Wildlife monitoring and sampling: when should we consider genetic data in wild populations?

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science in Wildlife Sciences

Auburn, Alabama
August 6, 2022

Keywords:
camera trap; density; disease; genetic; population; sampling

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Abstract

Conservation and management of wild populations requires management professionals to devise methods for collecting data that are efficient and accurate. For example, understanding population sizes and stability is important to predicting extinction risk but the lack of comparisons of costs and effectiveness of different methods limits efficient assessment of wild populations. Similarly, monitoring and tracking the spread of wildlife diseases is important for conservation and management, however disease samples and other data have to be collected efficiently and in ways that maximize our understanding of the disease-wildlife systems. In my thesis, we addressed these research gaps in three studies. First, we conducted a meta-analysis of peer-reviewed studies that compared two or more monitoring. This provided an insight into the quantitative differences that are expected among methods. Next, we developed a forward-time, agent-based model to compare different approaches for collecting wildlife data, including data needed to quantify population size or density or understand disease presence. We found that the stationary approaches have more detections or sampling events compared to the mobile approaches, but both approaches have equal number of unique detections (i.e. unique individuals detected or sampled). Our model also suggested that some sampling approaches may be better suited for very large populations compared to other sampling schemes. Finally, we reviewed our current understanding of avian malaria with particular focus on recent advances in understanding this disease and its effects on wildlife and future efforts to control further spread. The results of this thesis will allow conservation practitioners and managers to identify the most effective monitoring techniques for their species of interest, and consideration of more efficient sampling approaches for wildlife diseases.

Acknowledgments

I would like to express my gratitude to my advisor Dr. Janna Willoughby for her awesome mentorship and guidance. I would like to thank my committee members Dr. Zohdy, Dr. Gitzen, and Dr. Steury, for all their help and guidance. I would like to thank my colleagues in the Conservation Genomics Lab for all their help and support. I would also like to thank the Auburn College of Forestry, Wildlife and Environment. Finally, thank you to my parents, family, and friends for their endless support in my pursuit of higher education.

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Chapter 1: Comparison of minimally invasive monitoring methods and live trapping in mammals

Miranda Paez A, Sundaram M, Willoughby JR. 2021. Comparison of minimally invasive monitoring methods and live trapping in mammals. Genes 12:1949. doi:10.3390/genes12121949.

Abstract

The conservation and management of wildlife requires the accurate assessment of wildlife population sizes. However, there is a lack of synthesis of research that compares methods used to estimate population size in the wild. Using a meta-analysis approach, we compared the number of detected individuals in a study made using live trapping and less invasive approaches, such as camera trapping and genetic identification. We scanned 668 papers related to these methods and identified data for 44 populations (all focused on mammals) wherein at least two methods (live trapping, camera trapping, genetic identification) were used. We used these data to quantify the difference in number of individuals detected using trapping and less invasive methods using a regression and used the residuals from each regression to evaluate potential drivers of these trends. We found that both trapping and less invasive methods (camera traps and genetic analyses) produced similar estimates overall, but less invasive methods tended to detect more individuals compared to trapping efforts (mean = 3.17 more individuals). We also found that the method by which camera data are analyzed can significantly alter estimates of population size, such that the inclusion of spatial information was related to larger population size estimates. Finally, we compared counts of individuals made using camera traps and genetic data and found that estimates were similar but that genetic approaches identified more individuals on average (mean = 9.07 individuals). Overall, our data suggest that all of the methods used in the studies

we reviewed detected similar numbers of individuals. As live trapping can be more costly than less invasive methods and can pose more risk to animal well-fare, we suggest minimally invasive methods are preferable for population monitoring when less-invasive methods can be deployed efficiently.

Keywords: camera trap; census; density; genetic; hair; live traps; population; scat

Introduction

Methods of monitoring wildlife populations focus on measures of density or abundance of populations, which allow for the evaluation of the dynamics of populations over time and in response to management strategies [1]. Population estimates can also support the evaluation of the viability of a population, population size, hunting limits, and examining impacts of changes in the environment and system [1]. However, when it comes to rare or difficult-to-catch species, especially species from small populations or those that occur at low densities, trapping or otherwise capturing individuals can be extremely time consuming. Although there are many methods for monitoring wildlife such as camera trapping or minimally invasive DNA analysis, all methods have their limitations [2]. Identifying methods that provide accurate estimates efficiently is essential for monitoring species and managing ecosystems.

One traditional method for obtaining data to assess population size or density is live trapping [3]. With live trapping, individuals of the target species in a study are trapped and released between each sampling session, creating capture histories of individuals and providing information about the individuals captured, such as overall health, sex, and even reproductive status [4,5]. However, this method can be extremely labor intensive and, as a result, cost restrictive and time consuming. Traps are often checked in 12-h intervals but, depending on the species of study,

traps may be checked more often. For example, insectivore traps need to be checked much more frequently as their diet does not provide the energy to withstand long hours in a trap [5]. In addition, the potential to stress an animal and interfere with their daily activity is high, thereby limiting the quality of the data [3]. Although these effects can be limited by methodological choices cleared by animal ethics authorities [5], less stressful and more efficient monitoring methods are often desirable.

Since their development in the early 1980s, camera traps have been used to study population sizes especially for large carnivores with distinctive natural marks (e.g., *Panthera tigris*, *Panthera onca*, and *Lynx rufus*) [6]. Camera traps are noninvasive methods useful for species inventories, estimating population density, calculating home range, and monitoring population dynamics [7]. The advantages of using camera traps, compared to other methods, is that they have a relatively low cost and do not require physical or chemical animal restraint, avoiding capture stress [6,7]. Natural marks and fur patterns help identify individuals and establish capture history [8]. However, using camera traps is often restricted by the ability to identify individuals or species that do not have distinctive fur or marking patterns [9] but understanding where to place camera traps can be challenging when movement patterns are unknown [10].

One alternative to camera traps that is often less invasive than trapping is the use of genetic-based methods for individual identification [11]. With genetic sampling, capture histories can be constructed through the captured genotypes from samples of tissue, scat, or hair [4]. Genetic data captured can also reveal other patterns important to long-term population stability such as inbreeding rates, genetic diversity, population structure, and patterns of gene flow [12]. While scat samples can be collected opportunistically, hair snagging devices can also be deployed with

lures or bait [13]. As samples like scat and hair are often easier to obtain compared to the efforts related to trapping, reduced field costs can help to make this method more cost-efficient, enabling additional collection and larger sample sizes [4]. However, there are drawbacks to DNA-based techniques. When samples remain uncollected immediately after being deposited, the DNA will degrade. This can lead to genotyping errors such as false alleles and allelic dropout, inflating the number of unique individuals identified. Through repeated amplification of the genetic samples, these genotyping errors can be reduced, although the repeated genetic work comes with additional sample processing cost [4].

Minimally invasive sampling techniques are often preferred for endangered species because these methods generally pose less risk of injury or death compared to trapping or other approaches. Monitoring population densities using cameras or genetic analyses, especially in tandem, may provide insight into the trade-offs of capture methodologies. However, the overall pattern of detections that are made when using live trapping compared to those made using less invasive methods (i.e., those using camera traps or genetic data) across species is unknown. The primary objective of this study was to compile literature that compared individual detections using at least two common methods: live trapping, camera trapping, and genetic analysis. Using these data, we answered three questions: (1) “Does the number of individuals detected change when using live trapping methods compared to when less invasive methods (i.e., camera trapping and genetic analyses) are used?”; (2) “Do estimates from minimally invasive data collection methodologies have a similar sensitivity in identifying unique individuals?”; and (3) “What species and study-specific criteria are associated with differences in effectiveness of live traps, camera, and genetic data?” Our study focuses on the number of individuals detected because these are the data that were reported in the literature. However, detectability is an important part

of estimating population size and density. Despite this, our analyses are still useful because incorporating more detections can reduce error in population size estimates for most systems. Understanding how these methods differ in their ability to identify unique individuals will be important for targeting the correct methodologies for estimating population sizes in on-going management work.

