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Review

Environmental impact assessment for large carnivores: a methodological review of the wolf *Canis lupus* monitoring in Portugal

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The continuous growth of the global human population results in increased use and change of landscapes, with infrastructures like transportation or energy facilities being a particular risk to large carnivores. Environmental impact assessments were established to identify the probable environmental consequences of any new proposed project, find ways to reduce impacts, and provide evidence to inform decision making and mitigation. Portugal has a wolf population of approximately 300 individuals, designated as an endangered species with full legal protection. They occupy the northern mountainous areas of the country which has also been the focus of new human infrastructures over the last 20 years. Consequently, dozens of wolf monitoring programs have been established to evaluate wolf population status, to identify impacts, and to inform appropriate mitigation or compensation measures. We reviewed Portuguese wolf monitoring programs to answer four key questions. Do wolf programs examine adequate biological parameters to meet monitoring objectives? Is the study design suitable for measuring impacts? Are data collection methods and effort sufficient for the stated inference objectives? Do statistical analyses of the data lead to robust conclusions? Overall, we found a mismatch between the stated aims of wolf monitoring and the results reported, and often neither aligns with the existing national wolf monitoring guidelines. Despite the vast effort expended and the diversity of methods used, data analysis makes almost exclusive use of relative indices or summary statistics, with little consideration of the potential biases that arise through the (imperfect) observational process. This makes comparisons of impacts across space and time difficult and is therefore unlikely to contribute to a general understanding of wolf responses to infrastructure-related disturbance. We recommend the development of standardized monitoring protocols and advocate for the use of statistical methods that account for imperfect detection to guarantee accuracy, reproducibility, and efficacy of the programs.

Keywords: EIA, human infrastructures, imperfect detection, monitoring, wolves

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Introduction

Large home ranges, low densities, and human–wildlife conflicts makes large carnivore (LC) research a complex mixture of socio-ecological disciplines (Carter and Linnell 2016). The continued growth in the global human population, estimated to be between 9.4 and 10.0 billion people by 2050 (United Nations 2022), is creating an unprecedented pressure on biodiversity in general, and on LC in particular as predators and humans come into increasingly frequent contact (Johnson et al. 2023). Beside the historical threats of human persecution, habitat destruction, or depletion of prey (Ripple et al. 2014), the need for transportation routes and energy facilities poses additional concerns to wildlife, especially if built within their ecological strongholds (Ceia-Hasse et al. 2017, Palmeirim and Gibson 2021).

Human infrastructure impacts on wildlife have been the focus of much attention recently, especially in the context of the effects of linear features like roads, railways, and powerlines (de Jonge et al 2022). Obvious effects such as habitat destruction and increased mortality have been documented (van der Ree et al. 2015). However, the degradation of surrounding habitat by pollution, noise and edge or barrier effects, limiting gene flow and access to food resources has also been observed (Holderegeer and Di Giulio 2010, van der Ree et al. 2015, Skuban et al. 2017). Indeed, the effects of infrastructures can affect wildlife several kilometres from their physical installation (de Jonge et al. 2022) and collectively, the ecological characteristics of large carnivores with the potential environmental impacts of human developments mean that these species can be especially sensitive to new human constructions (Rytwinski and Fahrig 2011).

Being the most widely distributed of all large carnivores, probably no other animal has captivated humankind as much as the grey wolf *Canis lupus*. Wolves can live almost anywhere in the Northern Hemisphere, but just as in many other regions, the Iberian wolf subspecies *Canis lupus signatus* has historically declined in range and numbers to reach a minimum distribution area during the second half of the 20th century (Clavero et al. 2023), which corresponded to only 20% of its previous range in Portugal (Petrucci-Fonseca 1990). In response to this decline, the Portuguese authorities approved the 1998 Wolf Protection Law (Lei no. 90/88), classifying wolves as a fully protected species, outlawing the killing and capture of individuals, the destruction of favorable habitat, and disturbance of denning areas. The law also provided a legislative program for compensation for wolf-related livestock predation – the main source of human–wolf conflict in Portugal (Torres and Fonseca 2016). This national law, along with the Bern Convention (Council of Europe 1979) and Habitats Directive (Council Directive 92/43/EEC 1992) was the catalyst for contemporary wolf conservation in Portugal. Since then, there have been three wolf censuses nationwide: 1996–1997 (ICN 1997), 2002–2003 (Pimenta et al. 2005), and 2019–2021 (Pimenta et al. 2023), revealing an apparently stable population of approximately 300 individuals and 55–60 packs over an area of approximately 20 000 km^2.

Environmental impact assessment (EIA) is a decision tool for identifying and evaluating the probable environmental consequences of new proposed developments (Cashmore 2004). Ultimately, EIAs aim to predict environmental impacts at an early stage in project planning and design, find ways and means to reduce adverse impacts, shape projects to benefit the local environment, and present the predictions and options to decision makers (CBD 2010). In the European Union (EU), the EIA Directive (Directive 2011/92/EU 2011) states that all major development projects (e.g. nuclear power stations, long distance railways, motorways) are legally obliged to conduct an EIA before approval. In Portugal over the last three decades, there has been significant investment in new infrastructures such as highways, hydropower plants, or windfarms. In particular, the eolic industry has seen a remarkable growth, from less than 200 MW and 229 wind turbines in 2000 to more than 5500 MW and 2704 wind turbines installed in 2022 (e2p 2022). The highway network has increased from 579 km in 1993 to 3115 km in 2022 (Pordata 2023). As an EU Member State, Portugal had an obligation to ensure that appropriate EIAs were conducted prior to these developments, and from early 1990s to early 2000s a general improvement was seen in the quality of EIA produced, largely due to the increasing regulation by environmental authorities (Pinho et al. 2007).

Driven by the reliable wind and orographic constraints, the sites selected for wind energy development overlap extensively with the Portuguese wolf range, with more than 990 wind turbines installed within wolf habitat by the end of 2015 and overlapping with the territories of 22 (one-third) known wolf packs (Ferrão da Costa et al. 2018). Additionally, around the same period, more than 750 km of new or improved highways and, at least, five new major hydropower plants have been built within the wolf distribution area. The legal status of wolves, the increasing legislation related to EIA, and the proposal of locations for new infrastructure developments has led to many EIA-related monitoring programs since 2000 (Fig. 1).

Obtaining robust estimates of, and trends in, abundance and distribution is critical for quantifying environmental impacts and informing conservation and management decisions (Santos et al. 2018). The main challenge for such monitoring is collecting data at representative spatial and temporal scales that allow inferences to be made about ecological responses to change (Christie et al. 2019). While the notion that data collection methods and associated analysis should be explicitly linked and dictated by the desired inference objectives, this is often overlooked, leading to inefficient and ineffective use of conservation resources. Indeed, monitoring protocols should differ depending on the type of infrastructures under study and the expected impacts. For example, roads can impact wolves through habitat loss, traffic mortality, inaccessibility to required resources, and habitat and population fragmentation (Kohn et al. 2009), whereas windfarms can increase disturbance, reduce breeding site fidelity, and reduce local reproductive rates (Ferrão da Costa et al. 2018).

