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The review of the doctoral dissertation „ Coordination of male and female parental performance in the Little Auk, *Alle alle*" authored by Antoine Grissot

The dissertation presents the results of the study on several aspects of parental coordination in the Little Auk *Alle alle*. The manuscript containing 138 pages is written in English. First out of three chapters has already been published as a scientific article in an internationally recognized journal indexed by JCR. The two remaining chapters are presented as manuscripts prepared for publication.

The chapters are preceded by a summary in English and Polish language and a general introduction to parental care. This part of the dissertation proves that the Author is well versed in the concepts and hypotheses relating to the evolution of parental care and its different forms as well as the issues concerning sexual conflict and parental cooperation. My minor remarks refer to the summary in Polish, where typos and stylistic errors are noticeable. This, however, is acceptable as Polish language is not the Author's mother tongue. The remainder of the dissertation written in English does not raise any linguistic concerns. In some citations there are typos in authors' surnames or errors in the year of publication. Not all publications listed in the reference list are cited in the text.

The main part of the dissertation are the results of the research presented in the form of manuscripts of articles (already published or prepared to be published). At this point, it should be said that the Author approached his project very ambitiously, as Little Auks are not an easy research object, and conducting long-term observations in Arctic conditions is demanding both physically and financially. During the implementation of the project the Author collected a lot of valuable data, the analysis of which provided interesting results. He also used modern techniques for monitoring animal activity. The research topic itself, that is the cooperation between partners during breeding season, has been studied for a relatively short time, so the analysis of another species is a valuable contribution to a better understanding of this area of behavioral ecology. I will present my comments on the dissertation and the questions that arose while reading it in relation to the following chapters.

In **Chapter I** („*Effect of environment on Parental Coordination of Chick Provisioning*"), the Author analyzed the coordination of parental provisioning (short and long foraging flights) as well as the relationship between this variable and environmental conditions in five different breeding seasons and chick growth. The results of the research showed that the frequency of overlapping of short and long flights between partners was significantly greater than expected by chance, from which the Author concluded that Little Auks pair-members coordinate these behaviors. This in turn provides the chicks with a more even supply of food. Here is my first question for the Author. The presented analyzes show that the coordination of short and long flights covers 22.7% of their duration. Does this mean that the partners do not coordinate their flights for the remaining 77.3% of the time? If so, the vast majority of parental foraging flights were not performed optimally for the chicks, i.e. with minimization of long breaks between feedings when both parents were on long foraging flights simultaneously. Can parents' foraging flights really be considered coordinated if the coordination concerns less than a quarter of their duration? The Author assumed that if the coordination is significantly higher than expected by chance, then the actions are coordinated. There is some logic in such approach, however comparing the percentage of coordinated flights (22.7%) to uncoordinated flights (77.3%), it turns out that the former are many times less than the latter. In this approach, Little Auks behave rather in an uncoordinated manner, or to be precise: coordinated flights constitute a definite minority during foraging. I would like the Author to refer to such an interpretation of his results.

In the further part of Chapter I, the Author found no relation between the coordination of parental foraging flights and environmental factors as well as the condition of the nestlings. If coordinated flights constitute a minority of all flights, the lack of these relationships is not surprising, as the level of coordination may be too low to affect the development of chicks or to depend from environmental factors.

I would also like to ask some minor questions for this part of the research

- What was the coordination of long and short flights expected by chance (in %)?
- How many of the studied pairs showed any flight coordination and how many did not show any coordination? If I understand correctly Fig. 2 in Chapter I, it implies that about half of the pairs showed no flight coordination.
- How were the same pairs observed in consecutive seasons treated statistically (so that they do not constitute, for example, pseudo-replication)? How many such pairs were in the study?
- What color were the marks painted on the breasts of Little Auks with markers? Is it possible that such colorful "ornaments" could change the behavior of partners? For example, birds marked with specific colors may have looked more / less attractive to their partners, which could result in more / less parental investment for these partners. Some studies show that individuals may react to

additional colors, such as color bands. Other studies indicate that individuals who perceive their partners as more attractive may invest more in the offspring conceived with such partners.

- The coordination of long and short foraging flights did not increase the condition of the nestlings but maybe it improved parental condition. Were the more coordinated pairs in better condition? Has it been tested?
- The author states that some pairs were monitored in subsequent seasons - did such pairs increase coordination over the years?
- Why were 10-minute windows used in the analyzes instead of the total time when the parents were performing short and long flights?

