

Multiple methods increase detection of large and medium-sized mammals: working with volunteers in south-eastern Oman

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Abstract We compared the effectiveness of various methods for surveying medium and large wild mammals in southern Oman. Working with volunteers recruited by Biosphere Expeditions, wildlife professionals and local rangers, we used direct observation, camera traps, sign surveys (tracks and/or dung) and molecular scatology to study 66 sampling units of 2×2 km (grid cells) in an area of 32×36 km during a 4-week period in February–March 2011. Sixteen mammal species were recorded, and the largest numbers of species were recorded by sign surveys and camera traps (both $n = 9$); sign surveys, direct sightings and DNA scatology recorded species across the largest number of grid cells. For species with a sample size large enough for comparison (i.e. detected in ≥ 8 grid cells), DNA scatology proved most effective for detecting caracal *Caracal caracal*, signs for hyaena *Hyaena hyaena*, ibex *Capra nubiana*, porcupine *Hystrix indica* and hyrax *Procavia capensis*, and signs and direct sightings for mountain gazelle *Gazella gazella*. Clustering, in which records from multiple methods are either adjacent or overlapping, was highest ($\geq 76\%$) for the wolf *Canis lupus*, porcupine, ibex and gazelle. Our results indicate the best methods to detect and record the distributions of individual species in the study area, and demonstrate the advantage of using multiple methods to reduce the risk of false absences or partial detections. Our findings also highlight the potential of clustering as a means of cross-checking results of observations that are skill-dependent, which is particularly useful when employing a large workforce.

Keywords Citizen science, Dhofar, mammals, methods, Middle East, Oman, sampling, volunteer

Introduction

Knowing which methods are most efficient for recording target species is fundamental to the success of short-duration target research expeditions and surveys. Without such prior knowledge, efforts and resources may be wasted by using methods that are not appropriate for recording the species of interest. More broadly, failure to record species that are present may result in misleading descriptions of distribution and abundance. These potential biases have not been adequately addressed in the scientific literature, and most of the statistics used to infer density and presence of species have been developed using a single field method (e.g. Otis et al., 1978; Burnham et al., 1980; Boulonier et al., 1998; Karanth & Nichols, 1998; MacKenzie et al., 2002; MacKenzie & Nichols, 2004). More recently, models have been developed that incorporate data from multiple methods (e.g. Nichols et al., 2008), an acknowledgement that single-method approaches may not be ideal in all research situations, although not everyone agrees (Otto & Roloff, 2011).

Earlier use of multiple survey methods (e.g. Zielinski & Kucera, 1995) is now becoming more popular (Silveira et al., 2003; Gompfer et al., 2006; Nichols et al., 2008; Nomani et al., 2008; Ausband et al., 2014). Previously, particular methods were advocated for estimating the abundance and occupancy of particular species or taxonomic groups (e.g. Karanth et al., 2004; Balme et al., 2009; Mondol et al., 2009). However, sampling rare species (or populations) using a single method, such as camera trapping, necessitates increasing survey effort, often to a level that may be logistically unrealistic (Shannon et al., 2014). Furthermore, there is increasing evidence that different methods yield different detection probabilities (e.g. Nichols et al., 2008; Otto & Roloff, 2011) and may produce different estimates of abundance or presence (e.g. Gompfer et al., 2006; Nomani et al., 2008; Otto & Roloff, 2011). It is therefore possible that two methods may result in two different estimates, even when detection probability statistics are used, highlighting the relevance of analysing the efficiency of multiple methods.