Materials and Methods

We used a systematic literature search to identify existing data using the Web of Science. We conducted three searches using the following sets of keywords: (1) (hair OR scat OR gene*) AND (census OR density) AND (live trap*); (2) (camera trap* OR camera-trap* OR game camera* OR trail camera*) AND (census OR density) AND (live trap*); (3) (camera trap* OR camera-trap* OR game camera* OR trail camera*) AND (hair OR scat OR gene*) AND (census OR density). We included articles from 1900–present and our final list of papers included one non-peer-reviewed, preprint manuscript. From this set of papers, we identified studies that reported the number of individuals they detected in the focal species using at least two of the following methods to identify individuals: live traps, camera traps, or genotyping. These studies must have collected both of these sets of data over the same time period and for the same target population. A graphical representation of the filtering process is described using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses chart (Figure 1) [14]. From each paper that passed our filtering criteria, we extracted the focal species, location of study, size of study site, camera data analysis method, and type of genetic sample that was collected (Table 2). We also extracted study outcomes including the total number of individuals identified. When necessary, we used the study area size and density estimate to calculate the number of

individuals identified. Data depicted only in figures (this happened only once) was extracted using ImageJ (version 1.53i) [15].

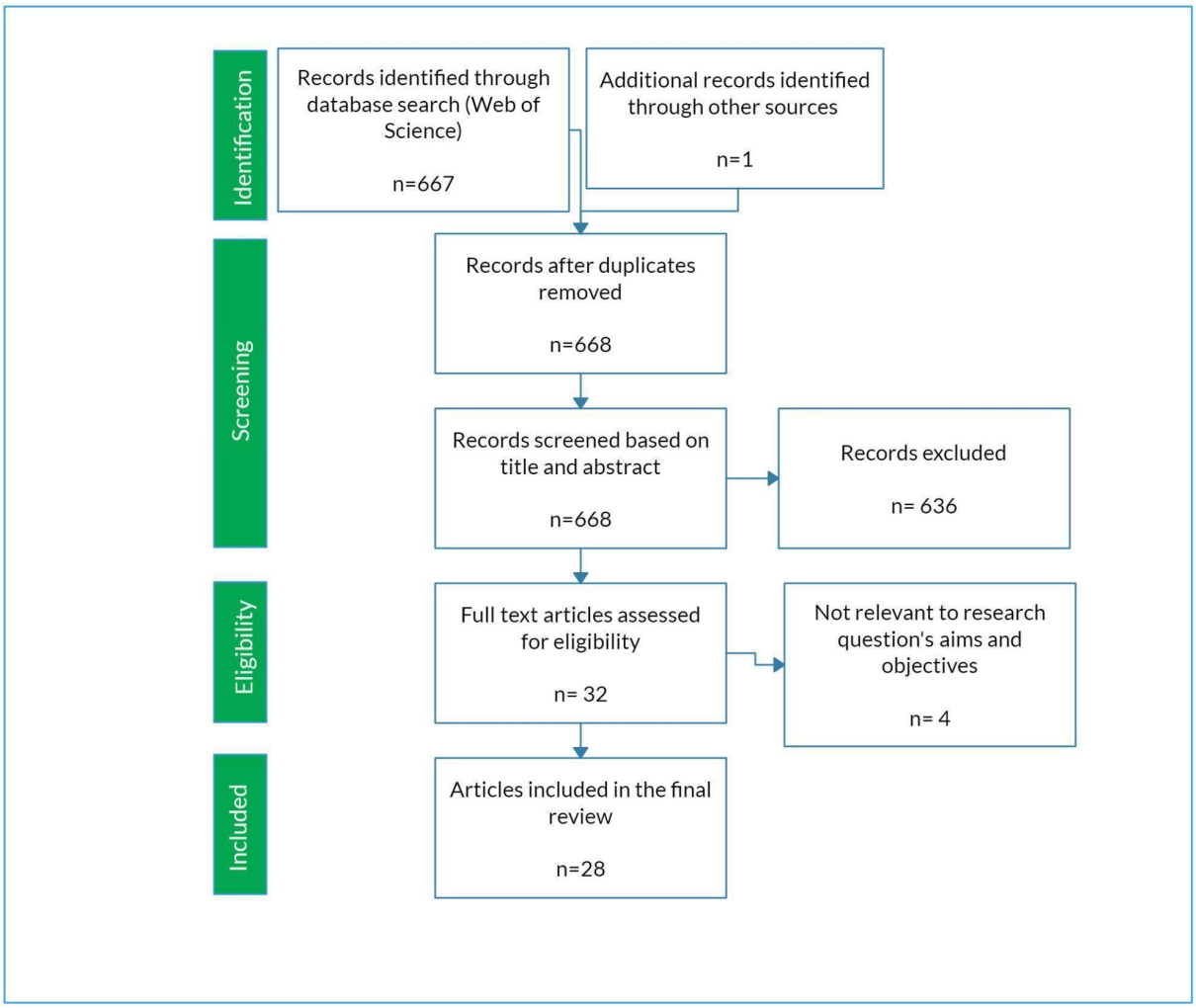

Figure 1. PRISMA diagram of our search protocol and results. Hundreds of papers were identified through database search using keywords mentioned previously. Title and abstracts were then reviewed as the first screening process. Papers that contained method comparison between invasive and non-invasive, and camera and genetic methods were retained. After screening, 32 full-text articles were reviewed for eligibility. 4 full texts were excluded from the comparison. Finally, 28 full-text meta-analyses were included in this meta-analysis.

We initially compared the number of focal-species individuals identified using live trapping to those made using less invasive methods (camera traps and genetic analyses) using a linear

regression. As we were interested in evaluating a 1:1 relationship between the two estimates, we predicted population size using less invasive methods to estimates made using live trapping data, setting the intercept to zero. To meet assumptions of normality, we log-transformed both estimates of population size. We then extracted the residuals for each population from the resulting regression line. We repeated these analyses to compare population size estimates generated from less invasive to each other, where camera trap estimates and genetic data estimates were the predictor and response variables, respectively. We subsequently used both sets of standardized residuals to understand which factors (see below) might be related to the discrepancies between the population size estimates. These and all subsequent statistical analyses were performed in R [16].

We calculated the mean number of individuals identified using trapping compared to less invasive approaches and the mean number of individuals identified using camera traps compared to genetic data analyses using a bootstrapping approach. Specifically, we estimated the mean difference between each group 1000 times, resampling 80% of the values with replacement. We then estimated the 95% confidence interval around the mean difference estimated from the data and compared this interval to zero.

We evaluated the extent to which the differences in number of individuals detected between methods were related to the phylogenetic relatedness of the species in the studies included in our analyses. We used the taxonomic categories of order, family, genus, and species to create a phylogenetic tree of species included in the studies from which we collected population size data, where all branch lengths were set to 1. We then used a phylogenetic least squares

regression to quantify the relationship between our phylogeny and the regression residuals described above using a linear model and assuming Brownian evolution [17].

Next, we tested the extent to which properties of each study were related to the differences between the number of detected individuals using a series of regressions where standardized residuals from our original regressions were predicted from study characteristics. First, we estimated the effect of camera analysis methods (spatial and random) on the differences between estimates. In the studies we examined, we categorized spatial methods as those that incorporated spatial locations into their analyses of camera data as "spatial" studies and "random" studies as those that did not incorporate this information into the analysis. Next, we quantified the effect of DNA source tissue (hair and scat) used on differences between number of detected individuals in the studies we analyzed. Finally, we considered how study site size contributed to the residual values using a regression where the log of study size, in kilometers, was used to predict the residuals from our initial regressions. For any of the identified comparisons that were significant predictors of the residuals, we calculated the mean number of individuals identified using the two different approaches, again using a bootstrapping approach. As before, we estimated the mean difference between each group 1000 times, resampling 80% of the values with replacement. We then estimated the 95% confidence interval around the mean difference estimated from the data and compared this interval to zero.