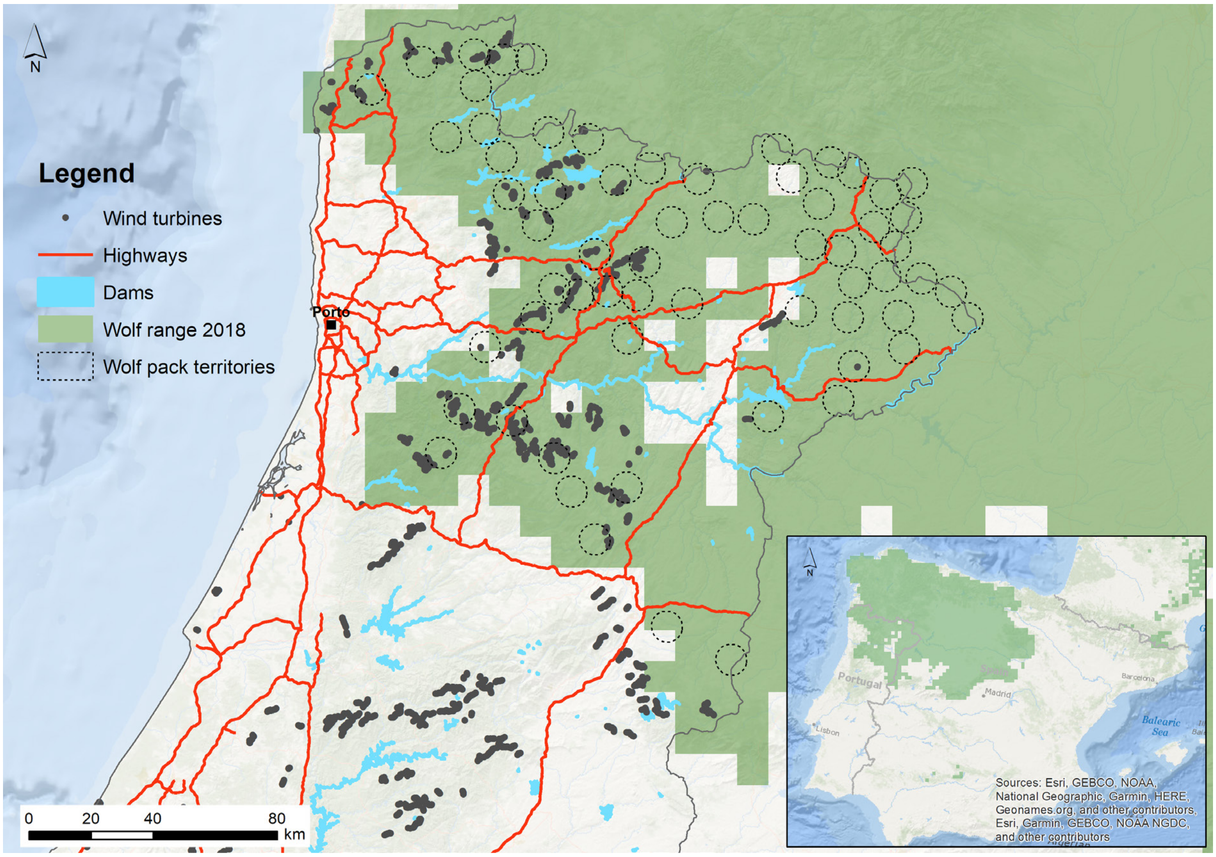

Figure 1. Infrastructures (highways, windfarms, dams) developed in and nearby the current wolf range in Portugal.

In Portugal, the initial motivation for wolf EIA monitoring programs was the generation of general ecological knowledge, with little consistency in survey design and focal parameters for assessing the effects of infrastructure construction and operation. Since there were very few national or international EIA guidelines or recommendations, Portuguese EIAs teams adopted the methodological approach used during the 2002–2003 wolf census (Pimenta et al. 2005), where scat surveys and howling stations were the primary methods used, sampled in a grid area around the infrastructure. In response to an apparent inconsistency in wolf monitoring program (WMP) protocols, the Portuguese Institute for Nature (ICNB then and ICNF now) established in 2010 a set of basic guidelines for wolf EIAs in an attempt to standardize WMPs (ICNB 2010, Table 1). These guidelines identified the major impacts that should be the focus of impact assessments, depending on the infrastructure under analysis: 1) exclusion effects; 2) barrier effects; 3) road mortality; and 4) changes in reproductive patterns (ICNB 2010). Other recommendations included the monitoring period for a before–after / control–impact (BACI) approach: one full year before construction, the construction phase, and at least five years post construction; the minimum spatial extent for sampling; and the basic field methods and subsequent analysis (Table 1).

In the 20 years since the first Portuguese wolf EIA (Ferrão da Costa et al. 2004), there has been significant advances in the technology related to all aspects of ecological monitoring, including electronics, non-invasive genetic procedures, and associated statistical methods. Considering these developments, we identified the need to review how previous and present monitoring efforts are meeting their objectives and align with existing guidelines. In doing so, we suggest updates to the current guidelines in light of recent monitoring and analytical developments, and draw attention to gaps that should be addressed to ensure that WMP can achieve their stated goals and contribute effectively to wolf conservation in Portugal and beyond. Specifically, we focus our analysis on try to answer four major questions:

1. Do wolf monitoring programs examine the adequate biological parameters to reach monitoring objectives and assess the expected impacts?
2. Is the study design suitable for measuring the expected impacts?
3. Are data collection methods and sampling effort collected in line with the intended analysis?
4. Do statistical analyses of the data lead to robust conclusions?

Material and methods

We reviewed all major wolf monitoring programs developed for environmental impact assessments in Portugal since 2002

Table 1. Summary of the Portuguese guidelines for wolf monitoring programs in Environmental Impact Assessment projects.

Infrastructure type	Potential effect	Parameters to monitor	Data collection methods	Grid for sampling site selection	No. of sampling sites	Quantification	Sampling frequency
All types	Exclusion effect	Wolf **presence** (and/or its wild prey, whenever justifiable) Wolf **frequency of occurrence** (and/or its wild prey, whenever justifiable)	Scat surveys. Camera trapping	Grid cells from 2 × 2 to 5 × 5 km, depending on infrastructure dimension	2 fixed sampling stations per grid cell (scat survey transects or camera stations)	No. of transects with wolf validated scats. No. of camera stations with wolf photos No. of wolf validated scats/km/month No. of wolf photos/month corresponding to different events (e.g. photos taken with a 12 h apart interval)	Each transect should be surveyed monthly For each camera station, the survey period should not be fewer than 12 days/month
All types	Changes in reproductive patterns	**Occurrence of reproduction** in the detected wolf packs **Breeding** site **location**	Camera trapping. Howling stations. Observation points	–	Mobile sampling stations per wolf pack (~ 100 km^2) (camera stations, howling stations, or observation points)	No. of wolf packs with confirmed breeding determined by: photos of cubs or wolves with evidence of lactation; howling of pups; direct observation of cubs or wolves with signs of lactation, alive or dead	No. of cameras, howling and observation stations should be as required to confirm reproduction or to conclude that information regarding this parameter is unlikely to be obtained
Roads Railways	Run over mortality	Occurrence of run over **mortality**	Route surveys	–	Whole route	No. of wolves and individuals of their wild prey killed per km every 15 days	Every 15 days
Roads Railways Big dams	Barrier effect	**Crossing rate**	Camera trapping	–	All passages that in the project phase were admitted being able to be used by wolf (at least one passage each 3 km)	No. of crossings / km / day	At least two cameras permanently per passage (one at each entrance)
		Degree of use of the passages which, at the planning stage, were considered likely to be used by wolves	Camera trapping	–	All passages that in the project phase were admitted as being able to be used by wolf (at least 1 passage each 3 km)	No. of crossings / passage / day	At least two cameras permanently per passage (one at each entrance)
		Evolution of **gene flow** between population clusters on either side of the infrastructure	Non-invasive sample collection (hair, scats) Genetic analysis	–	Whole study area. The sample must be representative of the existing population in the study area	–	–

(Supporting information). Given that the focus here is on the adequacy of targeted wolf monitoring for delivering conclusions about the effects of infrastructure development, we reviewed only monitoring programs that were specifically designed for wolves and not those concerned with general mammalian assessment.

