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Blood, sweat and tears: non-invasive vs. non-disruptive DNA sampling for experimental biology

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ABSTRACT

DNA data are becoming increasingly important in experimental biology. For example, it may be necessary to obtain DNA from an organism before using it in a bioassay or an experiment, to identify and distinguish between cryptic species, or when comparing different morphocryptic genotypes. Another example could be the assessment of relatedness between organisms prior to a behavioural study. In such cases, DNA must be obtained without affecting the fitness or behaviour of the subject being tested, as this could bias the results of the experiment. This points out the existence of a gap in the current molecular and experimental biology terminology, for which we propose the use of the term non-disruptive DNA sampling, specifically addressing behaviour and/or fitness, rather than simply physical integrity (invasiveness). We refer to these methods as "non-disruptive", and discuss when they are appropriate to use.

THE NEED FOR A NEW TERM

With the rise in popularity of molecular methods that has come from progressively cheaper and more user-friendly ways of accessing genomic information (Sboner *et al.* 2011), DNA data are becoming increasingly important in experimental biology (Suarez & Moyes 2012). Analysis of genetic material provides data for myriad uses. In addition to analysis of phylogenetic relationships or population genetics, DNA analysis is required to determine basic information about individuals of many taxa. When DNA analysis is performed prior to experimentation for purposes such as sexing and discrimination between cryptic species, it becomes extremely important to obtain DNA without affecting the fitness or behaviour of the subject being tested, as this could bias the results of the subsequent experiment.

While non-invasive methods of DNA collection focus on preserving the physical integrity of an organism (Waldner & Traugott 2012, Baumgardt *et al.* 2013, Brzeski *et al.* 2013, BreMiotto *et al.* 2014), the fitness or behaviour of the subject may still be affected. This uncovers the existence of a gap in the current molecular and

experimental biology terminology, specifically addressing behaviour and/or fitness, rather than simply physical integrity (invasiveness).

Non-invasive methods of collecting DNA are increasingly diverse, leading to confusion and misapplication of the term “non-invasive” in the literature. Misleading use of terminology in biology and ecology is a longstanding concern (Murphy & Noon 1991), and the phrase "non-invasive DNA sampling" is no exception. As with many other terms in biology (Hodges 2008, Herrando-Perez *et al.* 2014), it has been used in many different and inconsistent ways by various authors. With the aim of clarifying some of the existing discrepancies, we propose the introduction of a new term, “non-disruptive DNA sampling” (Table 1), that emphasises the effects of the sampling method not only on the physical integrity, but also on the fitness and behaviour of the organism from which the sample is obtained.

In order to make our intended meaning clear, we established a list of definitions for the terms used in this paper (Table 1) and the way they relate to one another (Figure 1). Rather than debating and refining existing terms, the essential point of Table 1 and Figure 1 is to distinguish between disruptive methods, which affect the fitness and/or behaviour of an organism, and non-disruptive ones, which do not.

NON-INVASIVE CAN BE DISRUPTIVE, NON-DISRUPTIVE CAN BE INVASIVE

Non-invasive DNA sampling methods that are non-disruptive

Many non-invasive DNA sampling methods are de facto non-disruptive because the DNA is collected without the subjects being aware of the researcher’s presence, or receiving any detrimental effects. For example, most eDNA sampling and DNA trapping methods do not require researcher and subject to be present at the same time and place. We dissociate eDNA from DNA trapping by the fact that DNA trapping involves the use of a device especially set up for the purpose of DNA sample collection, while eDNA sampling relies on the collection of DNA left behind by animals as they carry out normal behaviours (Table 1). Sampling methods that are both non-invasive and non-disruptive should be regarded as ideal (Figure 1), although they may be limited in their applicability.

An example of DNA trapping that is non-disruptive is remote plucking or hair trapping by means of unbaited barbed wire traps, placed at well-used runs. Remote plucking has been extensively used to collect DNA from mammals and estimate species abundance (Mullins *et al.* 2009, Sheehy *et al.* 2013). It is more reliable than direct observation (Frantz *et al.* 2004) or scat collection (Mullins *et al.* 2009), and often less time consuming. Examples of eDNA sampling include DNA collection from footprints in the snow, such as those from the Swedish Arctic Fox (Dalén & Götherström 2007), and from saliva on twigs, such as from ungulate browsing (Nichols *et al.* 2012). eDNA has many benefits for wildlife conservation as it often allows non-disruptive population and individuals monitoring.