Here we demonstrate how the efficiency of sampling methods varies by species, and that single sampling methods cannot be prescribed in a generalized way for all study situations. We also consider the potential for bias when

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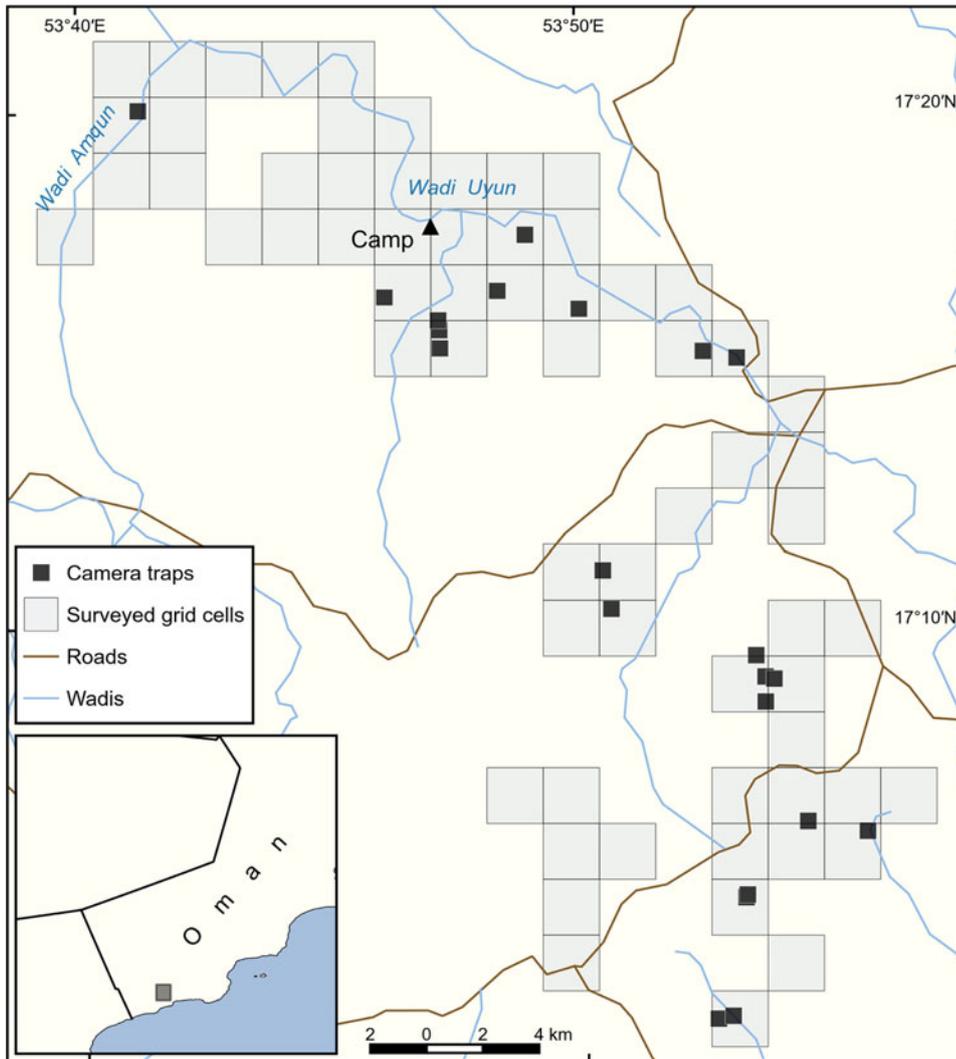


FIG. 1 The 66 2×2 km cells surveyed, by various methods (Table 1), for large and medium-sized mammals in south-eastern Oman, with the locations of camera trap stations.

there is a large team working in the field, a matter that may be of particular concern when volunteers are involved. When multiple sampling methods are used, particularly those that are less dependent on observer skill, the results may be cross-checked, and we examined how clustering of grid cells from multiple methods may be used to do this for individual species.

Study area

The 32×36 km study area in the south-west Dhofar Mountains (part of the Nejd or Jabal Al Qara region) in Oman, in the south-east Arabian Peninsula, was delimited by Wadi Uyun in the north and the cliffs above the Salalah plains in the south. The topography varies from wadis (seasonally dry riverbeds) to mountain ridges and escarpments. Vegetation coverage increases towards the southern monsoon-fed regions but consists of scattered bushes and does not hinder visibility.

