

5 “In my opinion this paper is not well written and sometimes confuse: manuscript contains a lot of methodological errors; moreover, particular parts, for example, the Introduction does not connect to the Methodology or Results. These types of results have been published many times before, but using much more accurate methods, and with unobjectionable sampling.”

The authors consider that the article does not contain methodological errors. The reviewer did not indicate specific errors, but only a general formulation about their presence in the article.

10 The authors disagree with the statement that "These types of results have been published many times before, but using much more accurate methods, and with unobjectionable sampling". No publications, apart from the work of Kłys, were found (again, the reviewer did not identify them) in which the description of bat behaviour during hibernation was based on an analysis of the relationship between variables (T, Rh, v).

Title:

15 1. i) “title does not link to manuscript content“

According to the authors, the title of the publication is not objectionable. But at the suggestion of the reviewers or the editor the title can be changed, for example to:

"Influence of subterranean refugiolclimate on the clustering of the western barbastelle bats during hibernation".

20 2. ii) “title and keywords repeat the same words”

There are different suggestions in different journals. On the website of the journal Biogeosciences in guidelines for authors no instruction on this topic was found. According to the reviewer's suggestions, authors can remove the repeated words *hibernation*, *western barbastelle*, *grouping*, *clusters* from the keywords in the title.

25

Introduction:

“this is more a review of existing research on bat wintering, but at a local level and without recent literature (a lot of self-citation!) than outlining hypotheses, for example:”

30 The authors agree that the introduction is a review of the state of knowledge on bat overwintering, but not just local knowledge. The literature so far, including recent items, does not discuss the influence of the relationship between the main physical parameters of air and its velocity in underground systems on bat clustering. The self-citations are due to the above-described deficiencies in the chiropterological literature.

35 “The grouping of individuals during wintering is variable both within the species and between species (Kłys, 2013).” – in fact this publication not contain any data about grouping”

In the opinion of the authors, the suggestion is unfounded. The author of the above publication describes hibernation strategies (including group or individual hibernation) in the monograph chapters listed:

- 40 - Chapter 6 Wintering strategies: pp. 47-49;
 - Appendix: p. Tab. 10, p. 139, Tab. 14, p. 143-144, Tab. 18, p. 148-149, Tab. 20, p. 151-152, Tab. 24, p. 155-156

Below is a link to the publication in question:

- 45 https://www.researchgate.net/publication/307899035_Multifactor_Analysis_of_Refugioclimate_in_Places_of_Hibernation_of_Chosen_Bat_Species

- 50 “... optimal values defining the so-called thermal comfort of selected species (KÅys, 2004; KÅys and WoÅoszyn, 2005; KÅys, 2008; KÅys, 2013)” - whether this is a thermoneutral zone or a thermopreferendum (in fact bats do not predict what the temperature will be in future, but rather choose their preferred parameters at exactly time, and change location when conditions change).”

A closer term is the thermoneutral zone, but it should be remembered that this does not refer only to temperature but to the relationships of many variables affecting, among others, bat clustering and its effects.

- 55 The authors are aware that bats do not predict what the temperature will be in the future, but rather choose sites where the relationships between the variables T, Rh and v create optimal conditions for hibernation.

“on the other hand basic literature is missing:”

The literature deficiencies of 2020-21 identified by the reviewer do not contribute relevant information to the reviewed article.

- 60 The authors refer to the scientific papers listed by the reviewer:

Boratyński, J. S., Willis, C. K. R., Jefimow, M., Wojciechowski, M.S. 2015. Huddling reduces evaporative water loss in torpid Natterer's bats, Myotis nattereri. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 179, 125-132. doi:10.1016/j.cbpa.2014.09.035

- 65 The article deals with the benefits of grouping bats, among others, in terms of reduced moisture loss (EWL). It was shown that EWL by evaporation in grouping individuals was lower than EWL of individually hibernating bats at both low and high relative humidity. The experiments were conducted in the laboratory. Hibernation strategies (individual, group) and preset of environmental conditions were planned. The reduction in EWL in grouped bats was due to a reduction in the contact area between skin and ambient air, rather than respiration. The effect of grouping is a
 70 reduced number of awakenings due to the need to restore water balance.

In contrast, the aim of the research in the peer-reviewed article was to identify the physical conditions under which clustering occurs in *Barbastella barbastellus* bats, not to investigate the consequences of clustering.

