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Corrigendum: Blood, sweat and tears: a review of non-invasive DNA sampling

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Abstract

In the original article, Box 1 was not printed in its entirety, and two references were badly quoted in page 3 of the pdf and were missing in the reference list.

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In the original article, Box 1 was not printed in its entirety, and two references were badly quoted in page 3 of the pdf and were missing in the reference list.

Box 1 should read as follow:

Box 1: The seven sins of non-invasive DNA sampling

Sin 1: Taxonomic bias

One conspicuous result from our review was that only 18 studies (~6% of the reviewed papers) focused on invertebrates compared to 356 focusing on vertebrates (Fig 2b). This striking imbalance implies that non-invasive methods are rarely considered for sampling invertebrate DNA. When authors claimed to use non-invasive DNA sampling on invertebrates, they failed to do so in 55% of the cases (Fig 2d), and even used methods that alter the physical integrity of the organism in 10% of the cases. For example, Rorat *et al.* (2014) collected individual earthworms, which they then electrified “lightly” to induce coelomic secretion. Yet, truly non-invasive methods exist for invertebrates, for example through field collection of insect exuviae (Nguyen *et al.*, 2017), pupal cases (Richter *et al.*, 2013), empty mummies (Lefort *et al.*, 2017), dust (Madden *et al.*, 2016), soil (Bienert *et al.*, 2012), or water samples (Mächler *et al.*, 2014). The misuse of the term non-invasive DNA sampling also varies in relation to the taxonomic group of interest within vertebrates (Fig 2d) ($\chi^2 = 190.69$, $df = 30$, $p < 2.2e-16$). For example, 27% of the studies on fish involved alteration of the physical integrity of the organism. These included fin clipping in eels (*Anguilla anguilla*) (Baillon *et al.*, 2016) and sting amputation in rays (*Aetobatus narinari*) (Janse *et al.*, 2013) which were both considered non-invasive because these body parts can regenerate, despite the fact that fin clipping is known to be painful for fish (Roques *et al.*, 2010). In comparison, less than 4% of the studies focusing on mammals, involved biopsies.

Sin 2: Misclassification of faeces as non-invasive DNA samples

The majority of the literature on non-invasive DNA sampling included the collection of faecal samples (62% of all studies reviewed here). Faecal collection is very prevalent in the field and assumed to be non-invasive by most authors. However, our analysis shows that 47% of the studies focusing solely on faecal sampling did not comply with the original definition of non-invasive DNA sampling. This included detection of animals and collection of faecal samples using aircraft (e.g. Roffler *et al.*, 2014), which may increase stress in animals (e.g. Ditmer *et al.*, 2015) or cases where animals were being held in captivity (e.g. Woodruff *et al.*, 2016), specifically captured to obtain faecal samples (e.g. Brown *et al.*, 2017). For example, Jedlicka *et al.* (2017) “extracted DNA from noninvasive fecal samples” of Western Bluebirds (*Sialia mexicana*) by catching adults and placing them in brown paper bags. Despite focusing on faecal samples, these procedures do not fit the definition proposed by Taberlet *et al.* (1999). The central misconception, here is that there is no such thing as “non-invasive DNA samples”. Rather than the type of sample, it is the method of sampling that needs to be scrutinized for its invasiveness. Another key issue with faecal sampling is that many animals mark their territory using faeces to dissuade potential intruders (e.g. in wolf communities, see Llaneza *et al.* (2014)) and also use such marks to recognise individuals from neighbouring territories, avoid unnecessary conflict and promote non-agonistic social encounters such as mating. Therefore, even when collected opportunistically after the animal has left, faecal sampling can in some cases affect the marking behaviour of territorial species (e.g. Brzeziński & Romanowski, 2006) (Fig 2a).