Methods

Two groups, of 13 and 21 participants, carried out surveys during 6–18 February and 20 February–4 March 2011, respectively. Each group comprised wildlife professionals, local rangers, and volunteers recruited by Biosphere Expeditions, who received training in data collection. An expedition leader (Paul O'Dowd), the expedition scientist (MM) and the national scientist (KH) were present throughout the expedition. Each group was divided into 3–4 subgroups to maximize the area surveyed.

Sampling and analysis of signs

We surveyed 66 2×2 km sampling units (grid cells; Fig. 1) for medium and large mammals during a 25-day period. The size of the area was determined by the capacity of the survey team to cover the area from the base camp, and the cell size was determined by the need to cover areas large enough to

TABLE 1 Large and medium-sized mammal species recorded in south-eastern Oman (Fig. 1), with their global and regional Red List status, the number of cells in which they were recorded (and the total number of records) by five methods (sightings, signs, camera traps, bones and carcasses, and faecal DNA analysis) and, for the seven most commonly recorded species (i.e. recorded in > 8 cells), the number of cells in which two or more methods recorded presence in two or more adjoining cells (i.e. cells that were clustered, with the percentage of the total number of cells in parentheses). Signs are mostly tracks and faecal samples (dung) identified by eye.

Species	Global status ¹	Regional status ²	No. of cells in which species recorded (no. of records) ³					Cell clustering (% of total cells)
			Sighting	Sign	Camera trap	Bones & carcasses	DNA analysis	
Carnivora								
Leopard <i>Panthera pardus nimr</i>	CR	CR					1 (1)	
Caracal <i>Caracal caracal</i>	LC	LC		1 (track, n = 1)	2 (2)		9 (10)	11 5 (45)
Gordon's wildcat <i>Felis silvestris</i>	LC	NT					1 (1)	
Striped hyaena <i>Hyaena hyaena</i>	NT	EN		26 (tracks, n = 40)	3 (20)		1 (1)	26 10 (38)
Grey wolf <i>Canis lupus</i>	LC	EN		7 (tracks, n = 9)	2 (2)	1 (1)	4 (10)	8 5 (63)
Red fox <i>Vulpes vulpes</i>	LC	LC		1 (1)		1 (1)		
Blanford's fox <i>Vulpes cana</i>	LC	VU			2 (2)			
Honey badger <i>Mellivora capensis</i>	LC	NT			1 (3)			
Small spotted genet <i>Genetta genetta</i>	LC	LC			1 (4)			
White-tailed mon-goose <i>Ichneumia albicauda</i>	LC	LC			1 (1)			
Artiodactyla								
Mountain gazelle <i>Gazella gazella</i>	VU		18 (31)	41 (tracks, n = 47; dung, n = 48)		1 (1)		46 41 (89)
Nubian ibex <i>Capra nubiana</i>	VU		1 (1)	23 (tracks, n = 17; dung, n = 34)	1 (1)	1 (1)		24 21 (88)
Hyracoidea								
Rock hyrax <i>Procavia capensis</i>	LC		1 (2)	28 (tracks, n = 10; dung, n = 40)	1 (17)	2 (2)		28 11 (39)
Lagomorpha								
Cape hare <i>Lepus capensis</i>	LC			2 (track, n = 1; dung, n = 2)				
Rodentia								
Indian crested porcupine <i>Hystrix indica</i>	LC			31 (tracks, n = 26; dung, n = 47; quills, n = 9)	5 (35)			38 29 (76)
Hedgehog <i>Paraechinus aethiopicus</i> or <i>P. hypomelas</i>	LC		2 (2)					

¹CR, Critically Endangered; EN, Endangered; VU, Vulnerable; NT, Near Threatened; LC, Least Concern; IUCN (2015).

²Mallon & Budd (2011).

³Blank cells indicate the species was not recorded by that method.

be relevant for describing the distributions of large and medium mammals.

Identification methods included recording mammalian signs (mainly tracks and herbivore dung), DNA analysis of carnivore scats, visual recording, and camera traps. Carnivore scats were not identified macroscopically because of the likelihood of significant error (Davison et al.,

2002; Harrison, 2002; Perez et al., 2006; Janecka et al., 2008; Vanstreels et al., 2010; Kelly et al., 2012; Mazzolli & Hammer, 2013), and therefore samples were collected for DNA-based species identification.

