PhD Thesis Acceptance Report
Research Discipline Council of Biological Sciences
Jagiellonian University in Kraków

Candidate's name and surname: Kyle Coughlan

PhD Thesis Title: Oxidative stress biology of nest-box breeding birds

Thesis Supervisor: Dr. hab. Ulf Bauchinger

Assistant Supervisor / Second Supervisor/ Co-supervisor (if applicable): Dr. Edyta Sadowska

Reviewer: Prof. Dr. Michaela Hau

1. The topic of the dissertation

Whether the topic of the dissertation is relevant to the development of the discipline of biological sciences (suggested approx. 50-150 words)

Oxidative stress, i.e., the presence of too many oxidating compounds relative to substances that provide oxidant protection (antioxidants), can have detrimental effects on organismal functioning by attacking critical structures such as DNA, proteins and membranes. One major hypothesis states that energy metabolism generates oxidative stress, in particular during periods with high metabolic rate. Metabolic rate is a key organismal trait tightly linked with an organism's performance and fitness. Thus, to understand how organisms cope with variations in energetic demands during different life cycle stages and environmental conditions, knowing the trade-offs that may be associated with an upregulation of metabolic rate are critical to illuminate the ecological costs and evolutionary implications of such responses. In his PhD thesis, candidate Kyle Coughlan tested whether a relationship indeed exists between periods characterized by high metabolic rate and measures of oxidative stress in two free-living Passerine species, blue and great tits.

2. The candidate's knowledge

An assessment, with justification, of whether the dissertation demonstrates the applicant's general theoretical knowledge in the discipline of the biological sciences (suggested approx. 50-250 words)

In his PhD thesis, candidate Kyle Coughlan demonstrated an excellent theoretical knowledge of his own research field as well as of the broader field of biology. In the Introduction, he provided relevant background knowledge on the biology of oxidative stress, the various kinds of damage it may inflict, and its proposed relationship with energy metabolism. I was impressed throughout the thesis by the clear lay-out of the general aims, hypotheses (both main and alternative) and predictions for his studies. He used this structure as a common thread in all parts of his thesis, which in turn highlights the rigorous scientific thinking that guided Kyle's research. I also really enjoyed reading the Discussion, in which Kyle provided a nicely balanced and broad assessment of his findings in the context of current work in this field. He touched on many topics including limitations of his study and possible alternative explanations of his findings by integrating knowledge from the fields of exercise physiology, circadian rhythms, stress endocrinology and behavioral ecology. Kyle's main hypothesis (which reflects the predominant thinking in the field) was not supported by the data, and while the alternative hypothesis received some support, his results were not entirely easy to interpret. This is often the case in field studies, where many unmeasured (or even unmeasurable) variables can change an individual's state. However, at the same time field studies are immensely valuable because they are conducted within the natural ecological setting of a population. Kyle really

did excellent work in the Discussion by concluding that while his data do not corroborate the hypothesis that periods with higher metabolic activity are those in which individuals also incur oxidative stress, they do indicate that the relationship between metabolism and oxidative stress is highly complex and still hardly understood. He then proceeded to highlight the areas where more knowledge is needed.

3. Independence of the candidate

An assessment, together with a justification, of whether the dissertation demonstrates the applicant's ability to carry out scientific work independently (suggested approx. 50-250 words)

Based on his PhD thesis, candidate Kyle Coughlan is clearly able to carry out independent scientific work. He is great at outlining the conceptual framework of his work by formulating aims, hypotheses and predictions and using them as a crucial backbone for his work. Kyle measured an array of variables as markers for the oxidative status of individuals and provided clear justifications for choosing these parameters. In the Introduction and Discussion sections, he demonstrated critical thinking by highlighting the strengths and weaknesses of the different hypotheses he tested. His thesis also provides evidence that he is able to gather relevant knowledge from different fields and to synthesize it into a coherent concept. Furthermore, throughout the thesis and especially at the end of the Discussion section, he outlined interesting avenues for future work.

Kyle has also been successful in publishing his work. Study 1 from his PhD thesis and related work on zebra finches not included in his thesis were published in peer-reviewed international journals with him as first author. He is also first author on two publications on work conducted prior to his PhD thesis. Kyle presented his work in oral and poster format at several international and national conference. Thus, the candidate is capable to successfully communicate and disseminate the findings of his work. Taken together, I am fully confident that he is able to carry out independent scientific work.