The main limitations associated with eDNA and DNA trapping include low DNA quantity and quality (Uno et al. 2012), as well as the contamination from nontarget species (Collins *et al.* 2012). Another limitation of DNA trapping might be the mixture of DNA from several different target individuals. In such instances, next-generation sequencing (NGS) or other post-PCR analysis (e.g. cloning, single stranded conformation polymorphism, high resolution melting, denaturing gradient gel electrophoresis) might be required to differentiate the DNA of each individual.

Non-invasive DNA sampling methods that are disruptive

There is a general assumption that if the physical integrity of an organism is not altered or only slightly altered, then the sampling method has no negative impact on the sampled organism. However, only few studies have endeavoured to test this assumption. The simple fact of capturing and/or handling individuals to obtain DNA samples can be extremely stressful, and therefore disruptive, for the animal. Examples include the capture of mammals for saliva swabbing, which induces more stress than instantaneous remote dart biopsy in seals or flipper notching (Emami-Khoyi A., personal observation).

Faecal and urine-derived DNA have been used, for species identification (Lefort *et al.* 2012) and to study the feeding behaviour of endangered species and species of

conservation interest (Deagle *et al.* 2010, Boyer *et al.* 2013). Despite their non-invasive nature, the collection of such eDNA sources could potentially be disruptive, particularly when the territory of the animal is taken into consideration. Many animals mark their territory using faeces or urine; sample collection from their territory boundaries must therefore aim to preserve territory delineation. As illustrated on Figure 1, removing a faecal sample from the environment might affect the marking of territorial species (Brzeziński & Romanowski 2006).

Non-disruptive DNA sampling methods that are invasive.

In most cases, eDNA and DNA trapping would be preferred over other non-invasive sampling methods. However, non-disruptive methods where the specimen is in hand, although more invasive, could present advantages. For instance, having the specimen in hand is likely to help retrieve fresher and better quality DNA.

The switch of focus from non-invasive methods, which emphasise avoiding breaches to the physical integrity of an organism, to non-disruptive methods, which are more concerned with minimising effects on behaviour and fitness, means that in some cases the most appropriate method will be invasive. For example, invertebrate antenna clipping in the natural environment may be less disruptive than collecting and taking a specimen back to the laboratory for faecal sampling or forced regurgitation. Under our definitions, antennae clipping in the field would be considered non-disruptive, despite being invasive. In such cases, a less disruptive, more invasive method may also yield more DNA of higher quality.

WHEN IS NON-DISRUPTIVE DNA SAMPLING REQUIRED OR PREFERRED?

DNA sampling methods should be chosen according to the research aims

Non-invasive DNA sampling provides a compromise between welfare and ethics, and obtaining a quality DNA sample. In circumstances where the subject is endangered, there may be welfare issues surrounding the use of invasive DNA sampling techniques (Mccarthy & Parris 2004). In other instances, such as when species are

afforded legal protections (Boyer *et al*. 2013), non-invasive DNA sampling techniques may be preferred. Additionally, the test subject may be required to be alive for further testing, or return to their natural habitat. If further tests involve capturing an animal for a laboratory experiment or for translocation (Waterhouse *et al.* 2014), then the effects of capturing and holding the organisms for DNA sampling are of less concern as individuals will need to be captured for these experiments anyway. However, the potentially stressful effects of capture and manipulation should not be further exacerbated by DNA sampling methods. If animals are to be sampled and observed in the wild, or if welfare or conservation is of concern, the sampling technique must also depend on how sampling could affect the fitness and behaviour of the subjects.

The disruptiveness of a particular method varies between species. For instance, Caudron *et al*. (2007) suggested that the degree of invasiveness in hair sampling and genotyping from hair follicles in pinnipeds and other marine mammals was influenced by three factors: the duration of sampling, the number of humans involved in the operation, and the sampling distance to the animal.

