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## **REVIEW**

of the doctoral thesis by Anne Norbertine Maria Andrea Ausems

**„Reconstructing important phases of the annual cycle of four species of storm-petrels  
using stable isotope analyses and ptilochronology”**

presented to the Biological Sciences Discipline Board

University of Gdańsk

and supervised by prof. dr. hab. Dariusz Jakubas

### **Topic of the thesis**

Doctoral thesis by Anne Ausems focuses on the ecology of four species of procellariiform birds (storm-petrels). In general, this is a difficult model to study, as storm-petrels usually nest on inaccessible islands spread across vast oceanic areas far from the mainland and they stay at the breeding sites only for a relatively short period required to lay eggs and raise offspring. For the rest of the year they wander across the oceans and are virtually impossible to study using direct methods. Thus, to gain information on the non-breeding ecology of storm-petrels, researchers use an array of indirect approaches, which can be effectively applied to the material collected during the breeding season. The aim of this doctoral thesis was to use stable isotope analyses and ptilochronology to obtain information on foraging niches and moulting period of four storm-petrel species. All these analyses were based on the samples collected from birds captured during the breeding period. The analysis of stable isotopes allowed to test for differences in trophic level and diet between different life stages (pre-laying females, chick-rearing adults and chicks), as well as it provided information on

the location of key moulting areas for the study species, while ptilochronology (assessment of feather growth bars) allowed to explain inter-specific variation in feather growth rate. What is important, all the research was conducted in a multi-species approach and focused on storm-petrels from both Southern and Northern hemisphere, which I find as one of the major strengths of the thesis. Southern and Northern storm-petrels are subject to much different ecological and environmental conditions, which may drive strong variation in annual cycles, physiology and behaviour. In conclusion, the choice of the model was ambitious and overall aims of the thesis were likely to provide valuable and novel information on the clearly understudied avian species.

### **Thesis structure and formal assessment**

The major part of the doctoral thesis consists of three peer-reviewed publications (henceforth also referred to as chapters):

1. Ausems, A. N., Wojczulanis-Jakubas, K., & Jakubas, D. (2019). Differences in tail feather growth rate in storm-petrels breeding in the Northern and Southern hemisphere: a ptilochronological approach. *PeerJ* 7: e7807.
2. Ausems, A. N., Skrzypek, G., Wojczulanis-Jakubas, K., & Jakubas, D. (2020). Sharing menus and kids' specials: Inter-and intraspecific differences in stable isotope niches between sympatrically breeding storm-petrels. *Science of the Total Environment* 728: 138768.
3. Ausems, A. N., Skrzypek, G., Wojczulanis-Jakubas, K., & Jakubas, D. (2021). Birds of a feather moult together: Differences in moulting distribution of four species of storm-petrels. *PLoS ONE* 16: e0245756.

The manuscripts were published in 2019-2020 in the international English-language journals from the Journal Citation Reports (JCR) with Impact Factors (IF) ranging from 2.379 to 6.551. This included two interdisciplinary Open Access journals (PeerJ and PLoS ONE) and one high-profile journal focusing on a broad spectrum of environmental sciences (Science of the Total Environment). The choice of high quality publication venue was likely to secure effective dissemination of results across a wide readership. What is worth stressing, the Ph.D. candidate had the leading role in manuscript preparation, as inferred not only from her first authorship in all three publications, but also from co-authorship statements. Based on this information, Anne Ausems was involved in all key stages of research, including conceptualization, development and validation of methodology, investigation,

data curation, analysis, and writing of the original drafts. She also participated in project administration. This allows to conclude that her role in the research presented within the thesis was of primary importance and that she demonstrated versatile skills required for an efficient completion of scientific process.

Apart from the publications, the thesis includes a summary part with two relatively short (3-4 page long) summaries written in English and Polish. While they present a concise background for all three chapters (introduction, aim and hypotheses, methods), the results and discussion largely overlap with abstracts of the chapters. However difficult it may be to avoid this kind of repetition, in my opinion more effort could have been invested to prepare a more holistic message across the chapters and provide a less fragmented picture of the results and discussion.

## **Chapter 1**

In this chapter, the Ph.D. candidate and coauthors used ptilochronology to assess the relative energy availability during moult in two Southern and two Northern storm-petrel species. They found faster feather growth in Southern than Northern species and this pattern was explained with differences in the annual cycles between these taxa – there is an overlap between breeding and moulting in the Northern (but not Southern) species, producing a trade-off in energy allocation and limiting feather growth. The results provide a novel insight into the moulting ecology of procellariid birds, but they were based on one important methodological assumption. The authors compared their own measurements of feather growth bars in storm petrels to the available data from other bird species and found them significantly outlying. Based on this observation and earlier information from albatrosses, the authors concluded that two growth bars are formed per 24 hours in their study species, contrasting with a single growth bar formed daily in other avian lineages. Thus, the authors adjusted growth bars accordingly prior to the analyses. Although the authors should be commended for checking how their data on feather growth rate fitted the global picture across bird phylogeny, I have one serious concern about their assumption. As can be judged from Figure 2, feather growth rates from the Northern species could be identified as much stronger outliers than feather growth rates of the Southern species, which actually were relatively close to the major point cloud at the scatterplot. So the question is how can we be sure that all four study species formed two growth bars daily, not only the Northern species? I am afraid that residual growth rate (against feather length) in the Northern species, as presented on Figure 2, would be smaller than for some non-procellariid species in which a single growth bar per 24 hours was assumed. In my opinion it is crucial to provide

strong and convincing support for the application of this adjustment across all study species – its exclusive application to Northern species could change the results entirely, either producing no significant differences between Northern or Southern species or possibly even showing an opposite pattern to the one reported (i.e. higher feather growth rates in the Northern species).