Chapter I is an interesting analysis of the parental behavior during breeding in difficult climate conditions. To my surprise, a relatively small percentage of long and short foraging flights were coordinated between the pair-members. Considering that Little Auks mates spend together many seasons, raise only one chick per season, and the conditions in which the offspring develop are not mild, I would expect the parents to coordinate their behavior at a much higher level, which should reduce the chick's long wait for food while both parents perform long foraging flights at the same time. However, this is not the case. It is very interesting that in conditions that definitely favor the evolution of parental coordination, it remains at a relatively low level (although, as the Author noted, this level is significantly higher than expected by chance). I think this part of the research would be more complete if the Author pointed out that most of the time Little Auks do not synchronize their long and short flights within a pair, despite numerous premises for coordination. It seems that the coordination of parental feeding flights in the studied species is not necessary for the development of the offspring, as about half of the pairs did not synchronize long and short flights at all, which did not affect the chicks. Perhaps the food provided is so reach in calories that the chicks tolerate longer breaks in feeding. Another reason for rather low flight coordination between parents may be the fact that any synchronization seems to involve the knowledge about the partner's actions. In the case of Little Auks it may be difficult, because their foraging flights last many hours and when absent at the nest, the parent does not know whether its partner was present at the nest at that time or not. Synchronization in most of the studied species of birds consists either in performing the same activity while the partners observe each other (e.g. synchronous provisioning, which reduces the risk of revealing the location of the nest to the predator) or in alternating activities, e.g. when one of the parents broods the nestlings and the other brings food, followed by swapping. In both situations, the partners are in regular contact and have knowledge of each other's activities. In Little Auks such a flow of information between partners during long-hour foraging flights seems unlikely. Can the Author propose a theoretical mechanism of parental flight coordination in the studied species?

In **Chapter II** (*Coordination of Parental performance Through the Whole Breeding Season*) the author analyzed parental coordination throughout the breeding season (as opposed to Chapter I, where the analyzes concerned only the middle part of the nestling period). The research showed that mates were characterized by high coordination during incubation (88% on average), which is not surprising because the environmental conditions force almost continuous incubation. Parental coordination during the nestling period was much lower (time of long and short flights overlapped in 27%) and this result was similar to that obtained in Chapter I (22.7%). The discrepancy of a few % could result from different methods (in Chapter I 10-minute time windows were analyzed while in Chapter II the continuous flight time, moreover, in Chapter I the coordination of parents was studied in the middle part of nestling period, and in Chapter II the data came both from the initial and middle part of nestling period). The author showed that the coordination of short and long flights within a pair at the level of 27% was significantly greater than expected by chance and thus considered parental behaviours during chick rearing as coordinated . However, I have the same question for these conclusions as in Chapter I: if 27% of short and long flights were coordinated in time, and the remaining 73% were characterized by a lack of coordination, should the parents' behavior be considered coordinated or not? What was the level of simultaneous long and short flights expected by chance? The author also found that the coordination of parental short and long flights was significantly higher in the initial part of nestling period than in the middle one. The study would be more complete if, apart from statistical significance tests, the author presented the real % of flight coordination in both stages of chick development.

As in Chapter I, when analyzing Fig. 2 in Chapter II, it seems that that about 50% of couples did not coordinate their flights at all. The results would be clearer if the Author provided the information what percentage of pairs coordinated their short and long flights and what percentage of pairs did not. From Figure 2 it can be concluded that the flights of some pairs are completely uncoordinated, while others coordinate a great proportion of the flights. I am not convinced whether averaging these results has biological justification. The Author summarizes the obtained results by stating that parents presented: "active coordination of the foraging pattern, leading to an avoidance of both partners performing long trips at the same time". I think that this conclusion is exaggerated, considering that in 73% of time the flights were uncoordinated - so it seems that in most cases the long flights of both parents overlapped.

The author also showed a quite complicated and difficult to interpret relationship between the coordination of the pair-members during the incubation period and the early and middle stage of nestling period (i.e. a higher amount of coordination during the early incubation was associated with a lower mid chick rearing coordination index, while a higher amount of coordination during the mid phase of the incubation was linked with a higher mid chick rearing coordination index). Given the low sample size ($n = 15$) and the fact that within the same pair the level of behavior coordination may change in subsequent days of breeding season, the relationship between the parental coordination during

incubation and different stages of nestling period demonstrated by the Author could be coincidental. I would suggest considering this possibility.