75 *Martínková N., Baird S.J.E., Kána V., Zima J. 2020. Bat population recoveries give insight into clustering strategies during hibernation. Frontiers in Zoology 17:26*
<https://doi.org/10.1186/s12983-020-00370-0>

This article does not add to the issues discussed by the authors. Marinkova et al. 2020 describe the grouping phenomenon of *Rhinolophus hipposideros* and *Myotis myotis* bats in relation to the temperature outside underground systems and the duration of winter. They present long-term observations of bats during hibernation by recording the observed condition (by means of photographs), the finding of group or single hibernation of individuals of a given species, and the size of the cluster. However, there is no reference to parameters describing the refugiolclimate or at least the microclimate of the underground.

85 Furthermore, the bat species described differ from *Barbastella barbastellus* significantly in their preferred underground microclimate/refugiolclimate conditions.

“In addition, text contain some misunderstandings: “In particular, the bats can decrease their body temperature almost to the ambient one (McNab, 1982) or in a short time increase it significantly above this value (even up to 40°C at the air temperature of only a few degrees) (Davydow, 2004).”
 90 In fact, arousal from hibernation even takes more than 30 minutes, which cannot be called a slow process “in a short time”. See also: Bachorec E., Bartonicka T., Heger T., Pikula J., Zukal J. 2021. Cold arousal - A mechanism used by hibernating bats to reduce the energetic costs of disturbance. *Journal of Thermal Biology*, 101: 103-107.”

Davidov 2004 describes the process of entering hibernation in detail. It is only debatable whether the time of 30 min. relative to the hibernation period can be called a 'short time'. The authors believe that it does.

What is incomprehensible is the reviewer's formulation of a slow process "in a short time" (a slow process "in a short time"), which is contradictory in its assumptions, i.e. the first part of the formulation contradicts the second part.

100 In the paper by Bachorec et al. 2021, they describe, among other things, the duration of cascade awakenings (in bat clusters). Although they were not able to detect the duration of the excitation, they found that there is a large individual variability in the bat's maximum temperature (Tmax) for both cold and normothermic excitations and in the duration of normothermic excitations.

„While we were unable to detect an effect of the number of disturbances, age or sex, and initial mass on arousal Tmax and duration we found that there was high individual variation in the Tmax of both cold and normothermic arousals and the duration of normothermic arousals”

iii) “the chosen model species is probably the worst for testing cluster size effect: the western barbastelle *Barbastella barbastellus* (Schreber, 1774) are: 1) psychrophilic/cryophilic bat species – hibernating mostly in transition zone in undergrounds (highly variable conditions); 2) its range extends from the large Mediterranean islands to southern Sweden and the southern British Isles (a very wide temperature range). Therefore, each visit can give different results.”

The bat species *Barbastella barbastellus* was chosen by the authors because of its special requirements in comparison to other species (much lower humidity, wide range of ambient temperature). As this species hibernates in highly variable environmental conditions, it is good for assessing the relationship between variables characterising the refugiolclimate (T, Rh, v). The western *Barbastella barbastellus* occurs in Europe and, according to the IUCN, has been declining since 2012. (Gotfrid 2020), so the research described in the peer-reviewed paper is important for understanding the conditions under which it may hibernate and therefore for its conservation. Furthermore, the authors clarify that they do not study influence of cluster size, as suggested by the reviewer ("*for testing cluster size effect*").

1. iv) "*no scientific hypothesis being tested here, only "evaluation" of microclimate parameters*"

Evaluation of microclimatic parameters was not the main aim of the study. At Line 115 the objective is formulated. At Line 116 a short explanation is provided. For evaluation of the relationship between the bats' grouping trend and the air parameters statistical model with structural parameters β_j related to the determined air parameters (or their combination) was constructed. The structural parameters were tested against null hypothesis $H_0: \beta_j=0$, in this way the reliable conclusions concerning changes in grouping in relations to air parameters can be drawn.

130 *Methods:*

1. i) "*each of selected hibernation places have been described in scientific paper – more properly than give sheet of protection (old data, without any additional information).*"

The authors' aim was to investigate the relationship between measured parameters describing the refugiolclimate in which bats hibernated. However, the location of sites with similar refugiolclimate conditions was not relevant.

The authors believe that the term 'old data' does not exist in scientific papers based on field studies. The methods used so far to describe bat behaviour during hibernation, according to the authors, do not take into account the relationship between key variables characterising refugiolclimate.

