

Monitoring vultures in the 21st century: The need for standardized protocols

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1 | A CONSERVATION CHALLENGE

Effective conservation relies on robust monitoring programmes to design and evaluate management actions (Nichols & Williams, 2006). Large vertebrates with slow life-history strategies present a challenge for monitoring given the necessary long-term investment of effort and resources. Furthermore, many of these vertebrates possess expansive habitat requirements, demanding large-scale monitoring approaches (Rudnick, Katzner, & DeWoody, 2009). For such species, localized monitoring efforts of limited duration can result in partial or even biased information, and delayed detection of threatened viability and population changes (Ogada et al., 2016). The development of practical, affordable and broadly applicable methods for monitoring vertebrates with slow life-history traits remains a challenge for applied ecologists globally.

The use of non-invasive genetic-based techniques, in particular, has enabled the estimation of demographic parameters for many long-lived species that are elusive, wide-ranging or rare. The identification of individuals by their multi-locus genotypes enables a 'capture-recapture' framework to estimate vital rates (such as survival and recruitment), and derive population size, growth and viability (Carroll et al., 2018). Rapid development of genetic techniques are making non-invasive monitoring more efficient and financially viable (Carroll et al., 2018). However, a 'conservation genetic gap' has emerged between genetic research and its

practical application, partially due to lack of access to expertise and funding by conservation managers, particularly in developing countries (Taylor, Dussex, & van Heezik, 2017). Despite best efforts to make genetic approaches accessible to wildlife managers, non-invasive genetic monitoring is generally conducted by researchers at academic institutions and rarely used in long-term monitoring programmes (Taylor et al., 2017).

Vultures – avian obligate scavengers – constitute a major conservation challenge for the 21st century. Sixteen of 23 extant vulture species are currently at-risk of extinction; vulture declines have been recent and rapid, and many species continue to exhibit range-wide contractions in both abundance and distribution (Ogada et al., 2016). These declines result from the confluence of the guild's intrinsic susceptibility to extinction (e.g. dietary specialization, extensive individual home ranges, slow demography) with anthropogenic impact (Ogada, Keesing, & Virani, 2012). Because of their strict dependence on ephemeral carrion resources, vultures are particularly vulnerable to dietary toxins such as lead pellets in hunted game, residual pharmaceutical compounds in livestock remains, and intentionally poisoned carcasses. The collapse of most vulture populations has raised awareness of the ecological, economic and cultural services these birds provide (Ogada et al., 2012). Vultures are a stabilizing force on the structure and dynamics of food webs, central in nutrient cycling and exchange, barriers to pests and disease epizootics, agents of carrion and waste disposal, important attractions for eco-tourism and

culturally iconic species (Donázár et al., 2016). The recognition that ecological services provided by vultures are generally declining triggered global efforts to preserve them.

Efforts to rescue vulture populations have focused on reintroduction and rehabilitation, and the establishment of food supplementation stations, so-called 'vulture restaurants' (Cortés-Avizanda et al., 2016). While many of these programmes have found success, a critical yet currently overlooked aspect of them are the scarcity of coordinated efforts to monitor vulture populations to obtain robust estimates of demographic attributes. Vultures present special challenges in monitoring as they often occur at low densities over expansive areas, breed and roost in sites that are remote and difficult to access, and possess long generation times (Donázár et al., 2016). Although coordinated cases of monitoring programmes exist (e.g. the International Bearded Vulture Monitoring network; <http://www.gyp-monitoring.com/>), these are limited to only a few localized areas, focused on particular species, and often methodologically inconsistent between programmes (Figure 1). Standardized monitoring programmes that wildlife managers can implement on the ground, across vultures' ranges and between species would strengthen the management of both highly endangered as well as larger and healthier populations.

Herein, we compared the common techniques used to study vulture populations using Andean condors (*Vultur gryphus*) – a large, widely distributed vulture of conservation concern – as a case study (Figure 2). From this comparison, we identified the use of shed feathers as the most robust method for long-term and large-scale monitoring of avian scavengers across species and political boundaries. Furthermore, we provide an effective, efficient and tested protocol for monitoring Andean condors via shed feathers, and call for its implementation by conservation practitioners at a continental scale.

