of the Belize marshes: *Eleocharis cellulosa, Cladium jamaicense* and *Typha domingensis.* The experimental setup was well designed; the question may only be asked **whether the 4-litre pots in which the experimental plants were cultivated were not too small** and thus limiting or unfavourably modifying rhizome growth towards the end of the experiment. The Appendix to the paper gives the data on the N and P contents in the aboveground shoots of the three plant species exposed to different levels of N, P and salinity. **How do these data compare with the results of analyses of plants growing under comparable conditions in the field? How do they compare with the N and P contents in other helophytes growing in similar habitats?** I would also like to remark that nutrient contents in the plants expressed as nutrient standing stocks (e.g., on a plant, pot or plot area basis) tell us more about the nutrient uptake by the plants than just the tissue concentrations of mineral elements. Their "dilution" in fast growing tissues can obscure the differences in the plants′ ability to use the nutrients for growth and vegetative propagation. **Why did the candidate not consider the evaluation of the experimental results also in this way?** This remark and question pertain also to the subsequent chapter.

Chapter V is a paper by E. Rejmánková, P. Macek and K. Epps, now in press, describing the changes in the vegetation structure and functioning in the Belize wetlands after three years of experimental phosphorus and nitrogen enrichment (artificial eutrophication) and differential salinity enhancement of the wetlands. The study consisted of a three-year manipulative experiment, comparing the effects of adding P, N and NP in combination with three levels of salinity, which clearly shows the deterioration of oligotrophic marshes under the influence of phosphate-rich effluents from agricultural areas. The nutrients were added in 2001 and 2002, and it is surprising that their effect was strongly expressed still in 2003 and to a lesser extent (possible fading out of the nutrients′ effect?) also in 2004. **Could the candidate explain why it was so?** The application of nutrients to limited areas in wetlands is always a tricky business. **Would the candidate explain** what is only referred to, i.e., **that a mere 2-day enclosure of the treated plot prevented the spread** (by local thermal currents or wind action) **of the applied soluble nutrients and salts to the surroundings?** The results of this study seem to confirm the general rule that removal of P-limitation of primary production is often coupled with a switch to N-limitation, in this case strengthened by the suppression of N-fixing Cyanobacteria. The decrease in species diversity in the marshes, brought about by the dominance of *Typha domingensis,* induced by the eutrophication and slight salinity, has also been proved. **I wonder if the candidate can agree,** on the basis of the experimental results, **with the statement that, along a gradient of trophy within a given ecosystem type, biodiversity is highest under mesotrophic conditions, rather than both oligo- and highly eutrophic ones.**

Chapter VI presents a study by P. Macek et al., submitted to press, on both quantitatively recorded and modelled spread of *Typha domingensis* in differentially eutrophicated (control and P-enrichment at two salinity levels) tropical wetlands. The study is thorough, though maybe a little speculative as concerns the extrapolation of the records of *Typha* spreading to a large area and the time interval of up to 9 years. With respect to the dynamics of vegetative propagation in *Typha*, I would like to put a **question about the minimum, maximum and average life span of individual ramets.** In other words, **to what extent has the ramet mortality been included in both the experimental records and the model? Could the annual biomass turnover factor be estimated on the basis of the results presented?** In a way, it is a pity that the spreading of the experimentally set up *Typha* clones could not be recorded more frequently than at one-year intervals. This would have enabled the dating of the growth of individual rhizome segments and of the emergence of individual shoots in a similar way as K. Fiala (1973, 1978, see also Westlake et al. 1998), dated it in his growth analysis experiments with *Typha angustifolia* and *T. latifolia.* **Was there recorded any thinning due to greater shoot mortality than emergence in older parts of the experimentally set up clones?** Apart from self-thinning, this phenomenon could have occurred especially in both elevated P treatments. On p.134 (top) there seems to be a discrepancy between the data on the mean aboveground biomass (0.47.2.62 and1.69 $kg.m^{-2}$, respectively) and the estimated annual net production (5.0 $kg.m^{-2}$). **Is this discrepancy the result of the biomass turnover in the *Typha* stands?**

Chapter VII contains a paper by P. Macek et al., submitted to press, on the limpkins´ (*Aramus guarauna*) feeding activity as a reason for a small-scale patchiness of wetland vegetation in the Belize marshes. The title of the paper suggests it will cover a broader scope of biotic interactions in wetlands. Nevertheless, the findings presented are original and interesting from the point of view of vegetation dynamics in wetlands. The experimental imitation of the eutrophication caused by limpkin droppings is ingenious. It would have been worthwhile also to assess the increase in $CaCO_3$ content in the soil containing fragments of *Pomacea* shells. This study deserves appreciation as one of the relatively rare instances of botanists taking animal activities into account in studies on vegetation dynamics.

Chapter VIII presents the candidate's short General Discussion of the results of all the papers (Chapters II to VII). The candidate deserves a high credit for summarising his results in such a concise way, though **some of his interpretations of the results may be too ge**neral, e.g., the application of the foraging theory and conclusions on the increase of branching in taller vegetation (p.164) to (all?) clonal plants without fully appreciating climatic and physiological factors affecting their growth and generative reproduction competing with vegetative propagation (photoperiodicity, thermoperiodicity, apical dominance, etc). The use of the term "invasive" (p.165) is not in agreement of the nowadays almost generally accepted concept of invasiveness of alien plants only. **Would not, e.g., the term "aggressive" be more appropriate?** A general remark: only very few of the references in the individual chapters of the thesis refer to literature published before 1990. I would, however, advice the candidate to look for relevant information also in older books and papers. The chapter ends with a brief and necessarily very general summary with conclusions. Among them, I only miss some reference to the relevance and applicability of the results obtained to the management of the Belize wetlands in particular and of tropical wetlands in general. **Could the candidate present them?**

On the whole, Petr Macek´s Ph.D. thesis represents an admirable achievement, especially in the study of tropical wetland plants and vegetation. The remarks made and questions asked in my review are only partly critical. For the most part, they have tried to stimulate the candidate's further and maybe more general considerations and appreciation of the value and use of the results presented. The amount of work this thesis represents is impressive and the quality of all the papers included in the thesis is high. For all the above reasons, I am hereby taking the liberty to recommend a positive evaluation of the doctoral thesis of Petr Macek. Consequently, I recommend that the degree "Philosophiae Doctor (Ph.D.)" be awarded to him after a successful defence of his thesis.

Třeboň, 3rd January 2009.

Jan Květ

RNDr. Jan Květ, CSc.
Opponent