Data and results presented in Chapter II do not indicate whether the pairs that were better coordinated at the beginning of the incubation were also better coordinated in the mid phase of the incubation and during the nestling stage (in Figures 2 and 3 the pairs were not numbered). It would be interesting to check whether the pairs which had greater coordination during incubation, also had greater coordination when feeding the chicks - in other words, for such a small sample size (n = 15) longitudinal analyzes might be more interesting. The graphs show that pairs considerably differ in coordination, so looking for common trends in such a small sample size with high between-pair variation may not be the best approach.

Since the research in both seasons analyzed in Chapter II involved the same breeding pairs, an interesting issue, but not raised by the Author, is whether specific pairs in consecutive seasons are characterized by similar coordination. Unfortunately, as mentioned earlier, the pairs in Fig. 2 are not numbered, so it is impossible to infer on it. It is a pity that the Author did not present the analyzes of the coordination of parental behavior of the same couples in the following years because it is emphasized that the species is monogamous and the pair-members stay together in subsequent seasons. So it is interesting whether, for example, the cooperation between partners increases over time.

I would also like to ask the Author why pairs whose broods were not successful were removed from the analysis. I am not entirely convinced by this approach. Perhaps the coordination of parental behavior has a significant impact on the hatchability of the eggs and/or the survival of the offspring. It would be interesting to present the coordination of behavior in pairs that failed to raise their chicks.

As the manuscript of Chapter II has not been submitted for the publication yet, I hope that the above comments and suggestions will help to improve it in a way that allows publication in one of the leading behavioral journals. The current results are interesting, but on the basis of the data collected by the Author it is possible to present a much more comprehensive analysis of parental behavior in Little Auks, which would compensate for the small sample size.

Chapter III of the dissertation (*Development Of A New Method To Look At Parental Investment And Coordination Using Miniaturised Light-Based Geolocators (gls)*) tests the applicability of GLS systems for the study of parental behavior in Little Auks. The study involved an experimental group equipped with GLS loggers and a control group, which is the optimal approach when testing a new method. Both groups were video recorded. The Author compared the data provided by geolocators placed on the birds to video recordings of the same pairs in order to investigate the accuracy of the tested system in recognition of individual behaviors (such as the presence of the parent in the nest, the presence of the parent in the colony and foraging). In addition, the behavior of the experimental group equipped with

geolocators was compared to the control group in order to determine whether the presence of geolocators affected the behavior of the birds.

The methodology used in this study is insightful and well designed. The analyzes revealed that the data obtained from geolocators enable identification of various bird behaviors. The level of accuracy decreased with the number of different behaviors, but it is likely that the system may be improved in the future so that it allows for effective recognition of a larger number of behaviors. The results obtained by the Author indicate interesting new possibilities of using geolocators to analyze bird behavior, in particular parental activity in species nesting in dark places, such as burrows or cavities.

The study also revealed that the placement of geolocators on birds was not indifferent to them and I significantly affected parental behavior. The changes concerned the pattern of incubation and foraging. Both incubation bouts of males and females as well as their foraging flights were significantly extended. The Author described these effects as fine-scale behavioral changes, with which I cannot agree. The results do not show precisely how much the average incubation bout and foraging flight time increased, but it can be concluded from Figure 2 that the incubation bouts increased by about 3 times (the median increased from approximately 1 hour to 3 hours). I would not call it a fine-scale behavioral change. To me the elongation of incubation bouts by a few hours constitutes a significant behavioral change.

The fact that the change in the incubation pattern did affect the hatchability of the eggs and the growth parameters of the chicks is not a basis for considering this change as minor. Sometimes even large behavioral changes do not adversely affect individuals, so the scale of the change should be separated from its impact. In my opinion, the behavioral changes caused by the placement of geolocators on the birds were considerable, despite the fact that they had no negative impact on tested parameters of breeding success. It should also be noted that the Author did not investigate the effect of geolocators on the condition of the individuals wearing them. Parents performing longer incubation bouts might spent less time foraging, which could (but did not have to) affect their condition.