It is useful to distinguish between three types of situations in which collection and use of non-disruptive DNA samples may be desirable. Below we describe: experimental studies, field behavioural studies, and capture- mark- recapture (CMR) research, which may benefit from using non-disruptive DNA sampling.

Experimental studies

Differentiating, sexing or genotyping individuals prior to experimentation would benefit from using non-disruptive DNA sampling if fitness and behavioural traits are to be subsequently assessed. For example, many species of tropical birds are monomorphic, and can only be sexed using molecular analysis (Vucicevic *et al.* 2013). Additionally, different species from cryptic species complexes can only be distinguished genetically (Hebert et al. 2004). Laboratory based behavioural or fitness studies involving cryptic or monomorphic species may therefore require DNA sexing or identifying of individuals before conducting research on them (Fumanal *et al.* 2005, Lefort *et al.* 2014). Even when species identification is not an issue, the

organisms being studied may comprise different morphocryptic genotypes (Fumanal *et al.* 2005) that need to be determined prior to experimentation.

Behavioural studies in the field

The second major use of non-disruptive DNA sampling is when relatedness between individual subjects needs to be determined prior to a behavioural study conducted in the field. For example, social interactions in mammals are often linked to kinship and can be mediated by the physiological state of individuals (Creel 2001). The capture and handling of animals can modify their physiology (Suleman & Wango 2004) thereby affecting their social behaviour. Recent studies also suggest that although behaviours observed shortly after release may appear 'normal', stress levels may still be high and impact activity budgets (Thomson & Heithaus 2014).

Capture Mark Recapture

The effects of DNA sampling on animal behaviour may also affect the results of studies that are not directly examining behaviour or fitness. The third case when non-disruptive DNA sampling is recommended is when doing Capture Mark Recapture (CMR) studies. CMR studies using DNA tagging are often used to estimate population size (Robinson *et al.* 2009), with the additional benefit of enabling population genetic analysis on the samples collected. Intrusive DNA sampling techniques may affect the survival rate of marked individuals, or introduce avoidance behaviours, which may cause marked individuals to avoid traps, and the population size to be overestimated. For example, toe clipping is commonly used to estimate population abundance of amphibians (Nelson & Graves 2004), but toe clipping has been shown to affect survival rates in some amphibian species (Mccarthy & Parris 2004), and stressful collection or capturing method may cause avoidance behaviour from experienced individuals. Such bias can be limited by the use of non-disruptive DNA sampling methods, which have minimal effects on animal fitness and behaviour. Although eDNA has been used in CMR studies and is in most cases non-disruptive, it can have some limitations. The presence of mixed DNA samples and the lower quality of the collected DNA can lead to false positives where animals not captured previously are believed to be recaptured due to their DNA profile being an indistinguishable shadow of previously captured animals (Lampa *et al.* 2013).

BEHAVIOURAL AND/OR FITNESS IMPACT OF DNA SAMPLING

Studies examining fitness effects of DNA sampling through non-invasive methods are very rare. Non-lethal and non-invasive DNA sampling can have unforeseen effects, and the degree of disruption caused to the sampled organism varies greatly with species. When investigated, the fitness consequences of DNA sampling methods have often been measured using survival as an proxy for fitness (Mccarthy & Parris 2004, Marschalek *et al.* 2013, Oi *et al.* 2013).

Mixed responses have been found depending on the DNA sampling technique and the taxa sampled. Sometimes responses vary strongly between closely related species and even between both sexes of the same species. For instance, a study performed by Vila *et al.* (2009) showed that neither leg nor hind wing clipping had an effect on the survivorship or reproductive behaviour of adult males of the protected moth *Graellsia isabelae,* while mid leg clipping had a negative impact on female mating success of the same species.