4. Originality of the dissertation

An assessment, with justification, of whether the dissertation provides an original solution to a scientific problem / an original solution to the application of the results of one's own research in the economic or social sphere (suggested approx. 100-250 words)

Candidate Kyle Coughlan's PhD work indeed provides an original solution to a scientific problem. Kyle's thesis draws attention to the fact that it has almost become a dogma in the fields of ecological and evolutionary physiology that high metabolic rates cause oxidative stress, which in turn should lead to damages at the cellular and organismal level and thus to reduced performance and decreased fitness. While there was already evidence and discussion of findings that are counter to this 'dogma' (which Kyle presents and acknowledges in his thesis as a critical starting point for his own line of work), the field still has not changed much. Hence, Kyle's multifaceted work on oxidative biology in two free-living species was very timely in testing the relationship between metabolic rate and oxidative stress. Since his results provide critical evidence in two free-living species that no such relationship exists, and in fact some findings indicate a relationship in the opposite direction, Kyle has added important data that will allow the field to move forward and begin to abandon the 'dogma'. Originality in Kyle's work also derives from his study design, in which he contrasts birds in different metabolic states during different seasons and life history states (non-reproductive/reproductive), times of day (night/day) and two species with somewhat divergent life history strategies (the blue tit being smaller and with a slightly faster pace-of-life than the great tit).

5. Questions and/or criticisms to which the Reviewer expects the candidate to respond during the defence

I really enjoyed reading the thesis in its entirety. It is well-written, concise and clear. The one part on which I would have loved to get more information is actual metabolic rates of the two tit species in the different states in which they were measured. For example, this information could have been included as a Table. Related literature was cited very well, but I had to go back to the original publications to assess these data, while a direct comparison of already existing work (including work in your own lab) would have been a great addition.

Questions:

- **ROS signaling (e.g., p. 17): what is the role of ROS signaling and how can we distinguish between actual signaling (which may require a receptor or other 'receivers' of sorts) and mere (side)effects on other molecules/substances/structures?**
- **Blue and great tits are related, use similar habitats, but also do differ in some of their life history, behavior and ecology. What was the main conceptual reason for combining the two species in your analyses?**
- **Are there measures of performance or fitness that you may have collected but were not able to include in your PhD thesis and possibly link to the oxidative status of individuals, to address questions on the organismal consequences of oxidative stress?**
- **Your thesis work was primarily observational/correlational. What kinds of critical experiments would you do, if you now had unlimited resources to do it again (or conduct additional work)?**
- **I am not entirely sure why you only included birds that successfully fledged a brood in your analyses. Could you not obtain samples from the other (non-successful) individuals? Or not at sufficient sample sizes? Because it could be interesting to see whether individuals that were not successful in raising a brood differed from those who did.**
- **In study 1 you only sampled individuals during their second clutch, which is a subset of the entire population. Could those individuals have been of superior quality because they could afford raising a second clutch, and could that have influenced your results?**
- **In most of your analyses you used models with only a few explanatory variables and covariates. Which makes total sense and adds to the clarity of the results. I am guessing that sample size issues also prevented further analyses. I wondered about the effects of sex, air temperature at sampling, body mass etc (factors that could affect the metabolic rate of individuals and thus your findings) – why you could/did not include them, and what in your opinion think their effects may have been?**
- **I am sure you have thought about the relationships among the different oxidative stress markers you assessed. Have you done any, even just exploratory, statistical analysis of their covariation and what that may mean?**

6. Other observations on the content or form of the dissertation (optional)

I, hereby, declare that the reviewed PhD thesis by **Kyle Coughlan** meets the criteria pursuant to art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended) and request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków accepts **Kyle Coughlan** for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences

YES/~~NO~~

I, hereby, request that the thesis is accepted with distinctions

~~YES~~/NO

Justification of the request (if YES is selected)

Apr 29, 2024
date

Prof. Dr. Michaela Hau
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Reviewer's signature

INFORMATION FOR THE REVIEWER:

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A duly signed original should be sent to:

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