2. ii) "*no data about number of visits in each hibernaculum, each year, etc. It's important because they are belonged to different climatic regions (see: Gottfried et al. 2019). Therefore, the results is completely questionable: if the species prefers a dynamic microclimate, then the choice of region determines the microclimate but not the bat itself. In addition, different winters can produce year effect and not preference.*"

The dates of the measurements at the individual hibernation sites can be completed by the authors in the data sheet on the University of Opole repository.

The microclimate of underground systems is not always related to climatic zones. The climatic conditions of the underground are influenced, among others, by the height of the opening above sea level, the exposure of the opening, the existence of vegetation in front of the opening, the morphology of the cave (length and shape of the passages and halls), the existence of heat-

150 transporting water streams, and heat energy from within the earth (e.g. Piasecki et al. 2001). Both
 'warm' and 'cold' underground systems can be found in each of the climate zones mentioned.

iii) *“what does 180 observations mean - is it measurements in all hibernation locations? No n
 observations is given for individual and for groups.”*

The authors treat n as the number of observations, in the article this means 180 different hibernation
 155 sites (refugioclimate) where T, Rh and v were measured.

These sites were described in an appendix located in the Opole University archives, available
 during on-line discussion period.

1. iv) *“devices from SENSOTRON (KÅys 2013)” – it sounds like it is equipment created by the
 Author, which is not the case; also, neither the accuracy nor the resolution of the instrument
 160 is given”*

The measurement methodology is included in the paper (Klys 2013) in APPENDIX on pages 130-
 132.

The authors felt that duplication of information was unnecessary, but at the suggestion of the editor
 or reviewers, they could copy the monograph pages mentioned and include them in the Supplement
 165 materials or add them to the article methodology.

2. v) *“... model describing an influence of the air physical parameters on bats' grouping ” – in
 this type of methodology, Authors can only describe preference of wintering places, but only
 at a given time (transition zone, dynamic microclimate)”*

This is also the aim of this article. Learning about these preferences will make it possible to protect
 170 bats, e.g. by designing artificial roosts or adapting existing ones.

3. vi) *“the use of PCA is incorrect here – are only 3 variables whose interactions can (and for
 sure, have) strong biological significance. Hence it will be correct to use one of a linear or
 linearized model (GLM/GLZ). It makes no sense to calculate quantile regression, because its
 175 only two groups (if I properly understood this paper).”*

In this article PCA was used for estimation of co-variability between explanatory variables and their
 structure assessment (possible clustering, outliers) not for a model construction. Collinearity in
 explanatory variables leads to increase in estimates variance, instability in a linear model (Weisberg
 2005).

180 Application of the GLM model for bats' clustering description with binomial or Poisson error
 distribution seem rational, but actually it is not. The statistical parameters presented in Tab. 1
 clearly indicate prevalence of hibernation in apart. The presented data imply at last 75 % individuals
 avoid grouping. Application of quantile regression reduces strongly bias resulting from numerous
 observations of single hibernating individuals.

185

Results:

1. i) *“very chaotic, in fact there is no results: first paragraph repeats data from tables below. Results are required descriptive statistics first (e.g.: a box-plot diagram), followed by a statistical test to test the hypothesis - here both parts are missing”*

190 It is quite common in scientific publications to add a short discussion to the presented data (Tab. 1). It helps a reader to realise specific data properties.

Usually in scientific articles the data are presented either in table or in a graph, but not in both.

There is no advantage of boxplot over table. Because of different units of the parameters

195 presentation of the variables require 4 separate boxplots. Presentation of our data in boxplot was simply inconvenient.

2. ii) *“for PCA – only the percentage of variance is given, but no real value, the effect of a variable on loadings also is needed, no test of co-linearity (highly correlated factors).”*

200 Only the relative variance of a PC contains interpretable information. In Fig. 3 the 2D projection of scores representing parameters T , v and Hr on the plane defined by the orthogonal PC1 and PC2 are shown. Vector coordinates of arrows represents contribution of the parameters in both PCs.

Co-linearity of variables can be simply assessed from the arrows direction.

- iii) *“equation - contributes absolutely nothing. Why is there no graph of the obtained regression? Why was PCA used if the variables analyzed partially?”*

205 Eq. 1 shows the model description actually used in calculations. We expect not all readers are acquainted with such formulation, so as an explanation Eq. 2. was presented. In Eq. 3 an algebraic expression describing the model with appropriate parameters β_i is shown.

A graphical representation of the data and the model must be drawn in 4D space (T , v , Hr and n). Authors don't know how to do it. PCA was used only for assessment of explanatory variables structure.