In addition to technical study properties, we were also interested in how biological variables of body size and dominant habitat may have contributed to the differences in detected individuals. To test this, we collected mean body size estimates from the list of Mammalian Species Account in the Journal of Mammalogy (by chance, all identified studies in our analysis focused on

mammals) and used the log of these values as predictors of the differences between number of detected individuals (i.e., standardized residuals from original regressions). To quantify the effect of habitat, we extracted the dominant habitat ecozone for each population from the Morrone biogeographic realms [18]. We then used these categories as predictors for the standardized residuals. All data used in our analyses are available and in Table 2. All analysis code available online via GitHub: https://github.com/andreamiranda26/Monitoring-Methods-Analysis.

Results

We screened a total of 668 studies that were returned from our Web of Science searches and ultimately identified 28 studies that used at least two methods for identifying individuals in a wild landscape. This included comparisons for 27 populations that compared live trapping to less invasive methods and 17 populations where camera and genetic data were compared. These studies focused on mammals and were conducted in North America, Europe, Asia, and Oceania (Table 1; all data included in Table 2).

Table 1. Regression coefficients and standard error (SE) estimates. The first two results describe the comparison of population size estimates generated using two different data collection methods. The remaining results analyzed the ability of several study and species-related predictors to describe the residuals from the initial two regressions. In the table header, F is F statistic, DF is degrees of freedom, and p is the p-value for the model.

Response Variable	Predictor Variable	Slope Estimate (SE)	Intercept Estimate (SE)	R^2	F	DF	*p*
Comparison of Population Size Estimates							
live trapping estimates	camera and genetic data estimates	0.976 (0.057)	–	0.919	296.1	26	<0.001
genetic data estimate	camera trapping data estimate	0.920 (0.145)	–	0.686	40.29	18	<0.001
Residuals from Live Trapping vs. Less Invasive Methods							
standardized residuals	phylogenetic tree	–	−0.192 (0.692)	–	–	26	0.783
standardized residuals	camera analysis (spatial or random)	1.547 (0.514)	−0.328 (0.383)	0.502	9.071	8	0.020
standardized residuals	genetic tissue source (hair or scat)	0.191 (0.476)	−0.313 (0.383)	0.000	0.161	16	0.694
standardized residuals	study site size (km^2)	0.066 (0.084)	0.046 (0.283)	0.000	0.622	23	0.439
standardized residuals	body size (kg)	0.010 (0.141)	0.056 (0.209)	0.000	0.005	25	0.946
standardized residuals	biogeographic realms (Australian, A; nearctic, N; palearctic, P)	–	A:0.102 (0.604) N: −0.146 (0.655) P: 0.233 (0.740)	0.000	0.294	25	0.748
Residuals from Camera vs. Genetic Methods							
standardized residuals	species phylogenetic tree	–	0.332 (0.923)	–	–	18	0.723
standardized residuals	camera data analysis (spatial or random)	0.012 (0.599)	0.433 (0.547)	0.000	0.000	17	0.984
standardized residuals	genetic data tissue source (hair or scat)	0.139 (0.598)	0.327 (0.546)	0.000	0.54	17	0.819
standardized residuals	study site size (km^2)	−0.822 (0.114)	0.761 (0.494)	0.000	0.519	17	0.481
standardized residuals	body size (kg)	0.114 (0.140)	0.087 (0.486)	0.000	0.673	17	0.424
standardized residuals	biogeographic realms (nearctic, N; neotropical, T; palearctic, P)	–	N: 0.672 (0.337) T: −0.410 (1.010) P: −0.412 (0.463)	0.000	0.416	17	0.667

We were interested in understanding how well minimally invasive approaches matched individual detection data generated from more traditional trapping efforts. Our initial regression, which forced the intercept through zero, suggests that these measures are well correlated with the 95% CIs of slopes overlapping 1 indicating a 1:1 relationship (Table 1; Figure 2). We also quantified the mean difference between these methods and found that minimally invasive methods were similar to population size estimates generated from live trapping efforts (mean difference = 3.19 individuals; 95% CI −8.150 to 15.602).

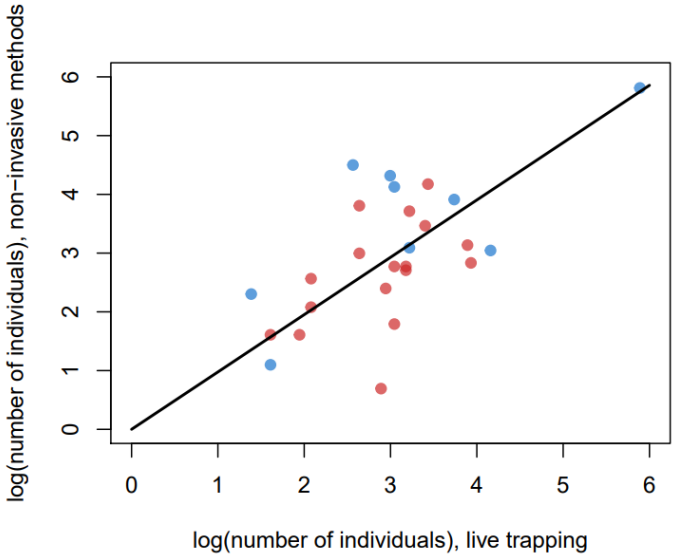

Figure 2. Comparison of the number of individuals identified using live trapping compared to less invasive methods (blue dots denote comparisons where the less invasive method used were camera traps whereas red dots used genetic data). Regression output is depicted by the black line slope = 0.976 ± 0.057, $p < 0.001$, R2 = 0.919, F = 296.1, degrees of freedom = 26). Less invasive methods were on average larger than the population size estimates generated from live trapping efforts (mean = 3.19 individuals).

We also compared identification estimates generated using two minimally invasive methods, camera traps and genetic identification of individuals. Using a regression that forced the intercept through zero, we found that these estimates were reasonably well correlated with the 95% CIs of slopes overlapping 1 (Table 1; Figure 3), even though the explanatory power in this regression was less than the regression comparing trapping to less invasive methods (i.e., $R^2 = 0.919$ compared to $R^2 = 0.686$, respectively; Table 1). We also found that these methods identified a similar number of individuals (genetic methods identified an additional 9.07 individuals compared to camera traps on average; 95% CI −3.323 to 24.212).

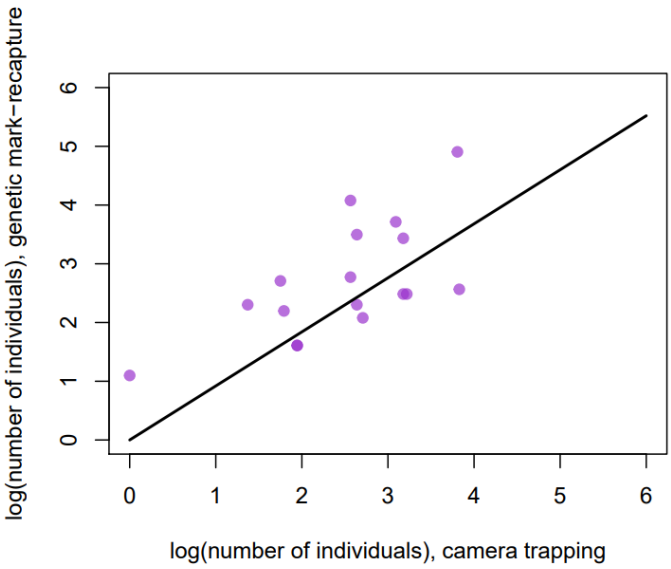

Figure 3. Regression results comparing population estimates generated using two non-invasive methods, camera traps and genetic identification of individuals. On average, genetic methods estimated an additional 9.07 individuals compared to camera traps (slope = 0.920±0.145, $p<0.001$, $R^2 = 0.686$, $F = 40.29$, degrees of freedom = 18

As the species in the studies we reviewed were distributed non-randomly across the phylogenetic tree (i.e., all mammals and many species in Carnivora), we considered how the potential confounding variable of shared evolutionary history influenced detection using a phylogenetic least squares regression. We found that phylogeny did not predict the difference in individuals detected using trapping compared to less invasive techniques. Similarly, phylogeny did not predict the difference in detection compared between the two less invasive techniques (Table 1).