2 | VULTURE MONITORING TECHNIQUES

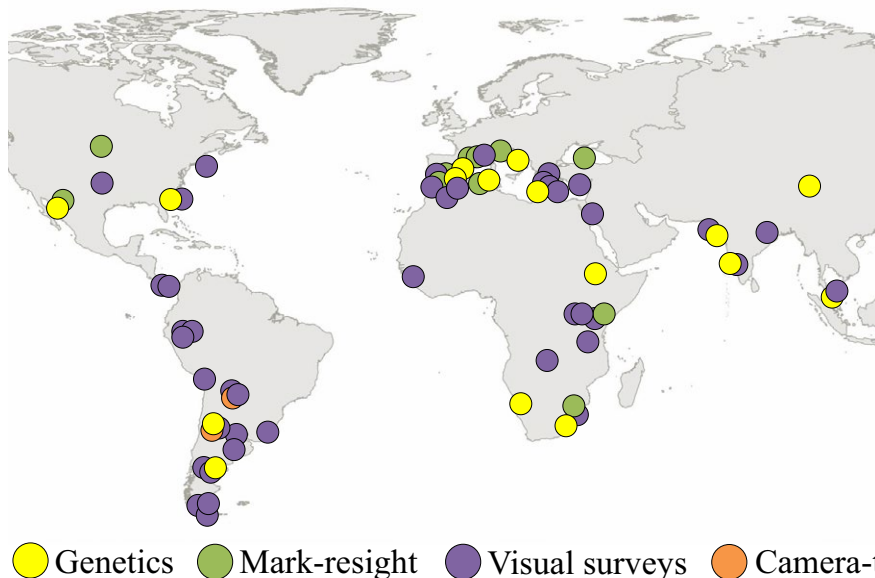
2.1 | Capture-based

For many longitudinal studies, mark-resight analyses of marked individuals via rings, wing bands, or bleached feathers are commonly employed (Figure 1). When sufficient sample sizes are obtained, analytical techniques that incorporate detection probability can yield demographic estimates readily comparable across studies (Hurley, White, & Cooke, 2013). Thus, mark-resight has proven effective for closely managed or studied populations – especially when rehabilitated or captive breeding individuals are tagged. However, this approach is difficult to apply over large spatial and temporal scales by managers given their expense, time requirements, and risks associated with capturing and handling wildlife, for both the vultures under study and researchers conducting the work (Rudnick et al., 2009).

2.2 | Count-based

Many vulture monitoring programmes currently rely upon field counts of unmarked individuals (visual surveys), including counts at roosting sites, colonies, provisioned carcasses, fix point counts, road counts on vehicle or foot and monitoring of occupancy of breeding sites (Figure 1). Given that these techniques can be implemented with little training by a large number of observers, they represent a useful approach for studies developed over large temporal and spatial scales, while allowing the involvement of citizen scientists for outreach and education. Despite being technically simpler than other approaches, these estimates are demanding in time, labour and logistics (Rudnick et al., 2009). Estimation of

(a) Sampling methods by location



(b) Sampling methods by species

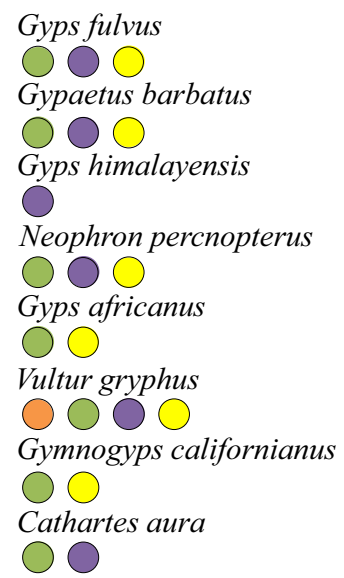


FIGURE 1 Non-exhaustive review of methods used to study vulture populations (a) globally (b) by representative vulture species. See Table S1 for references