## Chapter 2

This chapter contains the first results from stable isotope analyses and the Ph.D. candidate with coauthors investigated how stable isotope niches varied between different life stages in storm-petrels. This is the only chapter where investigation was restricted to two Southern species, lacking a comparison with Northern ones. Despite this limitation, the study is well designed and sampling scheme is smart – the authors collected adult blood, chick down and chick feathers to assess isotopes at the stages of chick-rearing adults, pre-laying females, and chicks, respectively. The major findings were as follows: i) pre-laying females had the widest isotope niches, likely because of larger foraging areas; ii) chicks' diet was at the higher trophic level than the adults' one, probably due to higher energetic needs during growth; iii) the level overlap between isotope niches at different life stages varied between the species (high overlap in Wilson's storm-petrel and low overlap in black-bellied storm-petrel), which was explained with variation in diet breadth (mostly krill in the first one, krill and fish in the second one); and iv) high overlap in isotope niches between the two species, suggesting a general similarity in main prey items and foraging areas. The results are generally well elaborated, and the discussion is thorough, providing extensive theoretical background for the results and introducing a range of alternative explanations. Also, advanced statistical tools were applied in the analyses and they certainly went well beyond what is traditionally considered a golden standard in the multivariate modelling. The one thing that was not clear to me in the methods section was whether analyses of isotope niches took variation in sampling effort into account. This would be especially important in the analysis of niche overlap – lower sample size could produce smaller niches and their smaller overlap between life stages. In fact, the black-bellied storm-petrel had much smaller sample sizes than Wilson's storm-petrel (38 versus 152 individuals, respectively), which could drive variation in isotope niche overlap, if not accounted for. Finally, I found some editorial issues with citations of tables and figures throughout the manuscript, e.g. Table 3 should be referred to in the caption of Figure 2, Table 4 should be referred to in the caption of Figure 4, Table 5 should be referred to in the caption of Figures 9 and 10, Figure 5 should be referred to in the results section while reporting results on hatching date, etc. It must be stressed, however, that while these

editorial errors decreased clarity of the manuscript, they did not undermine the overall scientific value of the chapter.

### **Chapter 3**

This is the second chapter which focuses on stable isotope analyses, but in the context of moult rather than diet. The Ph.D. candidate with coauthors aimed to investigate intra- and inter-specific variation in isotope niches of adult tail feathers grown during the last moulting episode. One of the major challenges of this work was to identify key moulting areas for each study species based on the isotopic composition of feathers. Intra-specific isotope variation was primarily explained with season and morphological characters of birds, suggesting that storm-petrels may change location of their foraging areas (or diet) based on their morphology. At the same time, location of moulting areas showed great inter-specific variation and I agree with the authors that these information could possibly be used to fine-tune conservation efforts targeted at storm-petrels, as it is always crucial to protect endangered species across their entire annual cycle, while the protection restricted to breeding sites is often deemed insufficient and ineffective. This is especially important for one of the study species, the Leach's storm-petrel, which was classified as globally threatened (category: Vulnerable) by IUCN due to rapid decline in population size over the recent decades. Although the authors acknowledge that their results may be of practical conservation value, I found no information on the unfavourable conservation status of their study species in this chapter and I think this could have been addressed more explicitly in the text. On the other hand, the authors admit that their methodology can only provide a rough picture of large scale movements of storm-petrels across the globe and that analyses of tail feathers cannot provide information about the entire moulting period. Although these limitations may seem to weaken the strength of the results and conclusions, I find it generally positive that they were thoroughly discussed in the manuscript.

### **Final conclusions**

In conclusion, the doctoral thesis by Anne N. M. A. Ausems is of high scientific value and reports novel results that broaden our understanding of certain aspects of bird ecology. In my opinion, the doctoral thesis meets the requirements by the Act of 14 March 2003 on academic degrees and academic title and degrees and title in art (Dz. U. z 2003 r. Nr 65, poz. 595; z 2005 r. Nr 164, poz.

1365; z 2010 r. Nr 96, poz. 620, Nr 182, poz. 1228; z 2011 r. Nr 84, poz. 455). On this basis, I request Anne Ausems to be admitted to further stages of the course of awarding a doctoral degree in the discipline of biological sciences.

A handwritten signature in blue ink, appearing to read "Robert Ausems". The signature is written in a cursive style with a large initial 'R'.