We examined our data for evidence of the technical aspects of the study design that may have explained the differences in the number of individuals detected. Although our data were somewhat limited in power, we found that the camera data analysis methodology was a significant predictor of the differences in detection when using the live trapping and camera methods. However, the camera data analysis method did not predict the difference in detection

when compared between the less invasive methods (camera vs genetic data analysis). The type of tissue collected for genetic analyses (hair or scat) had no predictive power for detection differences that occurred when using live trapping and genetic methods or when compared between less invasive methods. Finally, we found that study site size did not predict the differences in number of individuals detected in either of our comparisons (Table 1).

Following on the significant relationship between camera data analysis method and regression residuals, we quantified the difference in number of individuals identified using trapping approach and camera data analyzed assuming random movement of individuals or using spatial data information, using bootstrap analysis. We found that when spatial data related to detections was not used in the analysis, trapping approaches identified similar number of individuals compared to camera data (mean = 16 individuals, 95% CI −37.667 to 4.667). When spatial information was incorporated, camera-based approaches identified an average of 35 more individuals compared with trapping-based approaches (95% CI 3.750 to 66.000). However, we note that the number of studies that made these comparisons was small (four and five, respectively) and so these ranges should be interpreted with this limitation in mind.

Finally, we also considered how biological variables influenced the number of individuals detected using the various methods. We found that body size had no predictive power for detection differences compared between live trapping and less invasive methods or when compared between the minimally invasive camera and genetic data methods. We also found no significant difference in predictive power between the biogeographic realms in which these studies were conducted (Table 1).

Discussion

Overall, the number of individuals detected using trapping and less invasive methods were well correlated. However, on average, 3.19 more individuals were identified using minimally invasive methods than using live trapping and 9.07 more individuals were found with genetic-based estimates compared to camera data-based estimates. Although the boot-strapped confidence intervals around these estimates included zero, these differences may be important in management, particularly in the conservation of endangered species. For example, the California condor is a species of high conservation need that has undergone intensive management [19]. In 1990, less than 50 birds existed in the wild, meaning that an underestimation of close to 9 individuals would represent missing ~18% of the total individuals existing in the wild. These results support the use of minimally invasive methods of trapping, and in particular use of genetic identification-based methods, for quantifying population size particularly when missing a few individuals would substantially undermine conservation or management goals.

In addition to applications to species of extreme conservation concern, minimally invasive approaches may be preferable due to risk mitigation benefits as well as cost and time benefits associated with these methods. Compared to live trapping, less invasive methods offer protections to the focal populations because they are inherently less risky, as animals do not have to be handled. This provides a distinct advantage over live trapping for both animal well-fare and researcher injury risk. In addition, the total cost and effort for less invasive methodologies tends to be less than for invasive or lethal approaches, providing quantitative monetary and time advantages for these methods [20]. Combined with the relative similarity in detection of individuals that occurs with live trapping compared to less invasive methods, the advantages of

minimally invasive approaches suggests that these methods should be considered at least as often as live trapping when population monitoring is the goal.

One important limitation of our study was our comparison of the number of individuals detected. Although we would have preferred to compare estimates of population density or other metric that takes into account detection probability, these were not uniformly reported in the studies we identified. However, we suggest that the core ability to identify different numbers of individuals for the same population at the same time provides support for increased sensitivity of minimally invasive methods compared to trapping. Even without incorporating detectability (i.e. probability of detection), basing population size estimates on data from more individuals can increase the accuracy of population size estimates. In our data, we expect this to be true because all of the comparisons were made when two different approaches to monitoring populations and collecting information about individuals were made for the same population over the same time period. While, some of the differences between estimates within studies could have been accounted for by incorporating detectability, we suggest that, across species and study sites, using methods that detecting more individuals is likely to be beneficial to population size estimates, in most cases [21,22].

We interpreted the variance around the regression line comparing trapping to less invasive method counts of individuals not corrected for detectability, as well as the variance around the comparison between minimally invasive methods as a representation of the between-study differences influencing the overall trends. Most of the study parameters we analyzed had no significant relationship on the residuals, however, camera analysis was a significant predictor of the difference in individual counts estimated using live trapping and camera trapping methods.

Our analysis supports the idea that including camera trap location yields better individual counts, as evidenced by studies that used these data having identified 35 more individuals (95% CI 3.750 to 66.000) compared with counts from trapping data, whereas studies that assumed random movement when analyzing camera data identified 16 fewer individuals (95% CI −37.667 to 4.667) compared with trapping-based estimates. However, use of these kinds of models requires consideration of study-specific variables prior to collecting data, as analyzing data not intended for spatial models leads to biased count of individuals [23].

Although we advocate for the use of some of the less invasive methods available, consideration of species and habitat-specific variables are critical and may require in-field comparisons. For example, method comparisons could be beneficial for species that have low densities and low capture success, such as the southeastern fox squirrel (*Sciurus niger*), whose scarcity and difficulty in being detected requires a reliable method to survey and monitor their populations [24]. Likewise, for species sensitive to habitat loss and fragmentation such as the American marten (*Martes americana*), their density estimates are essential for deciding conservation strategies. American martens positively respond to baited camera traps, suggesting camera traps or baited hair snare traps for genetic analysis may be viable options [25]. However, the decline of marten populations may mean that, at least in some localized areas, genetic diversity is low, meaning that genetic analyses will require additional lab effort to produce individual identification information. Similarly, with Iberian lynx (*Lynx pardinus*) in Spain, where camera trapping is not financially or logistically possible [11], genetic analyses may represent a useful alternative. However, exclusion of wildcats (*Felis sylvestris*) scat, which is similar to Iberian lynx scats, would require extra investments [11]. Therefore, the decision to employ a particular data collection method requires species and ecosystem specific information.

Population estimates of wildlife populations are essential for proper research, conservation, and management. It is integral for the application of conservation and management strategies, such as establishing protections for threatened species, outlining sustainable harvest efforts, and mitigating human-wildlife conflict [26]. The effectiveness of wildlife conservation is heavily dependent on estimates that are accurate and precise to ensure proper decision making, since an inaccurate measurement can lead to a false signal of population stability [26]. Here, we show that minimally invasive methods, including cameras and many genetic-based identification methods, detect a similar number of individuals compared to trapping-based efforts. Because these methods offer advantages in animal welfare and cost, we suggest increased reliance on minimally invasive methods to generate reliable estimates of population size and density to support on-going management efforts.

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Table 2. Data extracted after screening each study. Species are separated alphabetically by the study comparisons made, camera trapping vs. genetic analyses, genetic analyses vs live trapping, and camera trapping vs. live trapping, respectively. The * highlights data that was extracted from figures using ImageJ (https://imagej.nih.gov/ij/).