In addition to fitness impacts, some studies have investigated behavioural impacts on species during non-lethal DNA collections. For example, remote biopsy sampling on marine mammals is known to cause little reaction from individuals when conducted correctly, and is unlikely to produce long-term deleterious effects (Bearzi 2000). Nonetheless, all biopsy sampling involves some level of risk (Bearzi 2000) and different individuals from the same species may react differently to similar stressful situations based on gender (Brown & Kraus 1991) and individual physiological and psychological factors (Barrett-Lennard 1996, Gauthier & Sears 1999). The impact of remote biopsy has been extensively studied in marine mammals, while not so much in other vertebrates. For example, Gemmell and Majluf (1997) studied the impact of remote biopsy on the behaviour of male New Zealand fur seals (*Arctocephalus forsteri*), and found that in most cases the seal recoiled from the impact and searched briefly for the assailant. Another study showed that bottlenose dolphins reacted equally to the darting process regardless of being hit or not, suggesting that the reaction is mainly caused by 'unexpected disturbance' rather than biopsy Krützen & Barré 2002). No sign of long term altered-behaviours was observed, and sampled individuals were still easily approached for systematic survey and individual tracking.

These examples suggest that no preconceived idea on the disruptiveness of a DNA sampling method should be established prior to testing. Therefore it is important to standardise sampling methods prior to defining their impact on a particular species.

TAKE-HOME MESSAGES

1. There is a gap in the current terminology, for which we propose the new term, non-disruptive DNA sampling, to specifically address behaviour and/or fitness effects of a sampling method, rather than physical integrity (invasiveness).

2. DNA sampling methods should be chosen according to the research aims; in particular, non-disruptive DNA sampling should be used prior to experimental or observational studies measuring fitness or behaviour, as well as studies using techniques such as CMR where fitness or behaviour may affect results.

3. Although eDNA and DNA trapping are often regarded as ideal to limit the impacts of DNA sampling on live animals, they are not always applicable, can present technical limitations and are often not compatible with experimental research.

4. If non-invasive methods are very disruptive or stressful to an animal, a more invasive but less disruptive method may be a preferred alternative.

5. More research is required on the fitness and behavioural consequences of different live DNA sampling methods in a variety of species and contexts.

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REFERENCES

Barrett-Lennard L (1996) A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. *Marine Mammal Science*, **12**, 14–27.

Baumgardt J, Goldberg CS, Reese KP, Connelly JW, Musil DD, et al. (2013) A method for estimating population sex ratio for sage-grouse using noninvasive genetic samples. *Molecular Ecology Resources*, **13**, 393–402.

Bearzi G (2000) First report of a common dolphin (Delphinus delphis) death following penetration of a biopsy dart. *Journal of Cetacean Ressource Managagement*, **2**, 217–221. Available:

Boyer S, Wratten SD, Holyoake A, Abdelkrim J, Cruickshank RH (2013) Using Next-Generation Sequencing to Analyse the Diet of a Highly Endangered Land Snail (Powelliphanta augusta) Feeding on Endemic Earthworms. *PLoS One*, **8**, e75962.

Brown MW, Kraus SD, D.E. G (1991) *Reaction of North Atlantic right whales* (Eubalaena glacialis) *to skin biopsy sampling for genetic and pollutant analysis*. Report of the International Whaling Commission Special Issue. pp. 1381–1389.

Brzeski KE, Gunther MS, Black JM (2013) Evaluating river otter demography using noninvasive genetic methods. *The Journal of Wildlife Management*, **77**, 1523–1531.

Brzeziński M, Romanowski J (2006) Experiments on sprainting activity of otters (Lutra lutra) in the Bieszczady Mountains, southeastern Poland / Observations des épreintes de la loutre (Lutra lutra) sur les montagnes du Bieszczady au sud-est de la Pologne. *Mammalia*, **70**, 58–63.

Caudron AK, Negro SS, Muller CG, Boren LJ, Gemmell NJ (2007) Hair Sampling and Genotyping From Hair Follicles: a Minimally-Invasive Alternative for Genetics Studies in Small, Mobile Pinnipeds and Other Mammals. *Marine Mammal Science*, **23**, 184–192.

Collins R a., Armstrong KF, Holyoake AJ, Keeling S (2012) Something in the water: biosecurity monitoring of ornamental fish imports using environmental DNA. *Biological Invasions*, **15**, 1209–1215.

Creel S (2001) Social dominance and stress hormones. *Trends in Ecology and Evolution*, **16**, 491–497.

Dalén L, Götherström A (2007) Recovery of DNA from Footprints in the Snow. *The Canadian Field-Naturalist*, **121**, 321–324.