The presence or absence and frequency of target species was recorded using the general location given by a grid consisting of 2 × 2 km cells, the code of which was displayed in

the global positioning system (GPS) of each surveyor. Once a species or signs of it were found in a given cell, it was scored as containing the species. Species were recorded only once for each cell during a given survey (i.e. there was no double counting). Signs not identified directly in the field were collected (in the case of scats) or photographed with a scale (in the case of tracks). Twenty passive infrared camera traps (Cuddeback, Green Bay, USA) were deployed pseudo-randomly in 15 cells, at locations such as waterholes and known animal trails, aiming for the widest coverage possible. Camera traps were active day and night, and set at 40 cm above ground, with the beam directed slightly downwards.

Training

Training of the survey group included an introduction to conservation issues, followed by training on practical aspects of the survey, such as species identification from tracks and dung, and the use of GPS and data recording sheets, which lasted 2 days. Before volunteers were allowed to carry out surveys on their own, experienced personnel accompanied them for at least 2 additional days during field surveys, to provide further teaching and to check their knowledge. To reduce identification error, group members were instructed to bring herbivore dung to base camp if they were unable to identify the species in the field. They were also briefed on how to photograph tracks (using a scale) for later identification.

DNA analysis of faecal samples

We used DNA analysis of scats to identify species of carnivores. Extractions were performed using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. The extractions were carried out in a UV-sterilized laminar flow hood dedicated to the analysis of DNA from non-invasive samples. Each batch of 10 extractions included one negative extraction control to monitor the occurrence of contamination with extrinsic DNA.

To identify species from each scat we used an assay that targets a short segment of the mtDNA ATP synthase subunit 6 (ATP6) gene, using the reverse primer ATP6-DR1 and the forward primer ATP6-DF3. We used polymerase chain reactions (PCR) for the ATP6 gene, following the protocols described by Haag et al. (2009, 2010).

The PCR products were visualized on a 1% agarose gel stained with GelRed (Biotium, Hayward, USA), purified with PEG 8000, sequenced using the DYEnamic ET Dye Terminator Sequencing Kit (GE Healthcare, Hatfield, UK) and analysed in a MegaBACE 1000 automated sequencer (GE Healthcare). Sequence chromatograms were edited and analysed using *FinchTV v. 1.4.0* (Geospiza, Inc.,

Seattle, USA). The ATP6 gene fragment obtained from each faecal sample was compared with reference sequence.

Geographical information system (GIS) and mapping

The mapping procedures and analysis were designed to be easily integrated and replicated across multiple expeditions by personnel with no formal training in GIS (Mazzolli & Hammer, 2013). The main reference map used was at 1:100,000 scale (Uyūn, NE 39-12F; National Survey Authority, Sultanate of Oman), prepared using aerial photographs from 1993 and field updates from 1999, with grid data in the Universal Transverse Mercator projection (zones 39 and 40, WGS 84 datum).

An image of the study area was imported and georeferenced in *TrackMaker* (Geo Studio Technology, Minas Gerais, Brazil). A grid of 2 × 2 km cells covering the area was uploaded into GPS units, to aid navigation and data collection. As the work progressed, additional features such as access roads, base camp, trails and camera-trap locations were added to the GPS units, and were later overlaid onto a topographic map in *TrackMaker*, which was then edited and redrawn in *Adobe Photoshop* (Adobe Systems Inc., San Jose, USA) to leave only the features of interest.

Cluster analysis

Maps were produced for each target species, with cells displaying their recorded distribution and the methods by which they were recorded. The number of overlapping or adjacent cells in which two or more methods recorded the presence of a given species was counted. If such clustered cells occurred in >70% of the total number of cells in which a species was recorded, we considered that two or more methods corroborated each other.