The starting point was a compilation from the 2019–2021 National Wolf Census (Pimenta et al. 2023), where every wolf monitoring program that occurred between 2014 and 2019 in Portugal was identified. The list was completed with projects that started before 2014 or after 2019 based on personal knowledge and enquiries to principal scientific teams, governmental agencies, and EIA consultants. Depending on duration, wolf monitoring programs can produce several, usually annual, reports that are not peer-reviewed and do not appear on standard search engines (e.g. Web of Science or Google Schoolar) but are publicly available from the Portuguese Environmental Agency (APA – www.apambiente.pt). We conducted an online search on APA's search engine (https://siaia.apambiente.pt/) and identified a total of 30 projects. For each of these projects, we were interested in the first and the last report to identify any methodological changes. If the last report was not present, we reviewed the most recent one. If no report was present, we requested it from the team responsible.

Our investigation centered on characterizing and quantifying four components of wolf monitoring programs that are interlinked and that should ideally be determined by the initial objectives: 1) biological parameters, i.e. what wolf parameters were studied to assess impacts; 2) study design, i.e. what sampling schemes were followed to collect and analyse data; 3) data collection, i.e. which sampling methodology and how much effort was used to collect data; and 4) data analysis, i.e. how data were analysed to estimate relevant parameters and assess impact.

Biological parameters were identified and classified under two categories: occurrence and demography, which broadly correspond to the necessary inputs to assess impacts like exclusion effect and changes in reproductive patterns. Occurrence-related parameters refer to variables used to measure the presence or absence of wolves, whereas demographic parameters refer to variables that intend to measure population-level effects such as abundance, density, survival, or reproduction. We also recorded whether any effort was made to quantify prey population distribution or abundance as recommended in the guidelines.

For study design, we reviewed the sampling design of each project, with specific focus on the spatial and temporal aspect of the study, such as the total area surveyed, the definition of a sampling site within this region (i.e. resolution), the duration of the study, and the number of sampling seasons. The goal here was to determine whether the sampling scheme used was appropriate for assessing infrastructure impacts on wolf distribution or demography, depending on what the focus was.

For data collection, we identified the main methodologies used and the corresponding sampling effort. By far the most frequent method used was sign surveys, and specifically scat surveys, and for these studies we recorded whether genetic identification of species or individuals based on faecal DNA was attempted. We compared how sampling effort varies by the various inference objectives and, as above, assessed which, if any, project or data collection approach is most likely to produce evidence of impact.

We divided the data analysis component into two groups: single-year and multi-year analyses. For single-year analysis we identified how monitoring projects used data to make inferences about the stated biological parameters of interest and discuss the associated strengths and weaknesses. For multi-year analyses, we recorded how differences or trends were quantified and associated with infrastructure impacts, commenting on the statistical robustness of the analyses used across the projects.

Results

We identified 30 wolf monitoring EIA programs conducted between 2002 and 2022 (Supporting information). The majority were established to assess the impact of windfarm development (n = 20) and the remaining were related to roads (n = 3), hydroelectric projects (n = 3), mines (n = 2), and powerlines (n = 2).

Biological parameters

The biological variables of interest varied across the projects (Fig. 2A). All projects intended to estimate wolf distribution as a parameter of interest. Use of space was investigated in 90% (n = 27) of the programs and wolf activity center's location by 67% (n = 20). In terms of demography, 90% (n = 27) of the projects intended to monitor the number of wolf packs present and their breeding success in the study area, and 43% (n = 13) proposed estimating the absolute number of wolves present or their density. Also 43% (n = 13) of the projects studied wolf prey parameters – presence and use of space of wild boar *Sus scrofa* and roe deer *Capreolus capreolus* – as a complement for infrastructure impacts on the wolf (the impact on its prey). While not strictly a biological parameter, all road monitoring programs (n = 3) proposed estimating whether roads were a barrier to wolf movement.

Study design

As per the guidelines, the majority of assessments followed a BACI design (93%, n = 28), where the impact zone of the infrastructure was compared to a control comprising the surrounding area, starting before the construction and following the development up to the first years of operation. Overall, few projects followed the proposed guidelines of a one-year reference situation followed by monitoring through construction phase and at least five years of operation (33%, n = 10). The typical (median) monitoring duration was five years and ranged from a single year to 16 years (and ongoing, Fig. 2B). The size of the areas studied ranged from 5.68 to 1648 km^2,

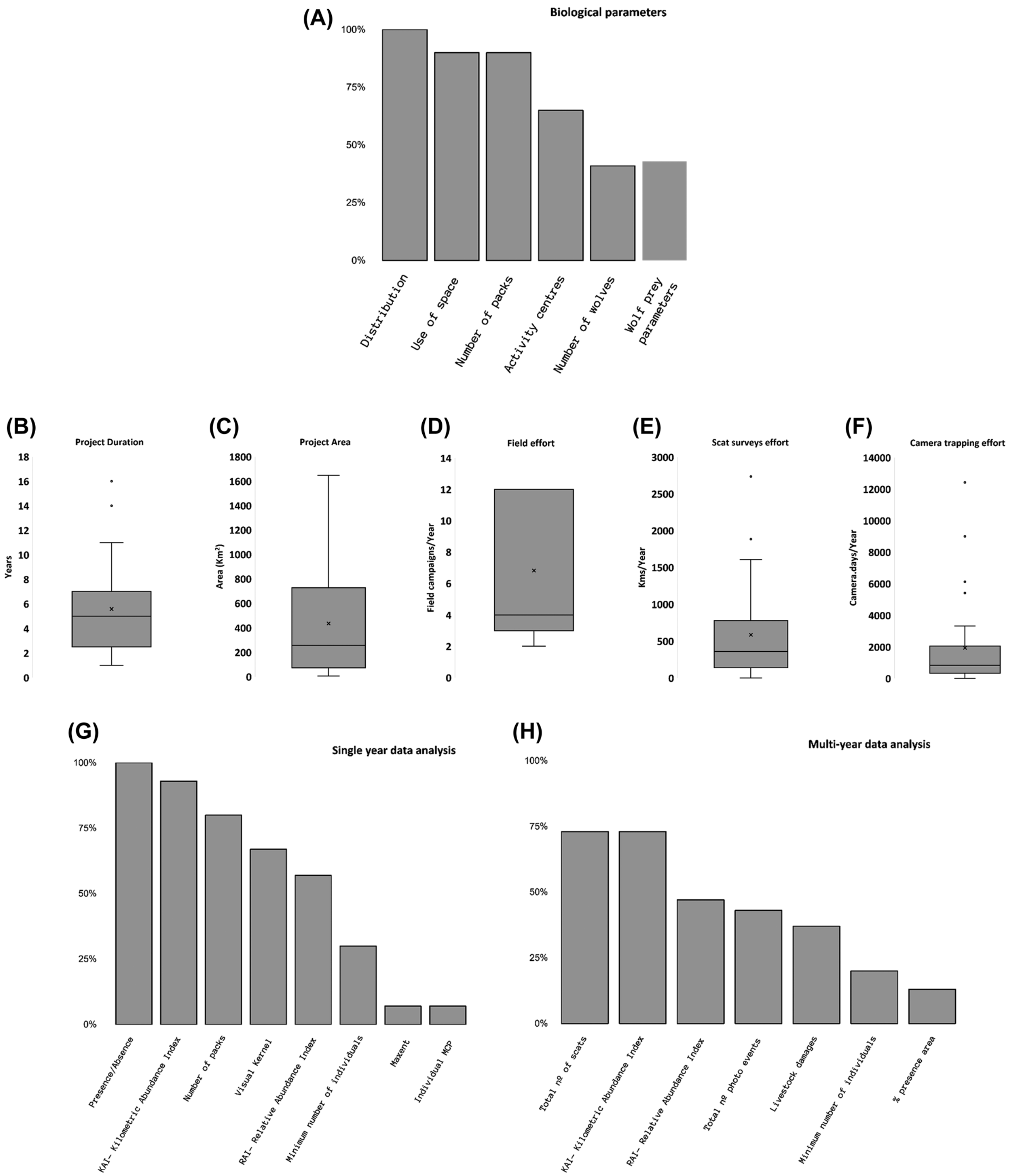

Figure 2. Descriptive statistics for: (A) biological parameters, (B) project duration, (C) project area, (D) field effort, (E) scat surveys effort, (F) camera trapping effort, (G) data analysis (single year), (H) data analysis (multi-year). In box-plots (B, C, D, E, F) mean values are represented with an (x) and outliers with a (·).

with an average of 438 km^2 (Fig. 2C). Ten of the 30 projects were considered to be 'regional scale' monitoring (i.e. more than 500 km^2, with several wolf packs territories and/or various infrastructures) and the remaining twenty were considered local projects focusing on a single infrastructure, with study areas smaller than 500 km^2, overlapping a single or a few wolf pack territories.