Species	Body Mass (kg)	Study Area (km2)	Camera Method	Camera Num. Indv.	Genetic Method	Genetic Num. Indv.	Live trap Num. Indv.	Citation
Canis lupus	115.5	85	spatial	46	scat	13		Galaverni et al. 2012
Felis silvestris	6.5	10.1	spatial	14	scat	10		Anile et al. 2014
Felis silvestris	6.5	10.1	spatial	3.93	scat	10		Anile et al. 2014
Leopardus pardalis	11.25	15.43	spatial	24	scat	12		Rodgers et al. 2015
Leopardus pardalis	11.25	1.54	random	25	scat	12		Rodgers et al. 2014
Lynx canadensis	12.65	300	spatial	22	hair	41		Doran-Myers 2018
Lynx pardinus	11.45	250	random	45	scat	135		Garrote et al. 2014
Martes americana	0.9	220.5	spatial	15	hair	8		Clare et al. 2017
Martes martes	0.89	12	random	5.76	hair	15		Balestrieri et al. 2016

Panthera onca	79	205	spatial	13	scat	16		Sollmann et al. 2013
Panthera uncia	32	108	spatial	7	scat	5		Janecka et al. 2011
Panthera uncia	32	655	spatial	1	scat	3		McCarthy et al. 2008
Panthera uncia	32	808	spatial	7	scat	5		McCarthy et al. 2008
Panthera uncia	32	813	spatial	6	scat	9		McCarthy et al. 2008
Puma concolor	64.5	10	spatial	0.09	scat	32		Loonam et al. 2021
Sus scrofa	175	11.2	spatial	24	scat	31		Davis et al. 2020*
Sus scrofa	175	8.1	spatial	13	scat	59		Davis et al. 2020*
Sus scrofa	175	5.43	spatial	14	scat	33		Davis et al. 2020*
Dasyurus maculatus	2.25	70			hair	16	21	Ruibal et al. 2010
Lepus americanus	1.49	1.8			scat	32	30	Cheng et al. 2017
Lepus americanus	1.49	1.8			scat	5	7	Cheng et al. 2017

Lepus americanus	1.49	1.8	scat	8	8	Cheng et al. 2017
Lepus americanus	1.49	1.8	scat	11	19	Cheng et al. 2017
Lepus americanus	1.49	1.8	scat	13	8	Cheng et al. 2017
Lynx canadensis	12.65	300	hair	41	25	Doran-Myers 2018
Martes caurina	1.8	40	hair	17	51	Slauson et al. 2017
Martes caurina	1.8	40	hair	23	49	Slauson et al. 2017
Martes martes	1.6	100	hair	15	24	Croose et al. 2019
Martes martes	1.6	100	scat	16	24	Croose et al. 2019
Martes martes	0.89	3.5	hair	5	5	Mullins et al. 2010
Meles meles	6.98	11	scat	20	14	Wilson et al. 2003
Microtus cabrerae	0.05	0.78	scat	65	31	Sabino-Marques et al. 2018
Microtus cabrerae	0.05	0.79	scat	45	14	Ferreira et al. 2018

Vulpes velox	2.2				scat	2	18	Schwalm et al. 2012
Vulpes velox	2.2				scat	6	21	Schwalm et al. 2012
Felis catus	3.6	6.4	spatial	10			4	Hansen et al. 2018
Lepus americanus	1.5	5	random	21			64	Villette et al. 2017
Lynx canadensis	12.65	300	spatial	22			25	Doran-Myers 2018
Pekania pennanti	4.53	317	spatial	90			13	Jordan et al. 2011
Pekania pennanti	4.53	317	spatial	75			20	Jordan et al. 2011
Pekania pennanti	4.53	317	spatial	62			21	Jordan et al. 2011
Sciurus niger	0.75	1.5	random	50			42	Greene et al. 2016
Tamiasciurus sciurus	0.34	5	random	334			361	Villette et al. 2017
Xeromys myoides	0.05	6	random	3			5	Kaluza et al. 2016

Chapter 2: Comparing two wildlife sampling approaches using an agent-based model and implications for their use in monitoring and management

Miranda Paez A, Willoughby JR. Comparing two wildlife sampling approaches using an agent-based model and implications for their use in monitoring and management. Target journal: Journal of Mammalogy.

Abstract

To efficiently conserve and manage wildlife species, proper and efficient sampling methods should be used. However, it can be challenging to establish an efficient sampling approach that may be suitable for a particular project because different project goals and focal species have different priorities. Using a forward-time, agent-based model, we compared two different approaches for collecting wildlife data: stationary sampling, where sampling locations were held constant over the simulated study period and mobile, where sampling locations were moved each simulated day. Samples or detections were broadly interpretable as individual detections, disease presence or absence, or other trait of interest that can be measured. We found that the stationary approach had significantly more detections than mobile sampling approaches above a small effort threshold, but stationary and mobile approaches had the same number of unique detections across all effort levels. This is important because repeatedly sampling the same individuals may not be needed for some study designs and repeatedly processing images or samples is costly in both time and resources. This pattern held when considering how these sampling methods compared when study duration was increased. However, we observed an important difference in the total number of detections and unique detections captured when using stationary compared to mobile sampling approaches: stationary sampling had disproportionally more detections (scaled

for population size) than mobile sampling. We interpret this as suggesting that stationary approaches may be more efficient for sampling very large populations although our model did not reach these population sizes. Overall, when repeatedly sampling individuals is needed or when population sizes are very large, we suggest a stationary sampling approach but when repeated samples are not needed, this approach would cause a drain on efficiency and resources, and a moving sampling location approach may be warranted.

Keywords: disease, agent-based model, mobile sampling, monitoring, stationary sampling

Introduction

Efficient and accurate sample collection is critical to the conservation of a species because management actions are often data driven. For example, understanding where individuals are found and how individuals move within a landscape can be used to inform wildlife corridor construction locations or translocation activities (Hromada et al. 2020). However, wildlife monitoring and sampling can be conducted in myriad ways that may or may not provide the desired data in the most efficient manner because it is difficult to predict efficiency before the system is already understood (Nusser et al. 2008). One example of this happens when we want to quantify population size or density: deciding where to place camera traps to set in order to capture sufficient data for estimating population density is predicated on our understanding of where the species will be found (i.e. unbiased camera placement;(Rowcliffe et al. 2008). This is further complicated for study questions that relate to characteristics of a population over time, such as the presence or prevalence of disease, because data needs to be collected from a representative sample of the population repeatedly and consistently to make accurate estimates and conclusions about the population trends (Nusser et al. 2008).

One common approach used for sampling and detecting wildlife is camera trapping. Camera traps are typically set up at a predetermined number of trap sites and remain in the same location for the duration of the study (i.e. the sampling locations are stationary). These cameras collect data by recording photographs or videos when movement is detected (Galaverni et al. 2012, Villette et al. 2016). These images can then be used to identify the focal species and sometimes to uniquely identify individuals in a study site (Anile et al. 2014, Davis et al. 2020). However, factors such as observer bias when interpreting images and camera angles can contribute to detection errors (Galaverni et al. 2012, Sirén et al. 2016). Furthermore, where a camera is placed in the landscape may also contribute to detection probability because a poor location, meaning one where individuals are not found or where the location is biased to a select few individuals, may also negatively affect the conclusions drawn from data that are collected using stationary sample locations.

Another common approach for understanding wild populations involves analyzing DNA to determine individual identification, disease status, or other characteristic of interest. Often, genetic analyses involve collection of tissues (e.g. scat or hair) noninvasively across the landscape and extraction of DNA from these tissues (Davis et al. 2020). While these tissues can sometimes be collected at pre-determined locations using tools like rub-posts or hair snares, collection of scat or tissue at kill sites is more likely to be done haphazardly across a study site (Ruibal et al. 2010, Cheng et al. 2017, Doran-Myers 2021), making collection efforts sometimes inefficient. This is problematic for quantifying population size or density and for tracking the spread of wildlife diseases, both of which are critical component conservation (Miranda Paez et al. 2022). Understanding how this haphazard and spatially mobile sampling approach influences our understanding of these population parameters is important to on-going management efforts.