We analysed clustered features without using automated GIS methods because the latter depend on data points or other features that represent an 'excess of events' in geographical space (Jacquez, 2008). Our data were not clustered in this sense. This was done to avoid autocorrelation, to approximate the format of data collected to that of the processed data (data were processed as clusters of cells, not as data points), and to cover as much area as possible by avoiding spending time on redundant recording of species in a single cell. Furthermore, there are concerns regarding the accuracy of automated GIS clustering (Hamfelt et al., 2011; Murray et al., 2012).

Results

We recorded 16 species of medium and large mammals (Table 1). The efficiency of the identification methods varied for each species. Seven species were recorded exclusively by a single method. Leopard *Panthera pardus nimr* and wildcat

Felis silvestris gordonii were recorded in single cells exclusively by DNA analysis. DNA analysis was also more efficient than other methods in detecting caracal *Caracal caracal*, and contributed substantially to detecting wolves *Canis lupus*. Wolf and hyaena *Hyaena hyaena* were recorded predominantly by tracks; gazelle *Gazella gazella* by tracks, dung and sightings; ibex *Capra nubiana* by tracks and dung; and porcupine *Hystrix indica* and hyrax *Procavia capensis* by dung (Table 1).

Camera traps also recorded species that were not recorded by other means, namely the honey badger *Mellivora capensis*, little spotted genet *Genetta genetta*, mongoose *Ichneumia albicauda* and Blanford's fox *Vulpes cana*. Camera traps yielded high recording frequencies for the hyaena, hyrax and porcupine, but they were localized in only a few cells compared with results from other methods. The hedgehog *Paraechinus* sp. was the only taxon exclusively recorded by direct observation.

Clustering of cells in the grid space was highest for wolf, gazelle, ibex and porcupine; i.e. $\geq 76\%$ of the distribution of these species were recorded by two or more methods in two or more adjoining cells. For the other species with a sufficiently large sample size ($n > 8$) for comparison (hyaena, caracal and hyrax), clustering was $< 63\%$ (Table 1).

Discussion

Comparison of methods

Our results show that the efficiency of detection methods varies by species, with one or two methods often outperforming others. For several species different methods produced different spatial distributions, suggesting a higher detection efficiency of multiple methods used in combination.

Previous studies have demonstrated differences in detection rates across methods, even at longer sampling intervals (e.g. Zielinski & Kucera, 1995; Gompper et al., 2006; Vine et al., 2009). Although camera traps are one of the tools most recommended for recording and monitoring wildlife (e.g. Silveira et al., 2003; Balme et al., 2009), in our study they did not detect six species, including the Critically Endangered Arabian leopard, and recorded the hyaena in only three of the 26 cells where the species was recorded, and the ibex in only one of 24 cells where it was recorded. Even if camera-trap sampling for hyaena and ibex were equalized for the whole study area (simulating their deployment in all 66 surveyed grid cells) by multiplying the number of grid cells recorded (camera traps were deployed in 15 cells) by 4.4 ($15 \times 4.4 = 66$ cells), and presuming the same recording rate, camera traps would have recorded ibex in six times fewer cells and hyaena in half of the number of cells in which they were recorded by other sampling methods.

Although gazelles were recorded in 46 cells using other methods, the species was not recorded by camera traps.

Thus there are cases in which meaningful parameter estimates cannot be obtained, regardless of one's statistical skills (Guillera-Aroita et al., 2014). Camera traps similarly returned a low sample size for ibex and leopards, and did not detect gazelles, in neighbouring Yemen (Khorozyan et al., 2014). Similarly, in Jabal Samhan recording rates for some species were found to vary greatly depending on the habitats sampled (Spalton et al., 2006); as other methods were not used we cannot know whether these findings are a true indication of the occurrence/absence of the species or a sampling artefact.