Deagle BE, Chiaradia A, McInnes J, Jarman SN (2010) Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? *Conservation Genetic*, **11**, 2039–2048.

Frantz AC, Schaul M, Pope LC, Fack F, Schley L, et al. (2004) Estimating population size by genotyping remotely plucked hair : the Eurasian badger. *Journal of Applied Ecology*, **41**, 985 -995.

Fumanal B, Martin J-F, Bon MC (2005) High through-put characterization of insect morphocryptic entities by a non-invasive method using direct-PCR of fecal DNA. *Journal of Biotechnology*, **119**, 15–19.

Gauthier J, Sears R (1999) Behavioral response of four species of balaenopterid whales to biopsy sampling. *Marine Mammal Science,* **15**, 85–101.

Gemmell N, Majluf P (1997) Projectile biopsy sampling of fur seals. *Marine Mammal Science*, **13**, 512–516.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences*, 101, 14812–14817.

Herrando-Perez S, Brook BW, Bradshaw CJ a. (2014) Ecology Needs a Convention of Nomenclature. *Bioscience*, **64**, 311–321.

Hodges KE (2008) Defining the problem: terminology and progress in ecology. *Frontiers in Ecology and the Environment*, **6**, 35–42.

Krützen M, Barré L (2002) A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Marine Mammal Science,* **18**, 863–878.

Lampa S, Henle K, Klenke R, Hoehn M, Gruber B (2013) How to overcome genotyping errors in non-invasive genetic mark-recapture population size estimation-A review of available methods illustrated by a case study. *Journal of Wildlife Management*, **77**, 1490–1511.

Lefort M-C, Boyer S, Worner SP, Armstrong K (2012) Noninvasive molecular methods to identify live scarab larvae: an example of sympatric pest and nonpest species in New Zealand. *Molecular Ecology Resources*, **12**, 389–395.

Lefort M-C, Boyer S, De Romans S, Glare T, Armstrong K, et al. (2014) Invasion success of a scarab beetle within its native range: host range expansion versus host-shift. *PeerJ*, **2**: e262.

Lefort M-C, Worner SP, Rostas M, Vereijssen J, Boyer S (2015) Responding positively to plant defences, a candidate key trait for invasion success in the New Zealand grass grub *Costelytra zealandica* (White) (Scarabaeidae: Melolonthinae). *New Zealand Journal of Ecology*, **39**, 128-132.

Marschalek D a., Jesu J a., Berres ME (2013) Impact of non-lethal genetic sampling on the survival, longevity and behaviour of the Hermes copper (*Lycaena hermes*) butterfly. *Insect Conservation and Diversity*, **6**, 658–662.

Mccarthy MA, Parris KM (2004) Clarifying the effect of toe clipping on frogs with Bayesian statistics. *Journal of Applied Ecology*, **41**, 780–786.

Miotto R a., Cervini M, Kajin M, Begotti R a., Galetti PM (2014) Estimating puma Puma concolor population size in a human-disturbed landscape in Brazil, using DNA mark–recapture data. *Oryx*, **48**, 250–257.

Mullins J, Statham MJ, Roche T, Turner PD, O'Reilly C (2009) Remotely plucked hair genotyping: a reliable and non-invasive method for censusing pine marten (*Martes martes*, L. 1758) populations. *European Journal of Wildlife Research*, **56**, 443–453.

Murphy D, Noon B (1991) Coping with Uncertainty in Wildlife Biology. *The Journal of Wildlife Management*, **55**, 773–782.

Nelson G, Graves B (2004) Anuran Population Monitoring : Comparison of the North American Amphibian Monitoring Program's Calling Index with Mark-Recapture Estimates for Rana clamitans. *Journal of Herpetology*, **38**, 355–359.

Nichols R V, Königsson H, Danell K, Spong G (2012) Browsed twig environmental DNA: diagnostic PCR to identify ungulate species. *Molecular Ecology Resources*, 12, 983–989.

Oi CA, López-Uribe MM, Cervini M, Del Lama MA (2013) Non-lethal method of DNA sampling in euglossine bees supported by mark–recapture experiments and microsatellite genotyping. *Journal of Insect Conservation*, **17**, 1071–1079.