For any kind of monitoring or sampling where it is impossible to collect data from every individual, the effects of errors in combination with other aspects of sampling design are important to quantify. For example, wildlife management requires accurately assessing population density, but there can be differences in number of individuals detected when varied wildlife detection methods are used (Miranda Paez et al. 2021). The spatial extent of sampling areas can differ when both a stationary camera trapping and spatially mobile DNA collections are used in the same landscape (Rodgers et al. 2014). As these samples are often obtained in ways that are not random, it can make it difficult to capture suitable standard errors and leading to a questionable sampling approach (Nusser et al. 2008).

Here, we used modeling to compare detections- broadly interpretable as individuals, disease, or other trait of interest- efficiency using two different approaches to collecting samples: 1. Stationary samples where individuals who move through a set sampling location are sampled; 2. Mobile sampling where sampling location moves for each sampling event and individuals that overlap these locations during the sampling event are sampled. In these models we also considered the effect of varied error rates, study duration, and the number of individuals in a landscape to understand how this may suggest different utility in wildlife conservation and management.

Based on our earlier meta-analysis (Miranda Paez et al. 2021), we expected to find that stationary sampling approach, which mimics camera trapping methods, would have fewer detections compared to the mobile sampling approaches that mimic common DNA analyses used for estimating population size and density. Furthermore, we hypothesized that error rates should result in similar estimates between these two sampling approaches, but that the simulated

species' population size and study duration would reduce any observed differences between the methodologies by increasing the number of detections in the stationary sampling approach.

Methods

We designed a forward-time, agent-based model to compare the number of detections or samples that could be collected as well as the number of unique detections and samples across two different sampling approaches: stationary and mobile. We defined the stationary approach as one that operates similarly to camera trapping, where all samples across the study duration are collected at a set of locations. As an alternative to this, we also simulated a mobile sampling location method, similar to the approach that is often used to collect eDNA or scat samples. Under the mobile sampling pattern, samples sites move to new locations each day and samples are collected from individuals who intersect with that location on that day. Below, we have outlined our model goals, parameters, and expected functions using the ODD protocol (Grimm et al. 2020). All modeling and analysis scripts as well as output data are available via GitHub: https://github.com/andreamiranda26/noninvasive_modeling.

Overview: Compare the effectiveness of stationary sampling methods, where sample location does not change, to mobile methods where sampling location moves around the landscape. We define effectiveness as collecting the maximum number of samples, collecting the maximum number of samples from unique individuals, or both. In addition to the two different sampling approaches, we also quantify the effects of error rate, study duration, and the number of target individuals existing on the study landscape.

Entities, state variables, and scale: Our models have two entities: individuals that move around the landscape and the landscape itself. The individuals are characterized by a unique ID and are

permitted to move a varied number of landscape grid cells (20, 50, 100, 500 cells/steps) in any direction. The landscape is comprised of a grid of 50 x 50 squares where the distance across each square is equal to the distance individual moves in a day. The landscape also includes stationary and mobile sample locations.

Process: Sampling locations were decided following two patterns: stationary and mobile sampling approaches. For the stationary sampling locations, a set number of sampling sites (5, 10, 25, 100 or 200) were randomly placed on the landscape at the first timestep of the model, and these locations remained constant through the end of each run. For mobile sampling locations, a set number of sampling sites that were equal to the number of stationary sites (5, 10, 25, 100 or 200) were randomly placed on the landscape at every time step. In both cases, no duplicate sampling sites were permitted within each sampling approach. When testing the effects of error rates, rates of 0.1, 0.01, and 0.001 were incorporated to both sampling approaches. Across this sampling landscape, we simulated individuals (20, 50, or 100 individuals) that traveled a specified number of steps (grid cells; 20, 50, or 100) each run in a randomly selected direction that could change between steps. Individuals could also remain in the same cell for two consecutive time points, where the probability of individuals moving to a new cell was 95%. At the end of each simulated time period, individuals that ended in or passed through sample locations were marked as 'sampled' for the sampling method corresponding to that sampling site. At the end of each simulated study, the total number of individuals and total number of unique individuals that were 'sampled' were stored for later analyses. Each unique set of model parameter values were repeated 100 times.

Functions/subroutines: Our model included functions that initialized the landscape, controlled movement of individuals, set sampling site locations (stationary and mobile), tracked individual movement, and recorded when individual movement routes intersected with sampling site locations.

Model output analysis: We compared the total number of samples collected and the number of samples collected from unique individuals across parameter values. Across all 100 replicates for unique set of parameter value combinations, we calculated the mean total number of samples and number of unique samples as well as the 95% confidence interval around these means. We then compared these values between the stationary and mobile sampling model outputs. https://github.com/andreamiranda26/noninvasive_modeling.

Results

We used modeling to compare the efficiency of stationary and mobile sampling approaches. We found that the total number of detections (e.g. samples collected, individuals detected) and total number of unique detections varied across the number of sampling events in the model (Figure 4). Although the total number of detections scaled linearly with sampling events irrespective of stationary or mobile sampling scheme, the number of unique detections increased quickly over small event increases and leveled off as the number of unique detections approached the number of simulated individuals. Additionally, the stationary approach had significantly more detections than mobile sampling approaches above a small effort threshold, but stationary and mobile approaches had the same number of unique detections across all effort levels (Figure 4).

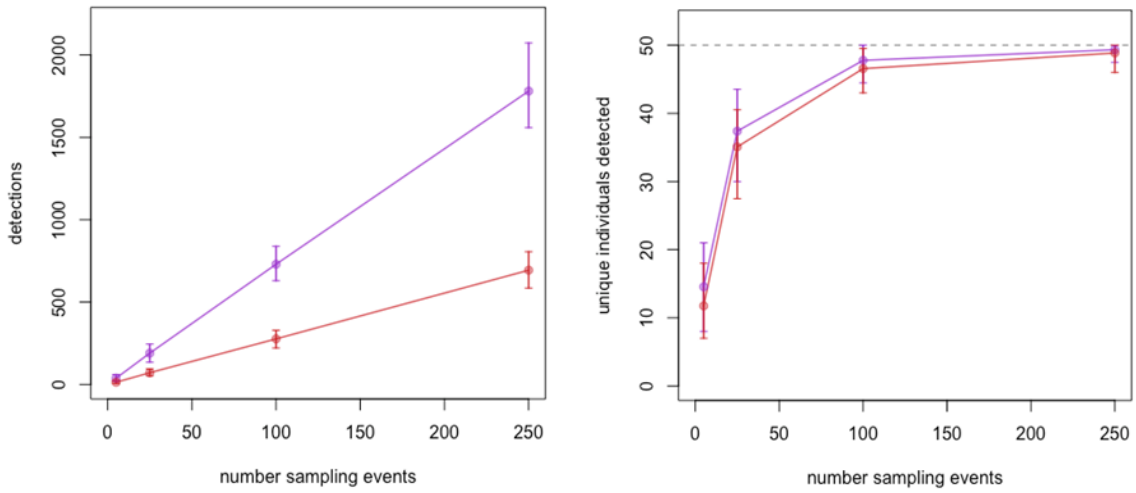

Figure 4. Stationary sampling methods (left) have more detections than mobile sampling methods, above an effort minimum threshold. Stationary and mobile sampling methods collect data from the same number of unique individuals when effort (right; number of sampling events) is the same. In both plots, stationary data is depicted by purple points, lines, and error bars while mobile data is depicted by red points, lines, and error bars. Dots depict means across 100 replicates and the error bars are the 95% confidence intervals around those means.

We also compared the effect of varied error rates on the total number of detections and the number of unique detections when using stationary and mobile sampling site approaches (Figure 5). Because of the generality of this model, these errors can be interpreted as errors in individual identification, ability to detect disease, or any other outcome of interest for a particular study. As we observed with a single error rate (Figure 4), the total number of detections scaled linearly with sampling events irrespective of stationary or mobile sampling scheme across all error rates whereas the number of unique detections increased quickly over small event increases and leveled off as the number of unique detections approached the number of simulated individuals again across all tested error rates. Overall, error rates of 0.001, 0.01, and 0.1 did not alter the effectiveness of stationary and mobile sampling approaches (Figure 5).