Data collection

The methods used to collect field data were broadly in line with the existing Portuguese guidelines. Of the methods used, scat surveys along line transects were the primary method for presence–absence surveys and were used in 97% (n = 29) of the projects. From these, 67% (n = 20) used genetic species verification of the scats with the remaining 31% (n = 9) relying solely on visual determination based on the size, prey content, or placement of the scat. From the projects with genetic validation, 45% (n = 9) attempted individual genotyping from scats, with the remaining 55% (n = 11) assessing solely species identity to distinguish between wolf, dog, and fox. Scat surveys were the only method used in 17% (n = 5) of the programs and, as expected, there was a strong positive relationship between project area and the scat survey effort (measured in km surveyed, Fig. 3A).

Camera trapping was used in 83% (n = 25) of the projects but there was not a significant relationship between project area and the number of cameras deployed (Fig. 3B), suggesting some non-standardisation of camera trapping methodology. Only one project used camera trapping as the sole field methodology, where the rest coupled cameras with scat surveys, and used them to assess wolf presence in specific locations (e.g. road passages, rendezvous sites) or deployed them near denning area to confirm reproduction.

Howling stations (human-simulated howling) were the prevalent method for evaluating wolf reproduction and breeding site location (80%, n = 24). The mean number of howling stations per year and per project was 15 (range: 0–150), having a mild relationship with the size of the study area (mean = 5.3 howling stations per 100 km^2, range: 0–52.8), or with pack presence (mean = 4.3 howling stations per pack, range: 0–31.1). The majority of projects had fewer than 20 attempts per year and only in a few instances reached above 100 sites/stations (Fig. 3C). Even if not required by the guidelines, 10% (n = 3) of the projects used telemetry as an auxiliary methodology for generating wolf data for impact assessment.

In terms of overall effort, the minimum number of field sessions reported was two per year and the maximum was 12 per year (i.e. monthly surveys, Fig. 2D). The median was four sessions per year (seasonal campaigns), but close to half of the projects (n = 16) conducted monthly surveys, which can result in around 60 surveys for an average project duration (five years) and more than 100 with 10 or more years. The median length of trails surveyed for scats by project each year was 586 km (range: 0–2736 km, Fig. 2E) and the mean camera trapping effort by project per year (number of camera days) was 1938 (range: 0–12 420, Fig. 2F).

Data analysis

Regarding single season data analysis (Fig. 2G), wolf distribution was assessed by all projects using one of several presence/absence analyses, where the study area grid (e.g. 10 × 10, 5 × 5, 4 × 4, or 2 × 2 km) or the survey points/transects were used as sites. If a sign was found (scat, picture, direct observation, etc.), presence was confirmed, and absence assumed otherwise. Some projects also gave an intermediate classification (probable presence or possible presence) if second-hand information was obtained for that area, for example a third-party sighting, reported damage to livestock, etc.

To infer use of space by wolves, 93% (n = 28) of the projects relied on a kilometric abundance index (KAI), which is a relative indicator of 'utilization', and it is represented by the number of scats found divided by the total length of transects surveyed (Martin-Garcia et al. 2022). KAI values were calculated for each study area grid cell or for a gradient of distances from the impact source, giving a relative indication of areas with more or less wolf use. More than half of the projects (57%, n = 17) also analysed use of space based on a relative abundance index from camera trapping. This index represents the number of independent wolf photo events divided by the total number of active camera days, where the time between events assumed for independence ranged from 60 s to 60 min. Use of space was also analysed with the aid of 'visual' kernels in 67% (n = 20) of the WMP (Quinn and Keough 2002). Kernel analysis uses the point locations of scats found to create a probability density surface, and the visual outputs help to better distinguish wolf high use areas than a 5 × 5 km or a 2 × 2 km grid.

The basic demographic parameter investigated was the minimum number of packs present. This was calculated in 86% (n = 26) of the programs using howling stations success, observations of two or more wolves together in camera trapping, or direct sightings. The minimum number of individuals present was assessed in 30% (n = 9) of the projects and conducted in three ways: 1) counting the total number of individual genetic profiles found in scats; 2) counting the maximum number of individuals present in a camera trapping event; and 3) counting the minimum number of wolves present in a wolf chorus from a howling station.

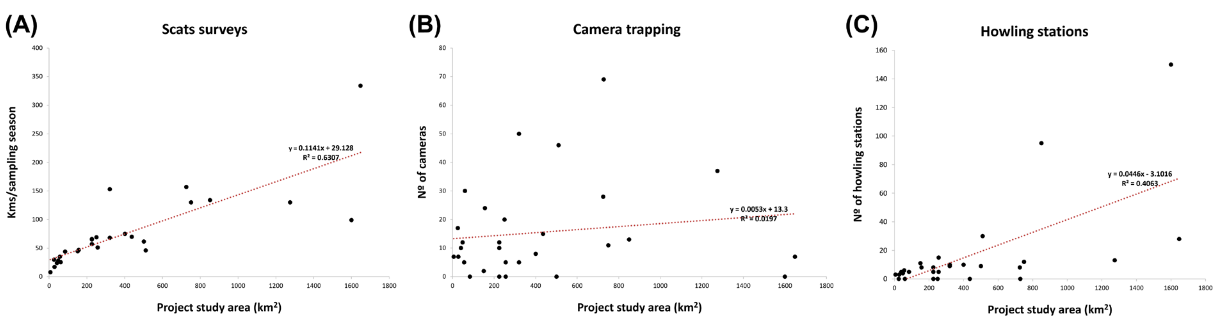

Figure 3. Relationship between field methods effort and study area dimensions. (A) Scat surveys, (B) camera trapping, (C) howling stations.

Other analyses that were used less frequently included the creation of a probability of occurrence map using maximum entropy (maxent; Phillips et al. 2006, 7%, n = 2) and the use of a minimum convex polygon (MCP: Worton 1987) from genetic profiles to access individual movements in the study area (7%, n = 2).

To compare wolf parameters through time, most projects used trends in either raw observations (90%, n = 27) or relative indices (87%, n = 26, Fig. 2H). A majority (73%, n = 22) used the total number of scats found each year as a proxy for wolf presence and distribution over time, ignoring variation in effort. This was done by comparing the total or average number of scats found, over time, between impact and control areas or within a distance from impact source (Fig. 4A). Note also that 73% (n = 22) of the projects used KAI (number of scats km^{-1}) which does account for effort, but not detectability (Fig. 4B). In some projects, KAI was calculated for each study area grid cell to infer spatiotemporal variation in wolf use of space. A similar approach was used for camera trapping data. The total number of wolf photo events was compared over time between impact and control areas in 43% (n = 13) of the total projects, whereas 47% (n = 14) used a relative abundance index (number of photo events/camera active days).