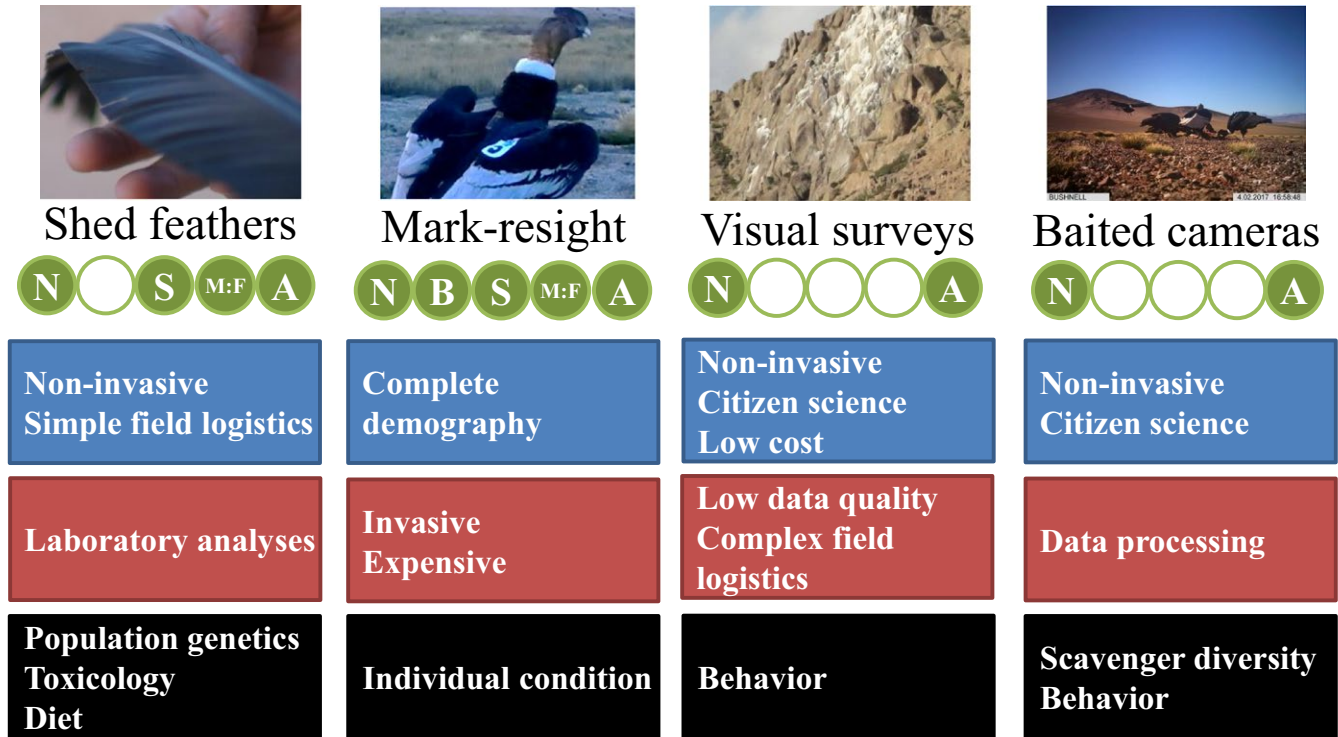


FIGURE 2 Comparison of sampling techniques used to monitor vulture populations based on data products (green; population size [N], proportion adult females breeding [B], survival rate [S], sex ratio [M:F] and age ratio [A]), advantages (blue), disadvantages (red) and additional data generated (black)]

sex ratios through this technique is unfeasible for monomorphic species, which include most species of vultures, potentially leading to overestimation of effective population sizes (Lambertucci, Carrete, Donázar, & Hiraldo, 2012). Furthermore, estimated population parameters can present important biases due to repeated observations of the same individuals during the survey, inconsistencies of monitoring efforts among sites and times, and spatial-temporal concentration of individuals. For example abundance estimates based on colonies are biased towards the breeding portion of populations (Rudnick, Katzner, Bragin, & DeWoody, 2008), and age structure of vultures using communal roosts vary with the location and characteristics of perching sites (Lambertucci, Jácome, & Trejo, 2008).

Vulture restaurants – stations where carrion is routinely delivered to vultures – provide a unique opportunity to simultaneously monitor a large number of individuals and species that aggregate to feed despite the presence of observers (Cortés-Avizanda et al., 2016). However, data collected at these sites can be biased towards the most social, dominant, and abundant vulture species, or towards a particular segment within the population (e.g. birds with lower body condition, or some age or sex category). Furthermore, vulture restaurants are inconsistently distributed and are intended to be a short-term management practice to recover a species (Cortés-Avizanda et al., 2016); thus, they represent an unsuitable approach for long-term monitoring programmes of vulture populations.