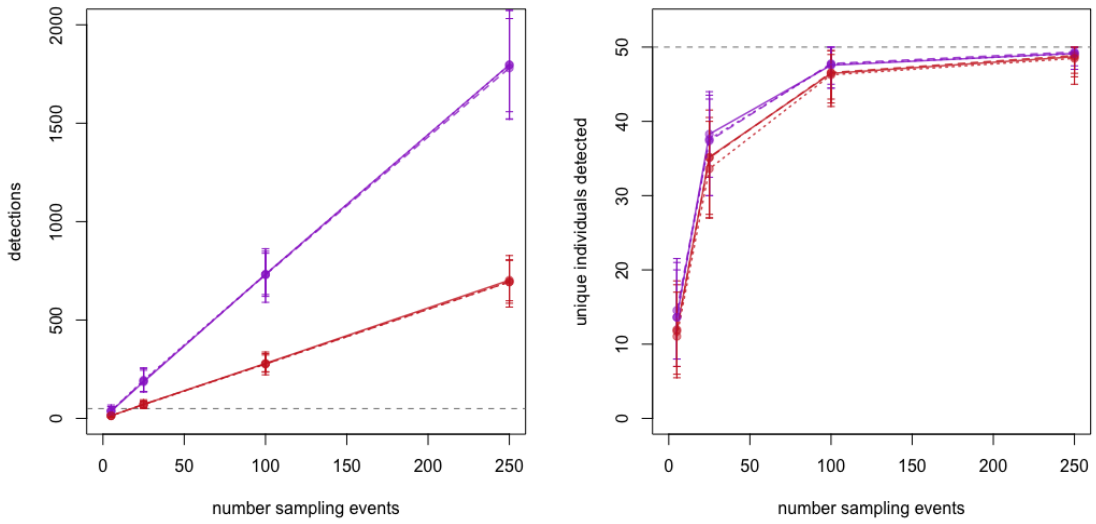

Figure 5. Error rate does not alter total detection (left) or unique detection (right) using either stationary and mobile sampling approaches. In both plots, stationary data is depicted by purple points, lines, and error bars while mobile data is depicted by red points, lines, and error bars. Dots depict means across 100 replicates and the error bars are the 95% confidence intervals around those means. Solid, dashed, and dotted lines depict 0.001, 0.01, and 0.1 individual identification error rates, respectively.

We assessed how population size influenced suitability of stationary and mobile techniques by varying the number of simulated individuals on the landscape. As we observed with varied efforts (Figure 4) and error rates (Figure 5), we found that the stationary sampling approach had more detections compared to the mobile approach but that there was no difference between the number of unique samples or detections within each population size level comparison (Figure 6). Here, we note two interesting trends detected in these data. First, the difference between the number of unique individuals detected and simulated population size increased as simulated population size increased (upper stationary estimates: 18, 45, 90; upper mobile estimates: 15, 38, 77). Second, even when these estimates were scaled by population size (upper stationary estimates: 7%, 9%, 10% unsampled individuals; upper mobile estimates: 5%, 14%, 15%), larger population sizes seem to be better sampled by the stationary sampling approach compared to the

mobile approach. Combined, these may mean that at even larger population sizes, stationary approaches may capture a larger proportion of the population compared to mobile sampling approaches (Figure 6).

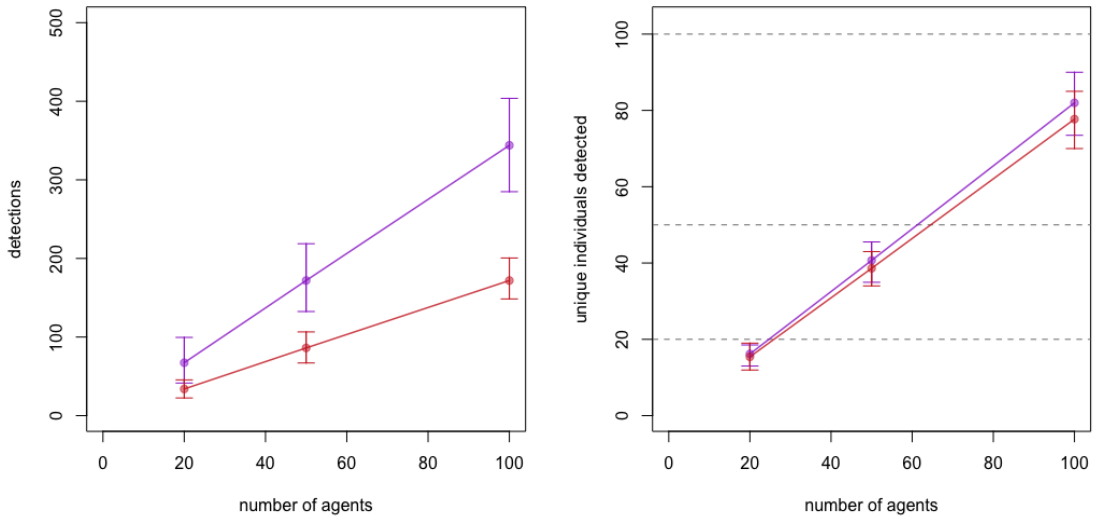

Figure 6. Population size does not alter effectiveness of total detection (left) or unique detection (right) using either stationary and mobile sampling approaches in the parameter space we examined. In both plots, stationary data is depicted by purple points, lines, and error bars while mobile data is depicted by red points, lines, and error bars. Dots depict means across 100 replicates and the error bars are the 95% confidence intervals around those means.

Finally, we considered how study duration can change the relationship between detections in stationary and mobile sampling approaches. Considering the breadth of estimates across different study durations, we found that more days collecting data lead to increased detections and that the rate of increase lessened as days increased (Figure 7). We found that when study duration was equal, the stationary method always had more detections than mobile method even when studies were relatively short. However, both stationary and mobile sampling approaches had the same number of unique detections (Figure 7).

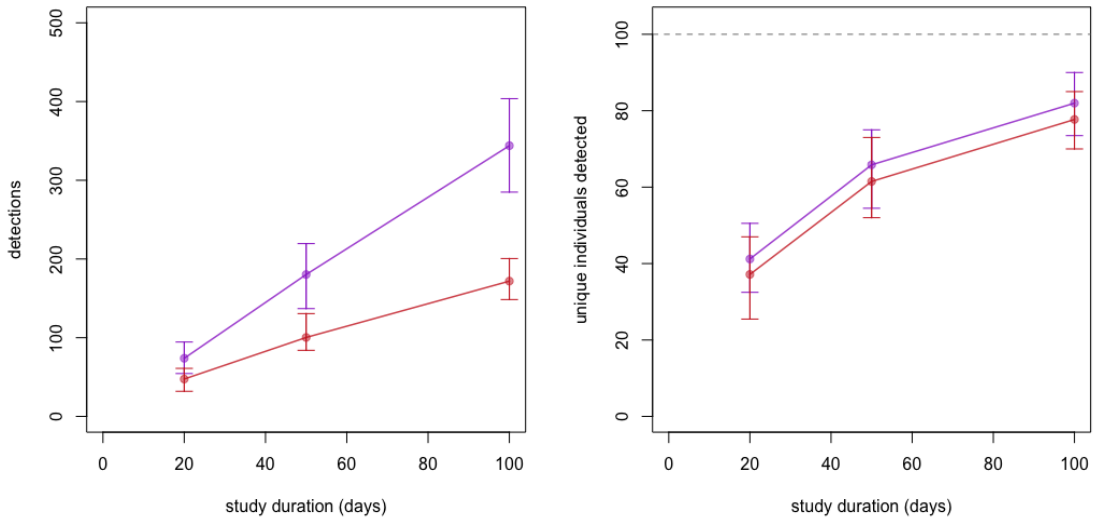

Figure 7. Study duration does not alter effectiveness of total detection (left) or unique detection (right) using either stationary and mobile sampling approaches in the parameter space we examined. In both plots, stationary data is depicted by purple points, lines, and error bars while mobile data is depicted by red points, lines, and error bars. Dots depict means across 100 replicates and the error bars are the 95% confidence intervals around those means.