Sin 3: Baiting DNA traps

In most studies using a DNA trapping strategy (90%), researchers employed bait or lures to increase the yield of their traps. Very few studies used non-lured DNA traps, for example, barb wire placed at sites used by brown bears (*Ursus arctos*) (Karamanlidis *et al.*, 2014; Quinn *et al.*, 2014) or modified body snares at otter (*Lontra canadensis*) latrine sites, to collect hair (Godwin *et al.*, 2015). Although it seems perfectly legitimate (and often essential) to increase the attractiveness of DNA traps with food (Cohen *et al.*, 2013), scent marks from other individuals (Anile *et al.*, 2012) or other attractants (e.g. Valerian essence for cats, (Steyer *et al.*, 2013)), the animal’s behaviour will obviously be modified as a consequence and therefore, these methods cannot be considered fully non-invasive sensu Taberlet *et al.* (1999).

Sin 4: Combining invasive and non-invasive methods

In a few examples the impact of the sampling strategy on animal behaviour is obvious from the article's title itself, for example when baited traps are mentioned (e.g. Steyer *et al.*, 2013). However, in many more papers (n=35) confusion arises because authors used the phrase "non-invasive sampling" or "non-invasive DNA sampling" while a variety of sampling techniques were actually applied, some of which were non-invasive and some of which were invasive *sensu* Taberlet *et al.* (1999). This lack of clarity about what is non-invasive and what is not can be misleading for the reader. Some authors clearly stated the invasiveness of the different methods used (e.g. Dai *et al.*, 2015; Yannic *et al.*, 2016; Cullingham *et al.*, 2016), however, most papers where mixed DNA sampling strategies were applied did not specify which of these methods were considered non-invasive.

Another facet of this issue arises when tools (e.g. new primers, extraction protocols, DNA conservation methods) are developed specifically for analysing samples collected non-invasively but are actually tested only (or partly) on samples that were collected invasively (n=17) for example by capturing animals to perform the sampling (e.g. Barbosa *et al.*, 2013; Malekian *et al.*, 2018). It is essential in such cases that authors fully acknowledge the invasiveness of the sampling method(s) they used. Often this is not clearly specified.

Sin 5: A bird in the hand is no better than two in the bush

Trapping and restraint of wild animals is recognised as a significant stressor that can result in distress, injury, and death (e.g. Ponjoan *et al.*, 2008). Capturing and/or handling animals for DNA sampling was observed in 24% of all articles reviewed here (Fig 2c), despite the clear definition given by Taberlet *et al.* (1999) that non-invasive DNA is "collected without having to catch or disturb the animal". Indeed, capture and/or handling of individuals to obtain DNA samples (e.g. saliva swabbing) can induce long-lasting stress effects (Harcourt *et al.*, 2010; Seguel *et al.*, 2014), and there are very few cases where capturing an animal might have no effects on its future behaviour. Therefore, when animals must be held captive, transported or restrained in order to perform DNA sampling, the method cannot meet the definition of non-invasive DNA sampling *sensu stricto* (Taberlet *et al.*, 1999). Skin swabbing of octopus (*Enteroctopus dofleini*) for example (Hollenbeck *et al.*, 2017), is unlikely to be possible in the wild without disturbing the animal and the potential negative impacts on animal welfare (see Fiorito *et al.*, 2015) for a review on cephalopod welfare) must still be recognised. Another common scenario where the animals are held during DNA sampling relates to the use of museum specimens or animals that were killed for other purposes (n=4). Whether they were legally hunted or poached and confiscated (e.g. Li *et al.*, 2017), this type of sampling does not qualify as non-invasive due to the disturbance and/or death of the animal through human activity. Often, a better term for such sampling is "non-destructive", which does not damage the specimen (Wisely *et al.*, 2004; Porco *et al.*, 2010) (Table 1). On the other hand, tissue sampling from animals that were found dead of natural causes is analogous to eDNA left behind by a free ranging animal and can be considered non-invasive (e.g. Koczur *et al.*, 2017). It should be noted, however, that opportunistic sampling from animals already killed for other purposes (e.g. culling, museum samples) may be an ethical option because it reduces the need to otherwise target living animals and conforms to the principle of Reduction (reducing the number of affected animals) under the 3Rs framework.