In projects initiated before 2010 (37%, n = 11), the number of livestock predation events by wolves was used as proxy of wolf presence and use of space – since livestock may represent up to 80% of the wolf diet in many Portuguese areas (Torres et al. 2015) – and was compared over time in the surrounding areas of the studied infrastructure. Other, less frequently, used methods for inferring trends included changes in the minimum number of individuals present in the study area over time (20% of the projects, n = 6) or changes in the proportion of area with confirmed wolf presence (13%, n = 4).

Despite the attempt to compare different parameters over time, only 60% (n = 18) of the projects used formal statistical analysis to assess the significance of these differences, typically using group comparison tests to support conclusions (e.g. Mann–Whitney, Kruskal–Wallis, Tukey or *t*-test). The remaining 40% (n = 12) drew (statistically unsupported) conclusions from patterns from the raw data visualization, making conclusive statements about effects or impacts difficult.

Discussion

Wolf monitoring programs created to conduct EIA largely focus on impacts like exclusion effect, barrier effect, or reproductive changes. Choosing the correct biological parameters to study is essential to produce the correct final assessments. Our review showed that emphasis is given more to occurrence parameters like distribution and use of space, but less to demographic parameters like abundance or density. Infrastructure impacts are felt in the short term at the individual level but may have mid-term implications at the populational level (Cook and Robison 2017), and hence monitoring both occurrence and demographic parameters will ensure a more robust assessment. However, a correct choice of biological parameters does not guarantee meaningful outcomes unless the whole process is properly linked in terms of study design, data collection methods, and subsequent analysis.

Our review showed that, overall, programs followed the Portuguese guidelines in terms of study design. The recommended BACI design uses a control and an impact area, and samples before and after the impact occurs. Its rationale is to explicitly account for pre-existing differences between the impact area (exposed to the impact) and the control area in the 'before' period, which might otherwise bias the estimate of the impact's true effect (Stewart-Oaten and Bence 2001). Applying a BACI design to a social, territorial, and low-density predator like the wolf poses several challenges, namely defining the control areas. The Portuguese guidelines

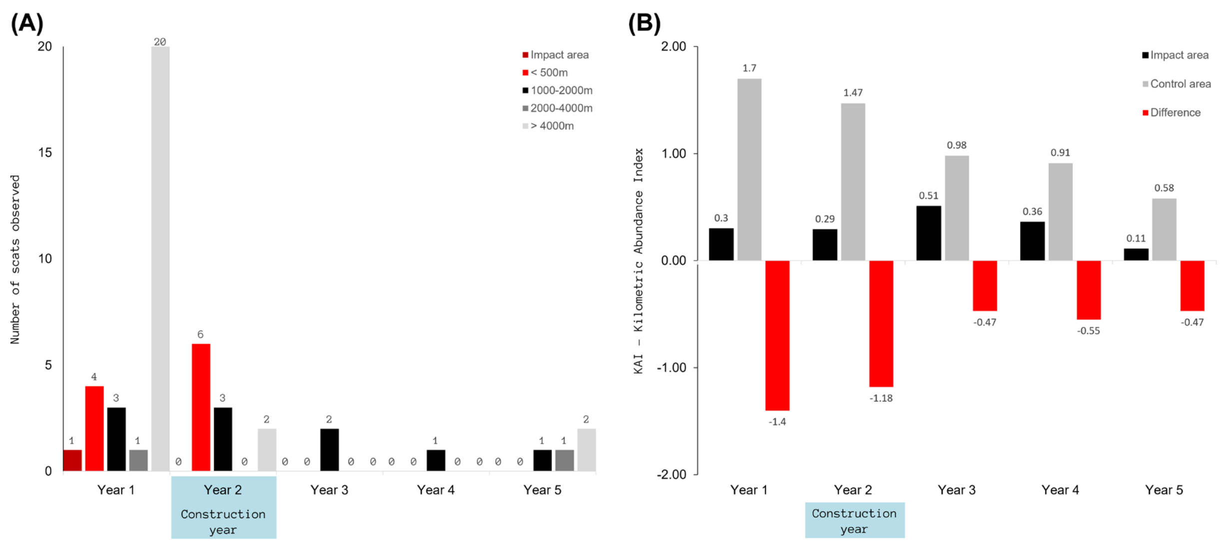

Figure 4. Examples of traditional graphs used in Portuguese wolf EIA reports: multi-year information graphs using (A) raw scat number found in a distance from impact area or (B) kilometric abundance index (KAI) assessments between impact and control areas.

state that control areas should contain landscape characteristics similar to those of the impact area, where no impacts are expected from the project, but close enough so that animals present will be subject to the same set of ecological constraints (ICNB 2010). Since most of the programs rely on a study area divided into grid cells (2 × 2, 4 × 4, 5 × 5, 10 × 10 km), usually research teams allocate some cells to the impact area and some cells to control areas. Given the size of the cells, we might be comparing areas that are part of the territory of different wolf packs. Since animals from distinct packs, even if contiguous, may have different dynamics (Rio-Maior et al. 2019), comparing areas that might belong to different groups can introduce an ecological bias to the analysis.

A before–after gradient (BAG) design might be a more appropriate scheme to adopt for wolves and other vertebrates. BAG designs require sampling at relative distances from the impact source in both pre- and post-construction periods (Ellis and Schneider 1997). They retain the ability to compare post-construction patterns to baseline conditions and have the potential to distinguish impacts from other dynamic factors operating in and around the project area when these data are also available, while not requiring the definition of a control area (Christie et al. 2020, Methratta 2021). Holding sampling effort constant, BAG has the potential to have greater statistical power compared to the basic BACI design because the variance associated with both spatial and temporal heterogeneity can be included in the explanatory terms of statistical models rather than being relegated to the error term (Methratta 2021).

The methods employed for wolf data collection followed the recommendations from the guidelines, based on non-invasive methodologies like scat surveys and camera trapping. EIA schedules are tighter than regular conservation projects and these methodologies allow population-level data to be collected and analysed in due time. Particularly for scat surveys, the current ability to derive individual-level information from genetics enables the analysis of second- and third-order resource selection by wolves (the distribution of individuals within their range and the use of habitat within individual home range territory, respectively, Royle et al. 2018). This would be the gold standard for environmental impact assessment. Camera trapping, even if it does not easily distinguish between individuals (but see Jiménez et al. 2023), is a method that provides information for long time periods on wolves and sympatric species, and delivers longer-term monitoring with greater spatial coverage and less human effort relative to scat surveys.

There was also a small percentage of projects using telemetry as an auxiliary methodology. Portuguese guidelines state that telemetry should not be used as the primary method for EIA given the risks of death and injury for the animals, the difficulties in having data in due time, the low sample size by project, and the fact that we might capture dispersing animals not representative of local population (ICNB 2010). In our review, all projects using telemetry were linked to parallel ecology/conservation studies occurring in those regions, and always corresponded to study areas larger than 500 km^2 and lasting for more than five years. None of the projects had more than two wolves captured in a single season/year and many of those captured were pups (hence not collared). The typical duration of the telemetry data was 6 to 12 months, depending on batteries, malfunctions, or mortality. Moreover, given this short duration, the comparison of use of space (i.e. before and after construction) is typically achieved by combining data from (only a few) different animals, which may highlight individual heterogeneity more than an actual area selection. With tight EIA schedules, the pre-construction phase rarely lasts more than one year; and, while telemetry offers interesting insights, at the sample sizes typical of a wolf monitoring program, we urge against relying solely on telemetry for impact assessment analysis. Telemetry data have, however, been shown to be of great value when formally integrated with, for example, non-invasive data (Tenan et al. 2017, Linden et al. 2018, Dupont et al. 2022). Ultimately, impact assessment should focus on population-level impacts using data representative of the population, and, if available, high resolution telemetry data can, and should be integrated into these analyses.