In my opinion this part of the study has brought very interesting results, but I do not fully agree with their interpretation - I got the impression that the Author diminishes the effect of geolocators on the behavior of Little Auks, which allows to present the tested solution as promising and worth recommendation. Meanwhile, the real value of the conducted research is to indicate that geolocators provide quite accurate behavioral data, however, they have serious limitations, as they significantly change the behavior of individuals. I am aware that currently there is a great emphasis on the applicability of conducted research, but unfortunately it often leads to highlighting the usefulness of a given solution while minimizing its evident flaws, with potential detriment to the objectivity of the research conducted.

Summarizing Chapter III, I think that in terms of the accuracy of monitoring the behavior of Little Auks (and probably other bird species), the GLS system is quite promising. On the other hand, I am skeptical about the usefulness of this method due to the behavioral changes caused by geolocators placed on Little Auks. The conducted research was methodically well planned and brought interesting results expanding our knowledge on the possibility of monitoring bird behavior with the use of new technologies, however, in my opinion, the interpretation of the results regarding the usefulness of geolocators for behavioral studies on Little Auks is improper. Any method that significantly changes behavior is not a good choice for behavioral studies. In my opinion this aspect should be much more emphasized in the discussion. Currently, the influence of geolocators on bird behavior is only briefly discussed and is not perceived by the Author as a serious obstacle in the implementation of the tested method. Noteworthy, a change in the incubation pattern may affect a number of other breeding parameters not studied by the Author, such as the time of embryo development or the aforementioned condition of the parents. These issues should also be raised in the discussion. Currently, the author concludes that "there are no apparent arguments against deploying GLS in the species," which I cannot agree with. My remark does not apply to the obtained results, which I consider valuable, but to the interpretation proposed by the Author.

Minor comments to Chapter III:

- I would like to ask the Author to explain the discrepancy between the sample size given in the methods and in the results. In the methods, the author states that the analyzes were carried out on 10 pairs equipped with geolocators, but the Table 1 in results section in indicates that 8 pairs were analyzed during incubation and 8 pairs during nestling period. In addition, when the nestling period is splitted into "early" and "late", the analysis concerns only 7 and 4 pairs, respectively. Why were analyzes not performed on 10 pairs during incubation as well as early and late nestling periods as described in the methods? Is the presented description of the methods correct and adequate to the performed analyzes?
- The way of presenting statistical results is inconsistent. In some places the Author presents the results in a classic way, i.e. the values of test statistics and the p-level, while in other places it is written that no relationship has been found without providing the statistics. For example, the Author writes that "the hatching success as well as all three chick growth parameters were not significantly affected by the carrying of a GLS", however, no statistical analyses support this statement.
- The graphs in figure 2 colored and in figure 3 black and white.

Conclusions

The comments presented in my review should be treated as questions addressed to the Author in order to initiate an academic discussion, which is the part of the defense of the doctoral dissertation.

Regardless of my aforementioned remarks, the dissertation written by Antoine Grissot makes a valuable contribution to the field of behavioral ecology. The Author demonstrated a broad and comprehensive approach to the issue of parental cooperation, which is quite a new and unexplored area of animal behavior. He used various research methods and obtained interesting results. He also has general knowledge on many aspects of the biology and ecology of the studied species. Noteworthy, conducting research in Arctic conditions is not easy and surely involves both determination and passion. A scientist who undertakes such research must properly plan and organize all tasks, become familiar with all necessary devices and finally resolve all current problems during a field study. All these elements were properly implemented in the doctoral dissertation presented to me for evaluation.

The doctoral dissertation, prepared by Antoine Grissot, provides an original solution to a scientific problem and demonstrates the candidate's general theoretical knowledge in the behavioural ecology in general and with the reference to parental cooperation in particular, as well as the ability to conduct scientific work. The dissertation presents interesting results in the framework of an experimental work and well-designed objectives.

In the conclusion, in the opinion of the reviewer based on the presented dissertation, Mr. Antoine Grissot should be awarded with the doctoral degree. His research meets the requirements set for doctoral dissertations by The Higher Education and Science Act dated 20 July 2018 (Polish Journal of Laws of 2018 item 1668, as amended). Thus I am asking the Biological Sciences Discipline Board of the University of Gdańsk to admit Mr. Antoine Grissot to the next stages of the doctoral dissertation procedure and to confer him the doctoral degree in the field of natural sciences and the discipline of biological sciences.

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