Robinson S, Waits L, Martin I (2009) Estimating abundance of American black bears using DNA-based capture-mark-recapture models. *Ursus*, **20**, 1–11.

Sboner A, Mu X, Greenbaum D (2011) The real cost of sequencing: higher than you think! *Genome Biology*, **12**, 125.

Sheehy E, O'Meara DB, O'Reilly C, Smart A, Lawton C (2013) A non-invasive approach to determining pine marten abundance and predation. *European Journal of Wildlife Research*, **60**, 223–236.

Suarez RK, Moyes CD (2012) Metabolism in the age of "omes". *The Journal of Experimental Biology*, **215**, 2351–2357.

Suleman M, Wango E (2004) Physiologic manifestations of stress from capture and restraint of free-ranging male african green monkeys (*Cercopithecus aethiops*). *The Journal of Zoo and Wildlife Medicine*, **35**, 20–24.

Thomson J a., Heithaus MR (2014) Animal-borne video reveals seasonal activity patterns of green sea turtles and the importance of accounting for capture stress

in short-term biologging. *Journal of Experimental Marine Biology and Ecology*, **450**, 15–20.

Uno R, Kondo M, Yuasa T, Yamauchi K, Tsuruga H, et al. (2012) Assessment of genotyping accuracy in a non-invasive DNA-based population survey of Asiatic black bears (*Ursus thibetanus*): lessons from a large-scale pilot study in Iwate prefecture, northern Japan. *Popululation Ecology*, **54**: 509–519.

Vila M, Auger-Rozenberg M a., Goussard F, Lopez-Vaamonde C (2009) Effect of non-lethal sampling on life-history traits of the protected moth *Graellsia isabelae* (Lepidoptera: Saturniidae). *Ecological Entomology*, **34**, 356–362.

Vucicevic M, Stevanov-Pavlovic M, Stevanovic J, Bosnjak J, Gajic B, et al. (2013) Sex determination in 58 bird species and evaluation of CHD gene as a universal molecular marker in bird sexing. *Zoo Biology*, **32**, 269–276.

Waldner T, Traugott M (2012) DNA-based analysis of regurgitates: a noninvasive approach to examine the diet of invertebrate consumers. *Molecular Ecology Resources*, 12, 669–675.

Waterhouse BR, Boyer S, Wratten SD (2014) Pyrosequencing of prey DNA in faeces of carnivorous land snails to facilitate ecological restoration and relocation programmes. *Oecologia*, **175**, 737–746.