The existence of two major seasons for analysis (May–October and November–April) – as it appears in the guidelines – makes sense in terms of wolf ecology (reproduction–pup-rearing period and non-reproductive period), but within each season, the number of field campaigns should be aligned with the inference goal. The guidelines recommend monthly scat surveys, and this advice was followed by only 50% of the projects we reviewed. In fact, how often to sample depends on the biological parameter we are trying to estimate. For example, if the goal is to assess breeding success and location, then winter surveys are not necessary, but if density estimation is the goal, then monthly or bi-monthly surveys are more appropriate, since more data are needed. Also, it is worth noting that for camera trapping, a minimum duration of 12 days is recommended by the guidelines, which is definitely too short for a wide-ranging, low-density carnivore – a minimum of 60 days is more appropriate for large predators (Loonam et al. 2021, Ausband et al. 2022).

Overall, wolf EIA monitoring programs have helped in sampling more than 5000 km^2 annually since 2006, representing around 25% of the wolf range in Portugal (20 400 km^2 – Pimenta et al. 2005, Torres and Fonseca 2016, Fig. 5). This effort has significantly increased the information available about wolf presence and distribution in Portugal, which have often served as surrogates for an institutional census. Despite all this effort, we have identified a range of inconsistencies between stated objectives and the conclusions drawn among monitoring projects. This is evident, especially when we reviewed the data analysis component of these plans. Every single project reviewed relied on naïve analyses or summary statistics to infer most of the focal wolf parameters and trend assessments. The direct use of detection/non-detection data for evaluating wolf distribution, or the utilization of kilometric abundance indexes to estimate use of space or infer population size, have several major shortcomings, including the assumption of a perfect detection in the observational process

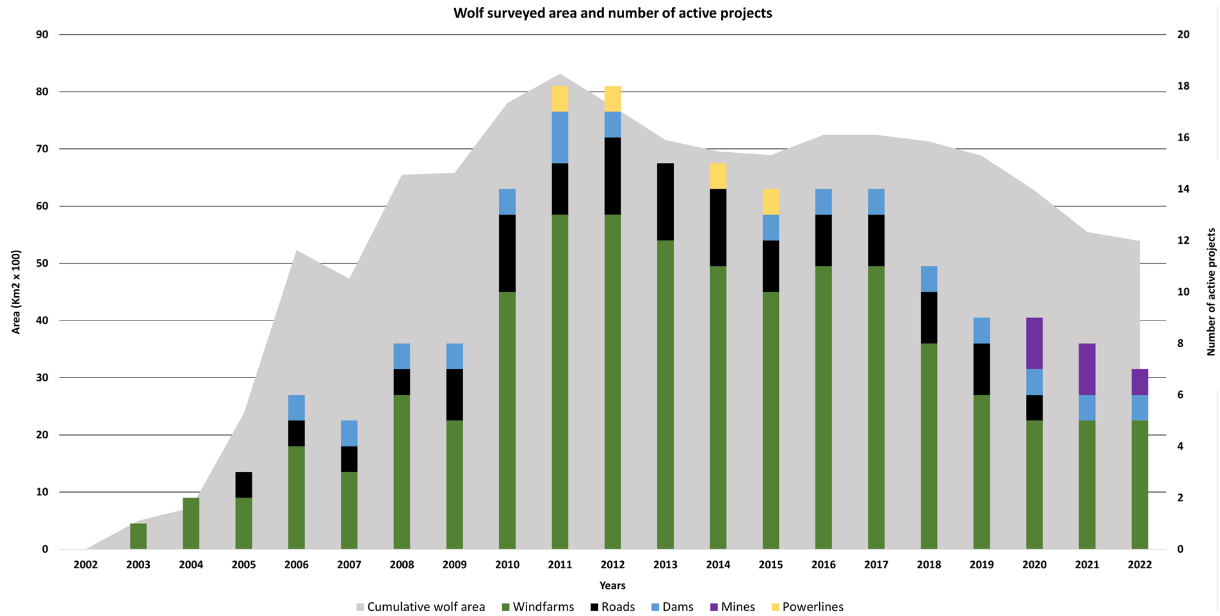

Figure 5. The cumulative wolf range surveyed yearly because of environmental impact assessment monitoring plans. The grey area corresponds to the overall area surveyed and the colored stacks correspond to active projects responsible for it (windfarms, roads, dams, mines, and powerlines) in the corresponding year.

to be reliable (Martin-Garcia et al. 2022). The collection of ecological data, especially in the field, suffers from many constraints, including species behavior, weather conditions, habitat coverage, or observer experience (Guillera-Arroita 2017). Being a social species, wolves use scats as scent marking for territorial defence (Mech and Boitani 2003), have preferred locations for depositing it (Barja et al. 2004), and may vary locations seasonally (Roda et al. 2022). This means that, even if present, wolves do not leave scats everywhere they pass, and using them directly to infer presence or use of space has limitations. Also, for example, sampling in a muddy substrate after the rain reduces scat detectability; detection in forest trails is lower than on open mountains trails; and sampling conducted by new volunteers may have different outcomes compared to an experienced professional. Indeed, there is a vast theoretical and empirical literature demonstrating that imperfect detection, and ignoring it, leads to biased inference about species distributions and overstated precision about estimated parameters (Guillera-Arroita 2017).

The issue of using uncorrected observations for inferences to inform conservation or to base impact assessments on has important implications. None of the methods used in the projects we reviewed made any attempt to account for imperfect detection. In essence, this assumes that variation in the data collected, that may be attributed to a wide range of factors that affect effort and survey efficacy, is interpreted as biological variation in wolf populations. Also, the lack of formal statistical analysis for making comparisons means that confidence in whether differences are biologically or statistically important is weak and not supported by the numbers. Indeed, this was the case for around 40% of the projects reviewed.

Conservation budgets are limited and EIA budgets even more so, thus it is critical that the enormous efforts and resources already allocated to wolf monitoring are effective, meet objectives, and can properly inform decision making. This is vital for correctly measuring impacts and effectively implement mitigation and offset measures. Our review of the current monitoring efforts has identified several areas where there is scope for improvement, particularly in the analytic procedures used by WMP, which will also have implications for study design and data collection (Fig. 6).

Monitoring strategies should be set (in this order) by the identification of the expected impact to assess, followed by the definition of what biological parameters to study and the data analysis needed to estimate those parameters. Only then we can build a study design and sampling strategy that will allow us to get the necessary data for the analysis, never the other way around. In this sense, we suggest that wolf monitoring programs should better align with a 'why? what? how?' process and argue, based on our review, that currently not enough time and thought is dedicated to these fundamental questions (MacKenzie et al. 2018). The 'why' we are doing wolf monitoring programs in EIA projects often lacks clarity and purpose, both from the teams and the authorities. The straightforward answer should be to help decision makers evaluate if a development project is expected to have a significant impact on local/regional wolf populations, create mitigation/compensation schemes, evaluate, learn from it, and improve future decisions. Many times, WMP are much less than this because of failures in giving robust assessments, and programs stick with updating wolf presence data over time. On the other hand, WMP could be much more effective if their design and conception led to an integrated workflow of