210

Discussion:

“This part is even more chaotic than the results; whole Discussion is rather speculation, not interpretation of obtained results. Many of cited articles reports completely different phenomena.”

- 215 1. i) *“...participation of individual heat transport mechanisms between the bat's body and the air (convection) or between the body and the ground (conduction) (KÅys, 2013)” – in this paper there are no results on these parameters.”*

The purpose of this citation was to indicate the mechanisms of heat exchange between the bat's body and the environment, which are presented in this paper.

- 220 2. ii) *“thermal preferences was study many times, but most of this research are giving only approximated temperatures – mainly temperature somewhere around the bat, not directly, except research with thermal imaging cameras (see: Boyles et al. 2008).”*

Temperature preferences have been studied many times. The authors are not interested in temperature preferences per se, but in the influence of the parameters describing the refugioclimate on bat's grouping.

iii) "... which simultaneously increases energy loss (Wermundsen and Siivonen, 2010)" – those Authors not described this parameter, only ambient temperature (T_a).

The authors believe that the suggestion is incorrect, and below are the citations in which the reference to energy losses occurs:

Chapter 4. – Discussion, page 271, col. 1, paragraph 1

„Evaporation increases loss of energy, and evaporative water loss is less at low than at high values of body temperatures [13,41]. At the beginning of the season bats used highest ambient temperatures but also highest humidity that in turn depresses evaporation, and consequently the loss of energy. Water loss may also trigger arousals [41], which in turn increases energy expenditure, because bats must raise their body temperature to euthermic levels [29]. At the end of the season *M. brandtii/mystacinus* and *M. daubentonii* used lowest humidity, but hibernated increasingly in crevices and clusters, that in turn reduces water loss and consequently the loss of energy.”

Chapter 1. – Introduction, page 263, col. 2, paragraph 1

„Our goal was to find out whether bats use different strategies (minimizing the cost of hibernation vs. minimizing energy expenditure) throughout the hibernation period. As body fat reserves get smaller and smaller towards spring, we predicted that at the beginning of the season bats would tend to use warmer areas (larger energy reserves) to minimize the cost of hibernation and at the end of the season they would use colder areas (smaller energy reserves) to minimize energy expenditure. By hibernating increasingly in clusters and crevices bats can decrease heat and water loss, so we further predicted that at the beginning of the season bats would hibernate in smaller clusters and less protected sites than at the end of the season when they would concentrate on minimizing energy expenditure. To test this theory we measured seasonal variation in temperature, humidity, clustering behaviour, crevice occupation, and location by *E. nilssonii*, *M. brandtii/mystacinus*, *M. daubentonii*, and *P. auritus* in southern parts of Salpa Line”

1. iv) "... the higher the degree of air saturation with water vapor, the more difficult it becomes to collect heat by evaporation (Thomas and Cloutier, 1992; Thomas, 1995; Paksuz et al., 2007)" – Paksuz et al., 2007 not writing in any part of article about water vapour!"

The authors meant relative humidity in the phrase "the degree of air saturation with water vapor."

In the papers mentioned by the reviewer, there are references to the loss of water (water vapour) by bats during hibernation:

- Thomas D.W, Cloutier D. 1992. *Evaporative Water Loss by Hibernating Little Brown Bats, Myotis lucifugus*, *Physiological Zoology*, Volume 65, Number 2, 443-456

The article is concerned with total water loss by evaporation (EWL) and with determining the route by which water is lost from the body of hibernating bats e.g:

- „We measured total evaporative water loss (EWL) for little brown bats (*Myotis lucifugus*) hibernating at 2° and 4°C. Total EWL was not influenced by body mass or temperature but was

directly related to the difference in water vapor pressure (AWVP) between the tissue surfaces and the atmosphere. Evaporative water loss (mg bat-E d-1) is 3763 AWVP (kPa). M

- 265 - „Because more than 99% of EWL occurs from the body surfaces, any reduction in the exposed surface will result in a proportional reduction in EWL. Thus, clustering may be an important active behavioral mechanism resulting in reduced EWL”
- Thomas, D.W.: *Hibernating Bats Are Sensitive to Nontactile Human Disturbance. Journal of Mammalogy*. 76, (3), 940-946, 1995.
- 270 There was an editorial error in the article. An article from the same year but with a different title should have been cited:
Thomas D. W. 1995. The physiological ecology of hibernation in vespertilionid Bats. Symp. zool. Soc. Lond. - No 67.: 233-244.
- Paksuz S., Özkan B., Postawa T. 2007. *Seasonal changes of cave-dwelling bat fauna, and their relationship with microclimate in Dupnisa Cave System (Turkish Thrace). Acta Zoologica Cracoviensia - Series A: Vertebrata, Volume 50, Numbers 1-2, 57-66*
- 275

The authors agree with the reviewer that this citation should be removed from the article.