2.3 | Feather sampling

Non-invasively collected moulted feathers combined with molecular techniques allows robust estimates of demographic parameters for a variety of rare and elusive species (Rudnick et al., 2009), yet only a handful studies have used naturally shed feathers to assess vulture populations (Figure 1). Feathers provide a reliable source of high-quality DNA for individual identification and molecular sexing (Bayard de Volo, Reynolds, Douglas, & Antolin, 2008; Rudnick, Katzner, Bragin, Rhodes, & DeWoody, 2005), appropriate for capture–recapture analysis (Kapetanakos, 2014; Rudnick et al., 2008) and estimation of minimum population size and sex ratios (Alcaide, Cadahía, Lambertucci, & Negro, 2010). Furthermore, moulted feathers can reveal a population's age structure for species that change plumage coloration while maturing (Alcaide et al., 2010). Since genetic tagging is permanent, in contrast to bleached feathers or wing bands that are temporary, it is a particularly useful method to monitor long-lived species (Rudnick et al., 2009). When using this technique, important considerations in sampling designs must be made. First, feather collection needs to account for behavioural differences in space use and foraging between individuals of different sex, age and breeding status to obtain a representative sample of the population under study (Katzner, Ivy, Bragin, Milner-Gulland, & DeWoody, 2011; Lambertucci et al., 2008). Additionally, the size of the feather and time since moulting affects DNA yields, with large and freshly moulted feathers being most reliable. Second, prescreening of DNA

yields and estimations of genotyping errors are required steps to ensure robust results (Bayard de Volo et al., 2008). Furthermore, approaches based on non-invasive sampling of DNA require laboratory analyses, which could be challenging for managers to implement themselves. Yet, molecular techniques continue to be improved for their implementation by personnel with limited training, and a large number of laboratories offer these services (see Data S1). Even with laboratory services, this approach is more cost effective when compared with live-capturing and marking individuals (Carroll et al., 2018).

2.4 | Baited camera traps

The use of remote cameras at baited stations can provide data on complete scavenger communities over large scales when standardized protocols are implemented by professional and citizen scientists (Jachowski, Katzner, Rodrigue, & Ford, 2015). Furthermore, single estimates of population densities can be obtained via capture–recapture models of uniquely identifiable individuals (e.g. via moulting patterns or natural marks such as crest shape), or from unmarked birds (Steenweg et al., 2017). This technique requires detailed standardization of camera configuration and station arrangement, and does not retrieve sex structure for monomorphic species. Furthermore, carrion size, location and season can influence the use of baited stations by scavenger communities (Turner, Abernethy, Conner, Rhodes, & Beasley, 2017). The most time-consuming step associated with this method is photo processing, but an increasing number of tools are available for the efficient management and sharing of camera trap data of large-scale studies, and citizen-science web portals can be used for crowdsourcing image analysis (Steenweg et al., 2017). It has been suggested that interconnected remote camera networks will be key for global monitoring of biodiversity in the near future (Steenweg et al., 2017). However, similar to DNA-based non-invasive approaches, this method remains largely unemployed to monitor vulture populations (Figure 1).

3 | ANDEAN CONDORS – A CASE STUDY

To compare the potential of emerging non-invasive approaches to estimate population parameters for vultures, we applied count-based, baited camera traps and feather sampling approaches to study Andean condors in a pristine area of the high Andes of Argentina, San Guillermo National Park (SGNP), where no previous estimates of condor population size were available. Surveys at communal roosts are commonly used to monitor Andean condors (see Table S1), but resulted an unfeasible approach for this area, as for many others in condors' distribution, due to the remote location and relatively small size of perching sites. Thus, we tested the efficiency of genetic monitoring of moulted feathers and remote camera traps to estimate Andean condor minimum population size, age and sex ratios.

We monitored via one camera trap eight standardized food patches offered simultaneously in open plains during March–April 2017. Each station consisted of carrion remains (obtained from a local slaughterhouse), and remained active for 7 days. All stations were used by facultative scavengers present in the area (mountain caracaras *Phalacrocorax macrorhynchos*, and culpeo foxes *Lycalopex gymnocercus*) while Andean condors used six of the eight stations in groups that ranged from 3 to 19 birds. Given that condor feeding groups are dynamic, we estimated mean sex and age ratio per station from the ratios registered in camera trap photos that allowed the classification of >70% of the birds present. We identified immature birds (juveniles and sub-adults) by their brownish-grey plumage and adult birds from their black and white plumage; males were identified by the presence of a crest. We found that the structure of the feeding groups differed across stations, with proportion of males ranging from 0% to 80% and proportion of adults from 0% to 93%. Overall, we observed large disparity in the use of standardized baited station by Andean condors. The type of carrion provided and the complexity of the area surrounding each station probably determined their use by condors, given that we had observed more than 60 individuals feeding upon a carcass of a native camelid in the area. Thus, spatial comparisons of demographic parameters obtained with this technique are likely unreliable even with standardized field protocols.

