

>> Dear editor,

Thank you for your comments. Please find below our responses in blue. We have uploaded the revised version of the manuscript on biorxiv. We hope that you will find this version suitable for recommendation by PCI ecology.

Thank you for revising your pre-print, "Blood, sweat and tears...", and resubmitting to PCI Ecology for review. I returned it to the original reviewer #2, who is satisfied with most of the revisions. However, he/she still has concerns about the issue of citing papers as being out of compliance with the definition of non-invasive DNA sampling when animals were killed for methods-development purposes, and even when there was no claim of non-invasive sampling in the paper itself.

In addition, I have the following comments for you to consider when revising:

Regarding Supplementary Table 1: Sorting by first author followed by a quick visual scan, I notice there are at least 7 duplicate entries in this table: entries 48/49; 50/51; 70/71; 80/81; 83/84; 119/120; 187/188. This will change the total number of papers sampled from 342 to 335, and the percentages, proportions, etc. will have to be recalculated. Please check closely and make sure there are no additional duplicates. In addition, for the duplicate pair 80/81, the entries in the "Sampling\_Method" column differ, so please revisit the paper and decide which is correct.

>> The review has been extended to all papers published until the end of 2018. This change as well as the removal of several papers following the reviewer's comment brings the total number of articles reviewed to 380 (see Supp. Table 1 for the complete list). All numbers have been updated throughout the manuscript, statistical analyses and figures were also updated accordingly to the new dataset (see track change document).

Also for Supp. Table 1, please include a column with the publication information for the paper (i.e., journal, volume, page numbers). And several places in the table, "Mixte" should be replaced by "Mixed."

>> Supp. Table 1 was modified according to the change in the list of papers, and is now including the proposed additional fields.

>> All the following suggestions have been implemented.

Line 103, consider "information to draw conclusions about the specific"

Lines 124-25, consider "section of each paper"

Line 145, "Marking" should be "Mark"

Line 186, consider "When the terminology for DNA sampling is misapplied as being non-invasive when it is not,"

Line 191, consider "for judging the validity"

Line 237, should be "encounters"

Line 239, should be "effects"

Line 261, should be "on animal"

Line 286, should be "adults"

For the "Sin 7" title (line 319), please consider changing to "Equating a non-invasive procedure with non-invasive sampling". This is a more descriptive and intuitive construction than the current title.

Line 362, "reproduction" should be "reproductive"; also, should be "as proxies for"

Line 434, should be "effects"

Line 458, should be "experimentation" (i.e., not plural)

Line 467 and 475, consider "that must be" (instead of "need to" or "needs to")

Line 495, should be "animals being more attracted"

L503, consider "being indistinguishable from that of captured"

L519, should be "to obtain"

Lines 863 and 878 indicate the literature review was through May 2018, but line 865 indicates it was conducted in July 2018. Which is correct? In the method section (line 100) it says July 2018.

Line 872, consider "width of the rectangles is proportional to the number of"

Line 880, consider "width of the bars is proportional to the number of"

After making these revisions, I will examine the paper one more time and consider for formal recommendation by PCI Ecology if the revisions are satisfactory. Please let me know of any questions. I look forward to seeing your revised version.

Best Wishes, --Tom Sappington

## **Reviews**

*Reviewed by anonymous reviewer, 2019-08-11 21:41*

I thank the authors for their responses to my original comments. I have only a couple of concerns that should still be addressed:

L222-228: "...did not comply with the original definition of non-invasive DNA sampling. This included ... cases where animals were ... even killed prior to sample collections[32]. For example, 17 of the faecal samples analysed by Kolodziej et al.[32] were obtained from the rectum of feral pigs (*Sus scrofa*) that had been hunted." I appreciate the authors' removing the Kierepka et al. reference in the original manuscript from this revised version. However, they have repeated the same mistake in substituting the Kolodziej et al. paper as being a case of authors claiming non-invasive DNA sampling despite killing the animal. Once again, Kolodziej et al. is a methods development paper; in this case the goal was to quantify genotyping error rates to determine reliability of future population size estimates based on non-invasive DNA samples. They approached this by comparing error rates between tissue and faecal samples taken from the same individuals, which were killed in "a driving hunt" in a forest in Germany. The paper does not indicate whether this was a hunt organized for the study, or whether the authors obtained the samples opportunistically from an independently organized hunt. Presumably the latter. Either way, the study is not relevant to the analysis here because it is about methods development, not about killing an animal to take a "non-invasive" DNA sample as implied in lines 222-228.