Our findings indicate that no single method should be relied on in all situations. The most appropriate method or combination of methods will depend on the target species, population and region, and on the parameters of interest. In a study in Slovakia, bears *Ursus arctos* were detected by tracks in 17 grid cells, but in only one cell by camera traps, and wolves and lynxes *Lynx lynx* were detected in up to 10 times more cells by tracks than by other methods (Hulik et al., 2015). We do not know how the detection probabilities of survey methods vary across the range of a single species, a variation that is probably associated with density. For leopards, for example, scrapes and tracks have been shown to be an efficient method to detect their presence in Jabal Samhan Nature Reserve in the east of Dhofar (Spalton, 2000), even during short surveys, whereas camera trapping is useful during longer surveys (Spalton et al., 2006; Spalton & Al Hikmani, 2014). However, short surveys of our study site in south-west Dhofar did not yield a high frequency of records, suggesting that leopards are rare in the area (Mazzolli, 2009; this study), and this has since been confirmed (Al Hikmani et al., 2015). Similarly, jaguars in the Atlantic Rainforest of Brazil now occur at such low densities that they were not detected by camera traps during a 6-year study, and were detected by tracks in only four instances (Mazzolli et al., 2013); in contrast, both camera traps and track surveys repeatedly recorded jaguars during an 11-day survey in Madre de Dios, Peru (Lee et al., 2010). We do not contest the value of camera trapping as a survey method (it successfully detected species that were not detected by other methods in our study) but it may not always be reliable in detecting species throughout their range.

Cross-checking results with cluster analysis

The role of volunteers in research has been widely recognized and is increasing (Brightsmith et al., 2008). Volunteers are particularly essential in large-scale monitoring programmes (Howe et al., 1995; Newman et al., 2003; Sauer et al., 2003; Kindberg et al., 2009; Schmeller et al., 2009). Participation of volunteers requires protocols that are easy to follow, and the data collected has to be scrutinized carefully and discarded if suspect or unreliable (Cohn, 2008).

Training is fundamental for securing unbiased data. In a deer monitoring study using pellet group counts volunteers received 5 hours of training using slides, yet they were only able to identify correctly 68% of deer droppings during the study (Buesching et al., 2014). However, in our study volunteers worked in groups, creating collaborative conditions that probably resulted in improved accuracy. Furthermore, they received 2 days of intensive training and had the opportunity to practise with scientists in the field for at least 2 more days before working on their own.

In such studies data quality also needs to be assessed, for which we used a post hoc evaluation with cluster analysis. The idea is that any inconsistencies will become apparent when using several methods for cross-validation. It would be reasonable to assume that contradictory findings from various methods could be an indication of observer error, but this is not always the case. The chance of surveyed cells clustering reduces when a single method notably outperforms others. In this case even if cells with records from different methods are overlapping or neighbouring, they will be a low proportion of all cells and therefore the measure of clustering will be low. This cluster analysis requires that at least two methods have records for a similar number of cells. A useful future improvement would be to incorporate an additional measure to corroborate the detection methods. We found a substantial spatial similarity of results among various methods for the wolf, gazelle, ibex and porcupine (i.e. for those species, cluster analysis showed that the various methods corroborated each other). For other species, however, the number of clusters of records from the various methods was low, which is attributable to a single method predominating in terms of recording success. This was the case for the hyaena and hyrax, which were predominantly recorded by skill-dependent methods (tracks and scats, respectively). The caracal was also predominantly recorded using a single, but non skill-dependent, method (faecal DNA). The hyaena's hind and front tracks differ in size and shape, and hyrax dung is usually found clustered near colonies and is markedly different from that of other herbivores in the region.

Our results show that methods vary in their ability to detect species of mammals. Some species would not have been recorded if the single method that detected them had not been used. The distribution of other species would have been underestimated (i.e. the hyaena, detected mainly by tracks; the hyrax, detected mainly from dung piles; and the caracal, detected from faecal DNA).

Conclusions

Our results indicated the best methods for detecting and recording distributions of individual mammal species in the study area, and demonstrated the advantage of using multiple methods to reduce the risk of false absences or partial

detections. Our findings also highlight the potential of clustering for cross-checking the results of observations that are skill-dependent, which is particularly useful when employing a large workforce.

Our findings have broad applications for surveying terrestrial mammals. Single methods often fail to detect target species, and thus a multiple-method approach is necessary to identify the most appropriate methods for the target species, region and habitat. We have shown that a combination of methods can produce information at a faster rate and result in a more complete mammal survey than any single method.

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Biographical sketches

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