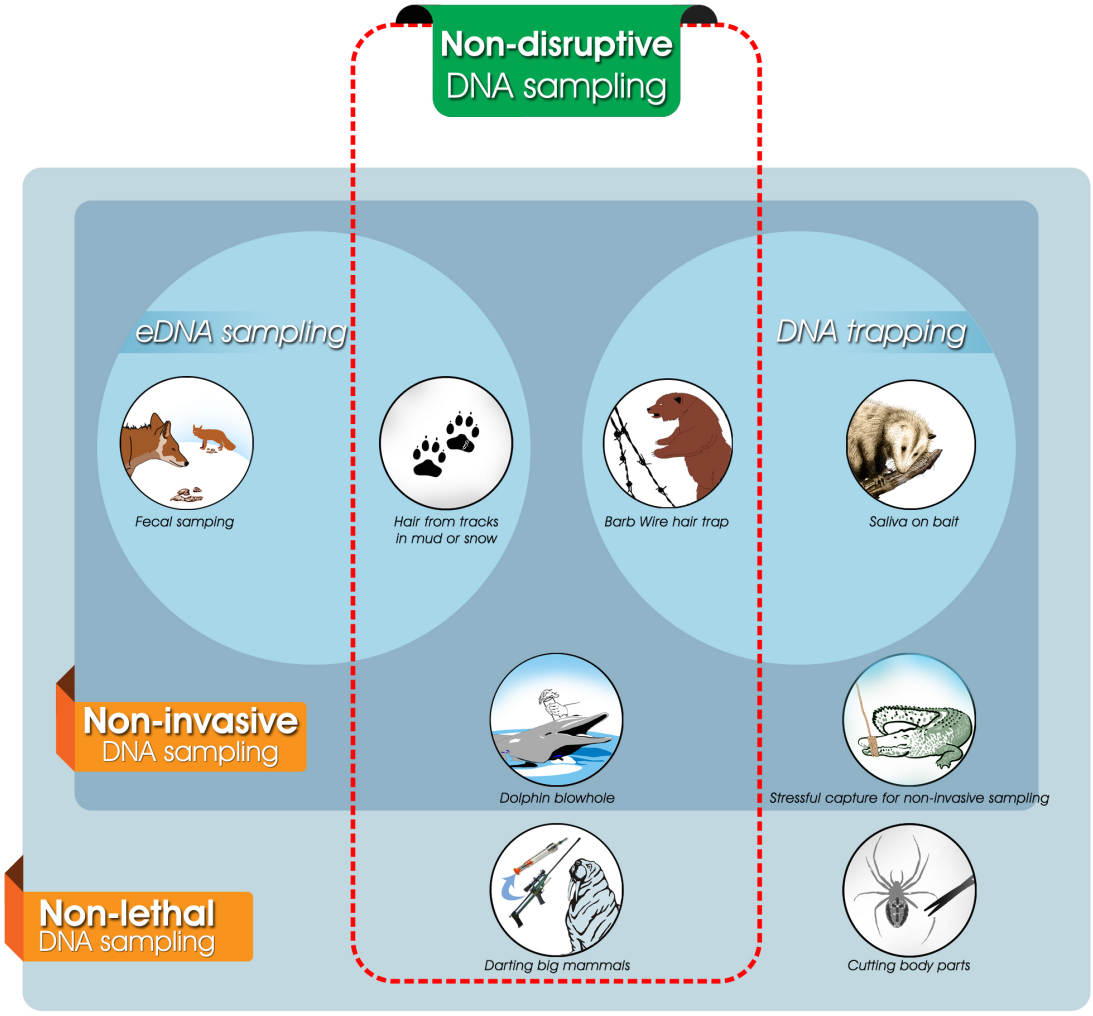

Figure 1. Non-disruptive DNA sampling methods, and their overlaps with non-invasive and non-lethal sampling methods.

Table 1. Non-exhaustive list of terms, and their definition inherent to the qualification of DNA sampling (See full references in Suppl. 1).

Term	Definition used in this paper	Example
Environmental DNA (eDNA) sampling	Obtaining trace DNA from one or more unknown organisms from the environment, when those organisms are no longer present.	Sampling fish DNA from stream water (Wilcox et al. 2013).
Noninvasive DNA sampling	Obtaining DNA using any method that does not affect the physical integrity of the organism (this includes eDNA; see above), but may affect fitness or behaviour.	Cloaca swabbing of lizards which does not physically harm them, but may affect their behaviour (Williams et al. 2012).
Nondisruptive DNA sampling	Obtaining DNA from a known individual organism without affecting its fitness or behaviour, but may affect the structural integrity of the organism (this differs from some (most) definitions of noninvasive DNA in the literature); this includes DNA trapping (see below).	Removing small parts of the butterfly hindwing does not affect survival or behaviour (Hamm et al. 2010).
DNA trapping	Remotely obtaining DNA from one or more unknown individual organisms by taking a sample while they are present. This usually involves some sort of trap or device, which may or may not be disruptive.	Remote hair plucking of badgers with barb wire traps (Frantz et al. 2004)
Noninjurious DNA sampling	Obtaining DNA from one or more known organisms through direct contact, but causing no physical injury or wound. This method may affect behaviour due to minor disturbance.	Skin swabbing of dolphins (Harlin et al. 1999).
Nonlethal DNA sampling	Obtaining DNA from a known individual organism in such a way that its fitness or behaviour are affected but it is not killed (we consider this to be inappropriate if these individuals will be used for experiments following DNA sampling).	Amphibian toe clipping affects survival rate (McCarthy & Parris 2004).
Nondestructive DNA sampling	Obtaining DNA from a known individual organism in such a way that the organism is killed, but not destroyed, so that it can be preserved as a voucher specimen.	DNA extraction from arthropods while keeping them as a voucher specimen (Paquin & Vink 2009).