Sin 6: All or nothing

Only 42% of the reviewed studies fully met the criteria of the original definition of non-invasive DNA sampling. In most cases, however, authors tried to minimise the impact of sampling, but the nature of the definition proposed by Taberlet *et al.* (1999) leaves no middle ground between invasive and non-invasive sampling methods. One potential solution to this is to use the term "minimally-invasive DNA sampling", which can be defined as obtaining DNA with minimised effects on the animal's structural/physical integrity, and potential impact on the behaviour and welfare of the organism (Table 1). In our dataset, this term was used in six studies to qualify skin swabbing of fish (Monteiro *et al.*, 2014), amphibians (Ringler, 2018) and bats (Player *et al.*, 2017), feather plucking of gulls (Jones & Kennedy, 2015), cloacal swabbing in rattlesnakes (Ford *et al.*, 2017) and ear biopsies in rodents (Barbosa *et al.*, 2017). A broader use of this term would lead to more accurate reporting, for which potential impacts of the sampling are acknowledged, while still emphasising the aspiration of the authors to minimise those impacts. The challenge associated with the

use of such a term would be to define where ambiguities fall between minimally-invasive and invasive sampling methods.

Sin 7: Equating a non-invasive procedure with non-invasive DNA sampling

The lack of perceived stress or pain experienced by an animal is often used as a criterion to support the classification of a method as non-invasive. For example, du Toit et al. (2017) stated that “*Pangolin scales consist of non-living keratin, therefore taking scale clippings is considered to be non-invasive*”. This statement relates to the common definition of a “non-invasive” medical or veterinary procedure, i.e. one that does not involve puncture of the skin or other entry into the body (Miller & Keane, 1983). This definition (rather than the one by Taberlet et al. (1999)) seems to be the one adopted by most authors (93% of the reviewed papers complying) (Fig 2d). This was also the case for several articles at the frontier between medical/veterinary fields. Kauffman et al. (2014) for example, called the sampling of vaginal swabs and urine from captive dogs non-invasive. Similarly, Reinardy et al. (2013) designated as ‘non-invasive’ a procedure consisting of “*lightly anaesthetizing fish and applying a slight pressure on their abdomen to expel sperm*”, which was then used for DNA analysis. These examples were rare in our dataset (n=3) probably because of our strict selection of articles from non-medical and non-veterinary domains (see selected fields in section 2). Nonetheless, as science becomes increasingly transdisciplinary and genetic methods developed in neighbouring fields are used in ecology, this type of confusion is likely to become more prevalent in the future. The discrepancy with the common definition of a non-invasive procedure comprises a significant limitation of the phrase non-invasive DNA sampling as defined by Taberlet et al. (1999), and importantly, could minimise the perceived impacts of sampling methods on animal welfare, even if these impacts are significant in reality. Although this issue was first highlighted in 2006 by Garshelis who stated that: “*the term noninvasive has 2 distinct meanings, 1 biological and 1 generic, which have become intertwined in the wildlife literature*” (Garshelis, 2006), the confusion continues to riddle the current literature.

Page 3, the sentence

"For example, invertebrates such as leeches¹⁰ and carrion flies⁹ were used to sample the DNA of the species on which they feed (Fig 1c)."

is now replaced by

"For example, invertebrates such as leeches (Weiskopf et al., 2018) and carrion flies (Calvignac-Spencer et al., 2013) were used to sample the DNA of the species on which they feed (Fig 1c)."

The following references should be added to the references section:

- Calvignac-Spencer S, Merkel K, Kutzner N, Kühl H, Boesch C, Kappeler PM, Metzger S, Schubert G, Leendertz FH (2013) Carrion fly-derived DNA as a tool for comprehensive and cost-effective assessment of mammalian biodiversity. *Molecular Ecology*, **22**, 915–24. <https://doi.org/10.1111/mec.12183>
- Weiskopf SR, McCarthy KP, Tessler M, Rahman HA, McCarthy JL, Hersch R, Faisal MM, Siddall ME (2018) Using terrestrial haematophagous leeches to enhance tropical biodiversity monitoring programmes in Bangladesh (S Diamond, Ed.). *Journal of Applied Ecology*, **55**, 2071–2081. <https://doi.org/10.1111/1365-2664.13111>