>> We removed this example and now only focus on the bird example, where authors state that they collect “noninvasive fecal samples” despite catching and holding birds captive in paper bags until they defecate. See our last response for more details.

L165-166: Why is application of "non-lethal...sampling methods" to non-invasive DNA sampling "surprising"? Should not all sampling methods for non-invasive DNA sampling be "non-lethal"? Judging from Fig. 1 and Fig. 3, the category termed "non-lethal" sampling is being applied in a way that I think means taking tissue or body-part samples from a live animal without killing it. At a minimum, this term ("non-lethal") must be defined in the paper. But I would suggest finding a different term to avoid confusion; because, for example, hair from a track in the snow would be assumed by a naïve reader to be a non-lethal sampling method in the sense that collecting it does not kill the animal. If changing the term will be too disruptive because it is already a well-established term for non-lethal tissue sampling, then I don't insist, but at least define it explicitly.

>> We agree that this sentence may sound confusing. Non-lethal DNA sampling is a broad category that includes non-invasive DNA sampling and invasive DNA sampling methods (as displayed in figure 3). Although this term is well established, it is generally used to refer to invasive methods only (possibly because authors prefer to write “we used non-lethal methods” rather than “we used invasive methods”).

In other words, it is true that all non-invasive DNA sampling should be “non-lethal”, but they should be more than that. We rephrased the sentence to avoid confusion. For clarity, we also added a definition of non-lethal DNA sampling in Table 1.

And what examples are there of "lethal" sampling methods being used for "non-invasive" DNA sampling? I see a few referenced in the supplementary table 1; but given the misapplied logic of the authors in categorizing both the Kierepka et al. paper in the original (although removed in the revised version), and now the Kolodziej et al. paper in the new version as non-compliant in use of the term "non-invasive", does not instill confidence that the authors of the other papers were truly guilty of killing an animal to take a "non-invasive" sample as these categorizations and wording in the text imply. Are the authors certain these were not methods-development papers like the Kierepka et al. and Kolodziej et al. papers? I do not think the simple use of "non-invasive" in a paper that clearly describes the purpose of the study to be non-invasive methods-development should be categorized in this way just because invasive sampling (possibly including the killing of the animal) is conducted to obtain the needed samples (L271-276 revised pdf). It is perhaps ok to recommend authors be more careful in this regard to alert naïve readers

who might get confused, but to classify papers like this in the literature analysis as being non-compliant with the definition of non-invasive is misleading rather than helpful.

>> Although this only concerns a handful of papers, it is an important point and a follow up on the first comment. Our manuscript is a review about how the term “non-invasive DNA sampling” is being used in the scientific literature. As such, we believe it should include method development papers that use this term.

We strive to provide the readers with an accurate view of how the term is being used but we are limited by the information given by the authors. Some papers are difficult to categorise because the authors did not clearly state in the main text whether they consider their sampling method as being invasive or not. In other words, some authors did not say, “we used a non-invasive method”, but they did not say either “for this to be non-invasive, the samples would need to be collected in a different way than what we did”.

When the term non-invasive was present in the title of an article but an invasive method was used without being clearly acknowledged as invasive later in the manuscript, we considered that the reader could easily be misled and the article was considered non-compliant. The same process was applied for articles where the term non-invasive was only listed in the keywords. We agree that this may have been too severe and lead to a misleading categorisation of some papers (Kierepka et al., Kolodziej et al. and few others). Rather than trying to guess what the authors meant or how the reader will understand, we are now acknowledging the fact that we do not have enough information to conclude on the compliance for these articles, and we simply removed them from the analysis.

For information, four examples of "lethal" sampling methods being used for "non-invasive" DNA sampling” remain. They correspond to studies where authors have used museum specimens. In these cases, the authors probably did not kill animals to collect DNA. As mentioned on line 354, “opportunistic sampling from animals already killed for other purposes may be an ethical option”. However, because the animals are killed, the sampling should not be described as non-invasive (but rather non-destructive) and the papers are therefore non-compliant with either definitions of Non-invasive DNA sampling.