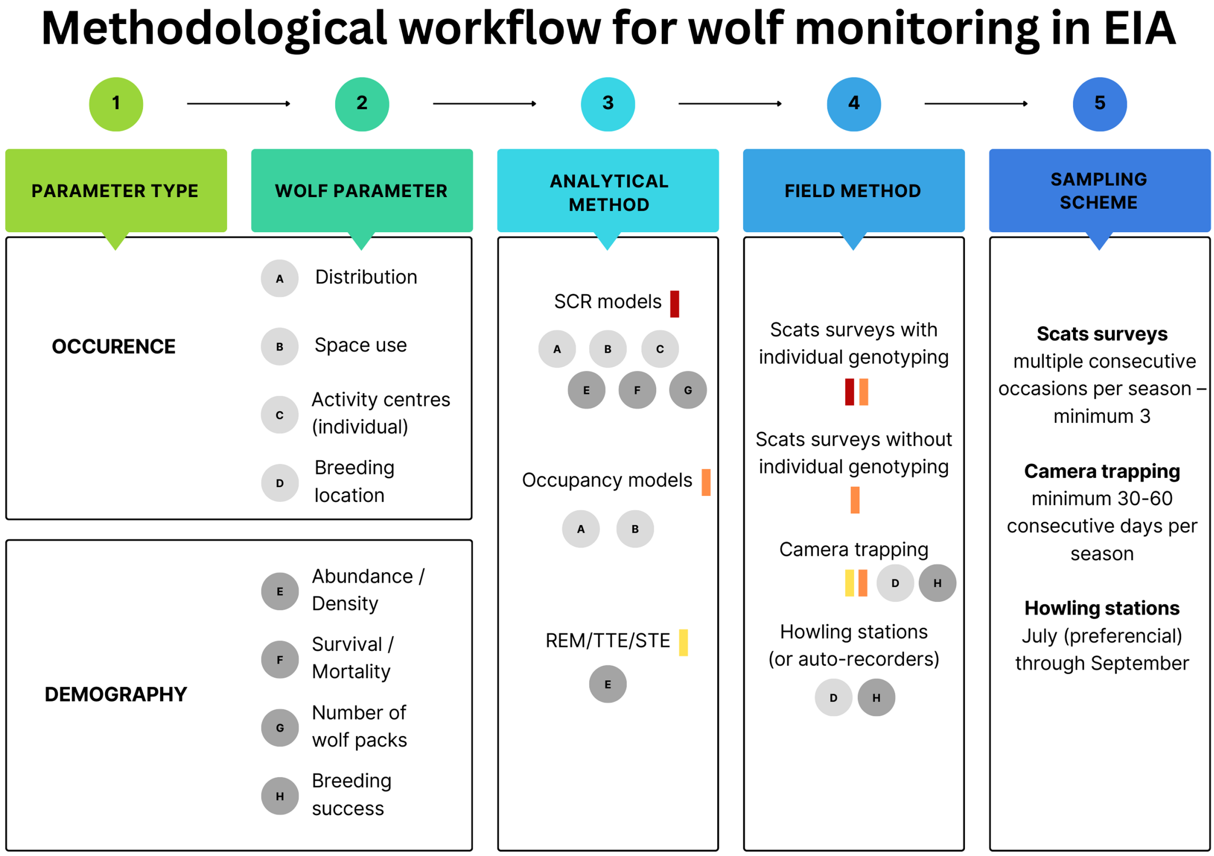

Figure 6. Proposed road map to use analytical methods that account for imperfect detection in wolf monitoring programs. Wolf parameters are represented by circles with letters, and appear under which analytical or field method will enable their estimation. Analytical methods are represented by colored bars (red, orange, yellow) and appear under which field methods allow their use. The minimum effective sampling effort for each field or analytical method is also presented. SCR – spatial capture–recapture; REM – random encounter model; TTE – time-to-event model; STE – space-to event model; EIA – environmental impact assessment.

robust monitoring and meaningful offset measures to ensure wolf long-term regional viability. But for mere current standard purposes, the 'why' can be related to the type of impacts we want to measure and, according to the Portuguese guidelines, it should be themes like exclusion effect, barrier effect, or changes in reproductive patterns.

So, 'what' to measure in order to assess the 'whys'? This is where the choice of the correct wolf biological parameters to measure impacts is essential. We can group wolf biological parameters in two groups: occurrence parameters (relating to spatial distribution) and demographic parameters (relating to abundance, survival, reproduction). One may choose one or several of these parameters to study wolf dynamics over time, depending on objectives, budget, logistics, and experience. Our suggestion is that, at least one parameter of occurrence and one of demography should be selected, since individual-level effects are best shown by occurrence, but population-level impacts are felt in demography. It is important that whatever the choice is, it will be clearly stated from the beginning and subsequent actions will be aligned with initial choices. Both group parameters can be used to answer initial questions and assess impacts, but the choice will have implications on study design, data collection, and data analysis: that is, the 'how?'.

For 'how?' to measure or estimate the biological parameters of interest over time we need to pay attention to two critical aspects of sampling animal populations: spatial variation and detectability (MacKenzie et al. 2018). Especially for species with large home ranges, complete censuses are not possible and therefore sample protocols should be representative of the population or area of interest. These are dictated by study objectives and selected to provide the best opportunity to discriminate between competing hypotheses and in a manner to allow inferences about unsurveyed locations (MacKenzie et al. 2018). Detectability refers to the fact that even in locations that are effectively sampled, signs, individuals, or entire species can be missed by surveys when present. Without information on the detection process, observations only reflect the combined effect of abundance/occupancy and detection (Guillera-Arroita 2017), which is mostly the case in Portugal.

The development of hierarchical models has fundamentally changed the landscape of ecological monitoring and analysis (Royle and Dorazio 2008) and we advocate for its use in wolf EIA monitoring. This family of methods jointly considers two processes: a state process representing the underlying ecological process of interest (e.g. distribution, abundance) and an observational process describing how the data were collected (Royle and Dorazio 2008). One example is capture–recapture that has, for many years, been the cornerstone of ecological statistics applied to population biology (Royle et al. 2018). It makes use of 'individual encounter history' data, obtained by capturing or encountering individuals (e.g. using camera traps, acoustic sampling, non-invasive genetic sampling, or direct physical capture), marking them, and observing them over time (Royle et al. 2018). However, standard capture–recapture methods overlooked an important aspect of population ecology: the spatial structure of populations and of the sampling.

Spatial capture–recapture (SCR) methods were developed to overcome some of these limitations, coupling a spatio-temporal point process, that reflects the latent activity centres of individuals, with a spatially explicit observation model, allowing for the inclusion of information about the spatial arrangement of the sampling devices and spatially referenced encounter histories (Efford 2004, Royle et al. 2014, 2018). SCR has proven to be a flexible framework for studying spatial processes such as individual movement, resource selection, space usage, landscape connectivity, population dynamics, spatial distribution, density, and inter- and intra-specific interactions (Royle et al. 2018). In the scope of environmental impact assessment on wolves, SCR methods can be very useful but have been largely overlooked (López-Bao et al. 2018). Specifically, SCR can be used to test hypotheses about density and therefore lend themselves to comparative investigations of infrastructure impacts. Likewise, SCR provides a formal framework for quantifying third- order resource selection – selection within an individual's home range – that can be modelled as a function of landscape characteristics in the vicinity of sampling locations as covariates that affect the probability of encounter (the observational model). For instance, using 'distance to infrastructure' as a spatial covariate, it is possible to test explicitly for the exclusion effect by quantifying how density changes over the gradient (Fig. 7). Moreover, the observational process model allows detectability to be modelled as a function of the variable hypothesised to influence detection (e.g. weather, observer, habitat). With sampling locations stratified throughout the study area, at different distances from impact source, SCR models would fit nicely within a BAG design and could even be used to test for cumulative impacts from other sources of stress (anthropogenic or not) that may appear in the region during the study timeframe.

In general, SCR requires spatially explicit encounter histories of known individuals (Efford 2004, Royle et al. 2014, Sutherland et al. 2016). For wolves, which do not have distinctive marks, data from camera trapping do not lend themselves naturally to SCR (but see Jiménez et al. 2023). Non-invasive genetic sampling with individual genotyping (mainly from scats), on the other hand, does. We found that approximately 69% of the WMP in Portugal used genetic sampling from scats, and therefore collected the information required for SCR. However, of these, only 45% used scat samples to go beyond species verification to identify individuals by genotyping. If the number of projects doing individual genotyping from wolf scats increased, the process of obtaining density estimates would be standardized resulting in consistent, unbiased, and comparable values for detection and density (Fig. 7).