References:

- 280 1. i) “a lot of self-citation of papers that are unavailable or very difficult to access”

The authors provide links to papers that the reviewer believes are difficult to access:

Kłys, G.: *Przyroda Podziemi Tarnogórskich*. Pyrzowice-Sosnowiec PTG. 2004. (18) (PDF) [Przyroda Podziemi Tarnogórskich \(researchgate.net\)](#)

- 285 Kłys, G., Wołoszyn, B.: The influence of weather and interior microclimate on the hibernation of common long-eared bats (*Plecotus auritus*). *Nature Journal*. 38, 57-68, 2005. (20) (PDF) [The influence of weather and interior microclimate on the hibernation of Common Long-Eared Bats \(Plecotus auritus\) \(researchgate.net\)](#)

- 290 Kłys, G.: Wybrane aspekty hibernacji nietoperzy, in. Kłys, G., Wołoszyn, B.W., Yagt-Yazykova, E., Kuśnierz, A. (Eds.), *Wpływ środowiskowych warunków na wybór hibernaculum przez nietoperze*. ZPW Plik Bytom. 2008. (17) (PDF) [Wybrane aspekty hibernacji nietoperzy \(researchgate.net\)](#)

Kłys, G.: Multifactor Analysis of Refugiolclimate in Places of Hibernation of Chosen Bat Species. T. 8 *Studia Chiropterologica*. Chiropterological Information Center, Institute of Animal Systematics and Evolution, Polish Academy of Sciences. 2013. (18) (PDF) [Multifactor Analysis of Refugiolclimate in Places of Hibernation of Chosen Bat Species \(researchgate.net\)](#)

2. ii) “some papers should not be cited, e.g.: *ĐjĐ¼Đ,ÑĐ½Đ¾Đ² et al. 1999;*
ĐčĐ¾Đ¼Đ,Đ»ĐμĐ½Đ°Đ¾, 2002 - it's faunistic not ecology/ecophysiology research;
Janicki et al., 2006 - is a handbook for vets”

300 The authors believe that scientific articles relevant to the issues discussed should be cited,
 regardless of the journal in which they were published. If the journal requires the use of the Latin
 alphabet rather than the Cyrillic alphabet, the authors will make corrections. The authors believe
 that the listed publication items are relevant to the issue discussed in the article.

- 305 iii) “the lack, in turn, of citation of fundamental/essential papers: *International Hibernation*
Symposium, Heldmaier, G., & Klingenspor, M. (2000). Life in the cold: Eleventh International
Hibernation Symposium”

The literature item indicated does not directly address the issues raised by the authors.

- Gottfried I., Gottfried T., Lesiński G., Hebda G., Ignaczak M., Wojtaszyn G., Jurczyszyn M.,*
Fuszara M., Fuszara E., Grzywiński W., Bączkowski G., Hejduk J., Jaros R., Kowalski M. 2019.
 310 *Long-term changes in winter abundance of the barbastelle *Barbastella barbastellus* in Poland and*
the climate change – Are current monitoring schemes still reliable for cryophilic bat species? PLoS
ONE 15(2): e0227912. <https://doi.org/10.1371/journal.pone.0227912>

Discussed in an earlier section, the authors' responses to the review.

- Boratynski, J. S., Willis, C. K. R., Jefimow, M., Wojciechowski, M. S. (2015). Huddling reduces*
*evaporative water loss in torpid Natterer's bats, *Myotis nattereri*. Comp. Biochem. Physiol. Part A*
 315 *Mol. Integr. Physiol. 179, 125-132. doi:10.1016/j.cbpa.2014.09.035*

Discussed in an earlier section, the authors' responses to the review.

- Martínková N., Baird S.J.E., Kána V., Zima J. 2020. Bat population recoveries give insight into*
clustering strategies during hibernation. Frontiers in Zoology 17:26

Discussed in an earlier section, the authors' responses to the review.

- 320 *McGuire L.P., Johnson E.M., Frick W.F., Boyles J.G. Temperature alone is insufficient to*
understand hibernation energetics. Journal of Experimental Biology (2021) 224, jeb239772.
doi:10.1242/jeb.239772

The authors will supplement the article with the work mentioned.