We collected moulted feathers (12–70 cm long) at the base of roosts and condor feeding sites during summer 2013. We extracted DNA from 151 feathers, and successfully genotyped 131 samples at three to five species-specific, neutral markers (probability of identity <0.01; Perrig, Donadio, Middleton, & Pauli, 2017). We identified 89 birds, of which 30% were males (Perrig et al., 2017). We aged remiges and rectrices feathers (>19 cm, $n = 49$) according to coloration: 70% of the samples were black, or black and white, and classified as belonging to adults, whereas 30% brown or grey feathers were classified as belonging to immature birds (Alcaide et al., 2010, Data S1). Overall, the use of shed feathers resulted in higher sampling rates than baited camera traps, reducing heterogeneous sampling due to behavioural differences between sex and age classes. We developed two multiplex reactions for six polymorphic markers (Data S1) that allow the identification of 99% of 278 feathers genotyped across central Argentina (Padr , Lambertucci, Perrig, & Pauli, 2018). We provide a detailed protocol on Andean condor feather sampling in Data S1. The use of this readily available and affordable protocol (~US\$15 per sample) on feathers collected from a variety of sources (e.g. roosting, feeding sites and rehabilitated or dead individuals) will yield transboundary estimates of Andean condor population demography.

4 | A UNIFIED PROTOCOL

The precipitous declines of most vultures globally stress the relevance of range-wide and long-term monitoring programmes for vulture conservation (Jachowski et al., 2015). Yet, we found that vulture studies

remain restricted to specific regions and species, and mostly brief in temporal scale. While transboundary conservation actions targeted at vulture populations have been established – like the international Multi-species Action Plan to Conserve African-Eurasian Vultures (vultures MsAP) approved in 2017 (Botha et al., 2017) – such agreements are dependent upon demographic parameters to evaluate the achievement of settled conservation goals, though no vulture monitoring protocol is provided. With the understanding that some monitoring techniques are particularly suitable for a species or region, standardized approaches that allow international collaboration and comparison of estimated demographic parameters are necessary for managing species as vagile and long-lived as vultures. We call on scientists and organizations focused on vulture conservation (e.g. Saving Asia's Vultures from Extinction, [SAVE], VulPro, Hawk Mountain Sanctuary) to promote and use standardized monitoring programmes. As we showed for Andean condors, non-invasive monitoring approaches based on moulted feather sampling provide robust, complete and cost-effective demographic data compared to traditional techniques. Similar species-specific laboratory and sampling protocols should be used to generate profiles of individual birds shared via web repositories. We see this as a promising approach for international collaboration towards continental vulture monitoring programmes.

Genetic analysis of shed feathers have been thoroughly tested, repeatedly validated (Rudnick et al., 2009), and previously shown to outperform traditional monitoring approaches (Katzner et al., 2011), but remain rarely used by practitioners for the estimation of demographic parameters. Indeed, shed feathers have been successfully used to monitor birds via 'mark-recapture' modelling (Kapetanakos, 2014; Olah, Heinsohn, Brightsmith, & Peakall, 2017; Rudnick et al., 2008) or via turnover rates of occupied territories (Rudnick et al., 2005; Selås, Kleven, & Steen, 2017). These studies generally require a large number of feathers to obtain a representative sample of the population, or sufficient recapture rates, and laboratory and analytical methods to control for genotyping errors (Kapetanakos, 2014; Rudnick et al., 2008). Simple non-invasive protocols, though, yield standardized and robust results, with affordable field efforts and laboratory cost. Thus, we encourage continued efforts to increase the accessibility of non-invasive genetic techniques beyond primary research, and for practitioners to adopt these approaches for monitoring large and long-lived birds.

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AUTHORS' CONTRIBUTIONS

P.L.P. and J.N.P. conceived the project and led the writing of the manuscript with inputs from S.A.L.; P.L.P., E.D. and S.A.L. collected the data; J.P. assisted in analysis. All authors critically reviewed drafts and have approved the final version.

DATA ACCESSIBILITY

Data available via the Dryad Digital Repository <https://doi.org/10.5061/dryad.v4v289p> (Perrig, Lambertucci, Donadio, Padró, & Pauli, 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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