SCR models are not the only framework that accounts for imperfect detection and that can be used in WMPs under EIA processes. Occupancy models (MacKenzie et al. 2018) use the same hierarchical approach to draw inferences about occupancy – the proportion of area, patches, or sampling units that is occupied by the species, while accounting for imperfect detection (MacKenzie et al. 2018). Occupancy models do not need information on individuals and can accommodate data from different sources (signs, camera trapping, direct observations, etc.). These models are better for estimating wolf occurrence and habitat associations than demographic rates as well as how detectability is influenced by environmental covariates.

In recent years, statistical methods to estimate abundance and density of 'unmarked' animals using only camera trapping have also been developed and tested (Gilbert et al. 2021). They include site-structured models (Kéry and Royle 2015), unmarked spatial capture–recapture models (Chandler and Royle 2013), random encounter models (REM – Rowcliffe et al. 2008), time-to-event models (TTE – Moeller et al. 2018), space-to-event models (STE – Moeller et al. 2018), instantaneous sampling (IS – Moeller et al. 2018), or distance sampling (Howe et al. 2017). While there is some scepticism on the current utility of these methods to provide precise and accurate estimates of abundance (Amburgey et al. 2021), they are being used to estimate the density of low-density and difficult-to-detect carnivores like the cougar (Loonam et al. 2021) or the wolf (Ausband et al. 2022). Even if there are some doubts about the power to generate precise estimates, the use of appropriate statistical methods will generate a more honest representation of uncertainty around the desired variables and observational process, which is extremely important for decision making and management.

Most Portuguese wolf research is conducted by very experienced teams using appropriate methodologies. We have, however, identified that there is a need to pay more attention to survey design and data analysis to make the most of the collected data. Current approaches to estimate spatiotemporal patterns of abundance, density, or occupancy, tend to overlook issues of imperfect detection which is contrary to a wholesale movement towards using hierarchical models in large carnivore monitoring and research more generally (Tourani 2022). Aside from providing rigorous estimates of population density, methods like SCR offer a means of modelling wildlife distribution in space, as well as the drivers of that distribution, of habitat use, and of connectivity (Royle et al. 2018, Tourani 2022). Parameters like density, abundance, use of space, dispersal, survival, recruitment, mortality, and growth rate can be and have been addressed in studies using SCR (Tourani 2022) in species like the wolf (López-Bao et al. 2018, Jiménez et al. 2023, Marucco et al. 2023), the snow leopard (Sharma et al. 2024), the African wild dog (Emmet et al. 2022), or the jaguar

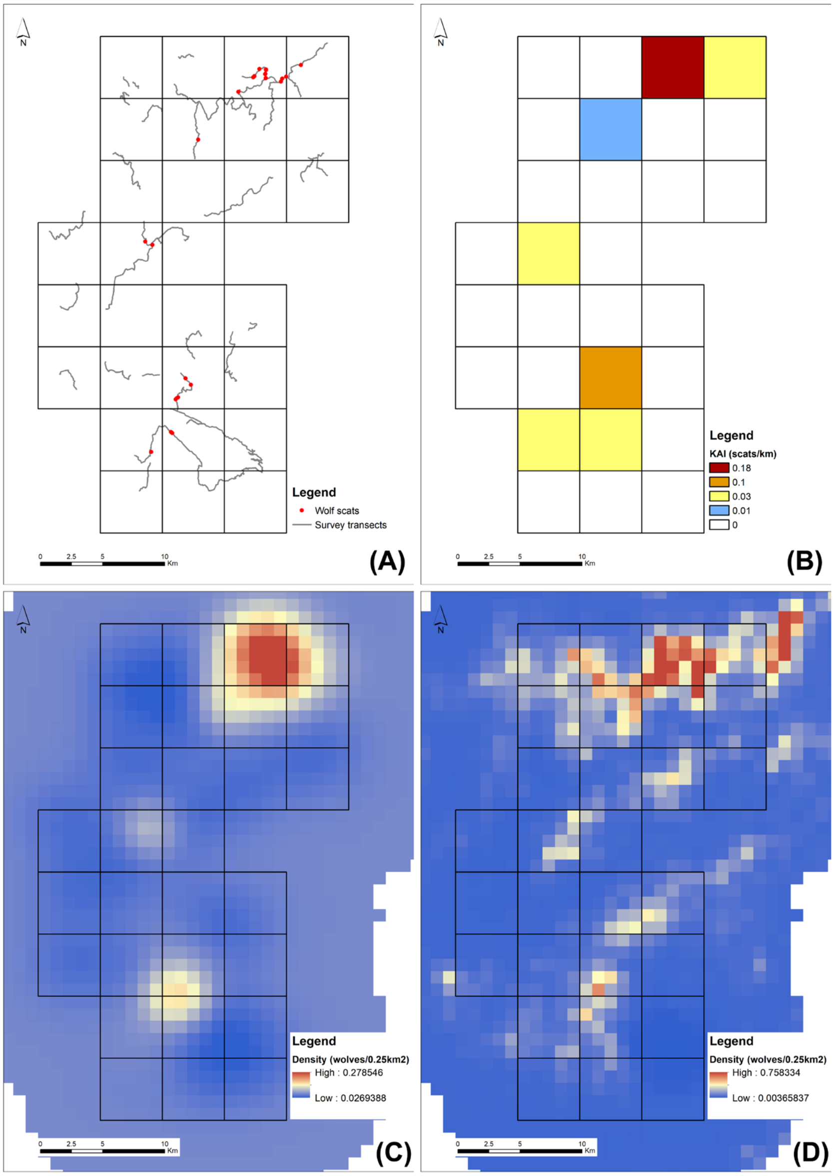

Figure 7. Example of spatial capture–recapture (SCR) outputs in comparison with naïve statistics analysis from a real dataset from Portugal (Bioinsight 2019 – Supporting information). (A) Spatial distribution of survey transects from a study area divided into 29 5 × 5 km cells, and location of 25 scats with wolf individual genotyping collected during the reproductive season of 2018. (B) Traditional report output, showing wolf presence cells and average kilometric abundance index (KAI – scats km^{-1}) within each cell. (C) SCR null model prediction (wolves/0.25 km^2), where no covariates were used to analyse its influence on distribution of individuals or the sampling process. (D) The best model prediction under an AIC evaluation (wolves/0.25 km^2), where the distribution of individuals is influenced by a habitat suitability index value, and the observational process is influenced by the sampling effort within each 5 × 5 km cell.

(Harmsen et al. 2020). The adaptation of these model frameworks (from study design to data analysis) for EIA monitoring is straightforward and will likely increase the effectiveness and value of the conclusions drawn. We argue that a reassessment of the guidelines to better align with recent field and laboratory innovations is required and we hope that this review will ignite the discussion around the necessity of an update on the Portuguese guidelines for wolf monitoring in EIA projects, and in particular, highlight the methodological pathways to do so.

EIA monitoring programs are fundamentally time-series projects, where there is the need to estimate species- and impact-relevant biological parameters before, during, and after an infrastructure development. Doing so in an efficient but robust manner requires improved standardisation and coherence which, if achieved, will ensure that decisions and mitigation strategies around construction projects will be evidence-based and support the continued persistence of healthy wolf populations.

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Author contributions

Gonçalo Ferrão da Costa: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (equal); Investigation (lead); Methodology (lead); Project administration (lead); Resources (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Miguel Mascarenhas:** Conceptualization (equal); Funding acquisition (equal); Writing – review and editing (supporting). **Carlos Fonseca:** Supervision (supporting). **Chris Sutherland:** Conceptualization (supporting); Funding acquisition (equal); Supervision (lead); Writing – review and editing (equal).

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Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.t1g1jwt87 (Ferrão da Costa et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

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