Discussion

In our model, we considered how spatial structure of sampling individuals from wild populations (stationary or mobile approaches) influenced the number of detections and the number of unique detections. As expected, when the number of sampling events per day increased, this increased the total number of detections as well as the number of unique detections in both stationary and mobile sampling schemes. Interestingly, there were more total detections when using stationary sampling approaches than mobile approaches but both approaches detected the same number of unique individuals (or took samples from the same number of unique individuals; Figure 4). This distinction is important because repeated sampling of individuals can be a burden on analyses because it is time consuming to processes these data and storing large amounts of repeated data can also be costly (Adams et al. 2017). For example, combing through repeated images from

camera traps or processing extra DNA samples for genetic analyses can result in increased labor costs, data storage, and reagent use. However, repeat sightings and samplings can be useful; estimating probability of detection for particular areas requires repeat sampling as does tracking parasite burden and clearing rates (Steenweg et al. 2019, Shearer and Ezenwa 2020). Because of this, planning for sample collection requires consideration of the number and type of repeats necessary and, when the number of replicates needed is low, it may be worthwhile considering more efficient mobile sampling schemes over station efforts.

We assessed how population size influenced suitability of stationary and mobile techniques and found that the stationary sampling approach had more detections compared to the mobile approach but that there was no difference between the number of unique samples or detections within each population size level comparison (Figure 6). However, two interesting trends were present in our data: increasing difference between the number of unique individuals detected and simulated population size increased as simulated population size increased and that larger population sizes seem to be better sampled by the stationary sampling approach compared to the mobile approach. Although the 95% confidence intervals overlapped at each population size we used, we speculate that as population size increases the number of unique detections will continue diverge. This suggests that at very large population sizes, stationary approaches may capture a larger proportion of the population compared to mobile sampling approaches. However, balancing this potential benefit with the costs of increased data collection, storage, and analyses (Figure 4) will require careful consideration of species and habitat specific variables.

A limitation of our study was the movement rules for the agents on the landscape. The difference in the total number of detections from both sampling approaches could have been impacted by

the agent's movement as the ability to move in any direction would allow the possibility for the agent to return to the same stationary sampling location. This could have resulted in multiple detections counted when the agent would return to that stationary sampling station, or when the agent decided not to move from that particular cell for one or two consecutive runs (caused by the 5% chance of the agent staying in the same cell). This could potentially be incorporated into the model by using variable distance values that an agent can move throughout a landscape. Alternatively, future models could incorporate spatial clustering of agents (e.g., home ranges) or preference in landscape use to see if this affects the differences in detections between sampling methods.

As research and knowledge of wildlife diseases grows so does the opportunity to control and prevent diseases, and monitoring of wildlife populations and disease is an important part of disease ecology (Wagnon and Serfass 2016). Improving disease surveillance and sampling of wildlife populations could lead to better detection, understanding, and preparedness to protect wildlife species across a wide range of zoonotic disease (Nusser et al. 2008). Importantly, our ability to detect disease early depends on our ability to efficiently collect and analyze samples to inform our understanding of the potential spread of the disease in a given space and time (Wagnon and Serfass 2016). Therefore, adequate sampling effort and sampling groups is needed to properly monitor disease (Wagnon and Serfass 2016). Based on our simulations, we suggest a stationary approach to collecting samples for disease analyses. For many situations and species, sampling in set locations, is more likely to generate the time series data needed to understand infection and clearing rates because it will provide the more data on single individuals compared to sampling locations that move across the landscape. However, this may only be true for species that move over somewhat small geographic areas, as wide-ranging or migratory species were not

considered in our models. Despite these unknowns, monitoring for these diseases is important as understanding disease can lead to better knowledge and have a positive effect on animal health, public health, and conservation (Wagnon and Serfass 2016).

Conclusions

We developed an agent-based model to simulate the differences in detection when using stationary sampling approaches compared to sampling locations that are moved throughout the landscape. These models have applications to understanding wild population demographics such as population size and density as well as understanding population characteristics such as disease prevalence. We found that the stationary approaches have more detections or sampling events compared to the mobile approaches, but both approaches have equal number of unique detections (i.e. unique individuals detected or sampled). Therefore, when repeatedly sampling individuals is needed, we suggest a stationary approach but when repeated samples are a drain on efficiency and resources a moving sampling location approach is warranted.

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Chapter 3: Management of avian malaria in populations of high conservation concern

Miranda Paez A, Chalkowski K, Zohdy S, Willoughby JR. 2022. Management of avian malaria in populations of high conservation concern. Parasites & Vectors 15:208. https://doi.org/10.1186/s13071-022-05327-2

Abstract

Avian malaria is a vector-borne disease that is caused by *Plasmodium* parasites. These parasites are transmitted via mosquito bites and can cause sickness or death in a wide variety of birds, including many threatened and endangered species. This Primer first provides contextual background for the avian malaria system including the life cycle, geographic distribution and spread. Then, we focus on recent advances in understanding avian malaria ecology, including how avian malaria can lead to large ecosystem changes and variation in host immune responses to *Plasmodium* infection. Finally, we review advances in avian malaria management in vulnerable bird populations including genetic modification methods suitable for limiting the effects of this disease in wild populations and the use of sterile insect techniques to reduce vector abundance.

Keywords: Avian, Malaria, Mosquito, *Plasmodium*

Transmission cycle of avian malaria

Avian malaria is a disease that infects various tissues and blood cells of birds and is caused by >50 parasite species within the genus *Plasmodium* [1]. Malaria parasites require an invertebrate vector and a vertebrate host species to complete the life cycle. Vectors for *Plasmodium* include mosquitoes from the genera *Culex*, *Aedes* and *Culiseta*, and *Plasmodium* species are capable of infecting and completing their life cycle in > 400 species of birds, covering 11 orders [2]. The >

50 parasite species that cause avian malaria differ in characteristics such as host range, geographic distribution, competent vectors and pathogenicity [3]. Because the parasite's vectors and host species are so numerous and varied, the potential for avian malaria to negatively impact species as it spreads to new areas is high, resulting in the decline and even extinction of avian species in these newly invaded ecosystems.

The avian malaria life cycle (Figure 8) starts when a feeding mosquito infects an avian host with *Plasmodium* sporozoites, and sporozoites develop into exo-erythrocytic meronts (i.e. cryptozoites) in reticuloendothelial cells [4, 5]. This is followed by the development of merozoites into the second pre-erythrocytic exo-erythrytic stage, producing metacryptozoites. Further generations of metacryptozoites can be formed from previous generations, or alternatively merozoites from metacryptozoites can enter the bloodstream, infect erythrocytes and become meronts to continue into the erythritic cycle [5]. Merozoites can also develop into the next exo-erythrocytic form, the post-erythritic phanerozoites, which can develop into further generations of phanerozoites or develop into merozoites. From merozoites, the erythrocytic cycle continues with the development of male and female micro/macrogametocytes. These gametocytes are then capable of infecting another mosquito to begin the process of sporogony in this next host [5]. Once inside the mosquito, the gametocytes develop into gametes in the midgut. These gametes then come together to form a zygote, which develops into ookinetes that travel to the epithelium and develop into oocysts. Within the oocysts, infective haploid sporozoites form and, once mature, burst through the oocyst wall. These haploid sporozoites then invade mosquito salivary glands, where they can be transferred to another bird host when the mosquito feeds (Figure 8).

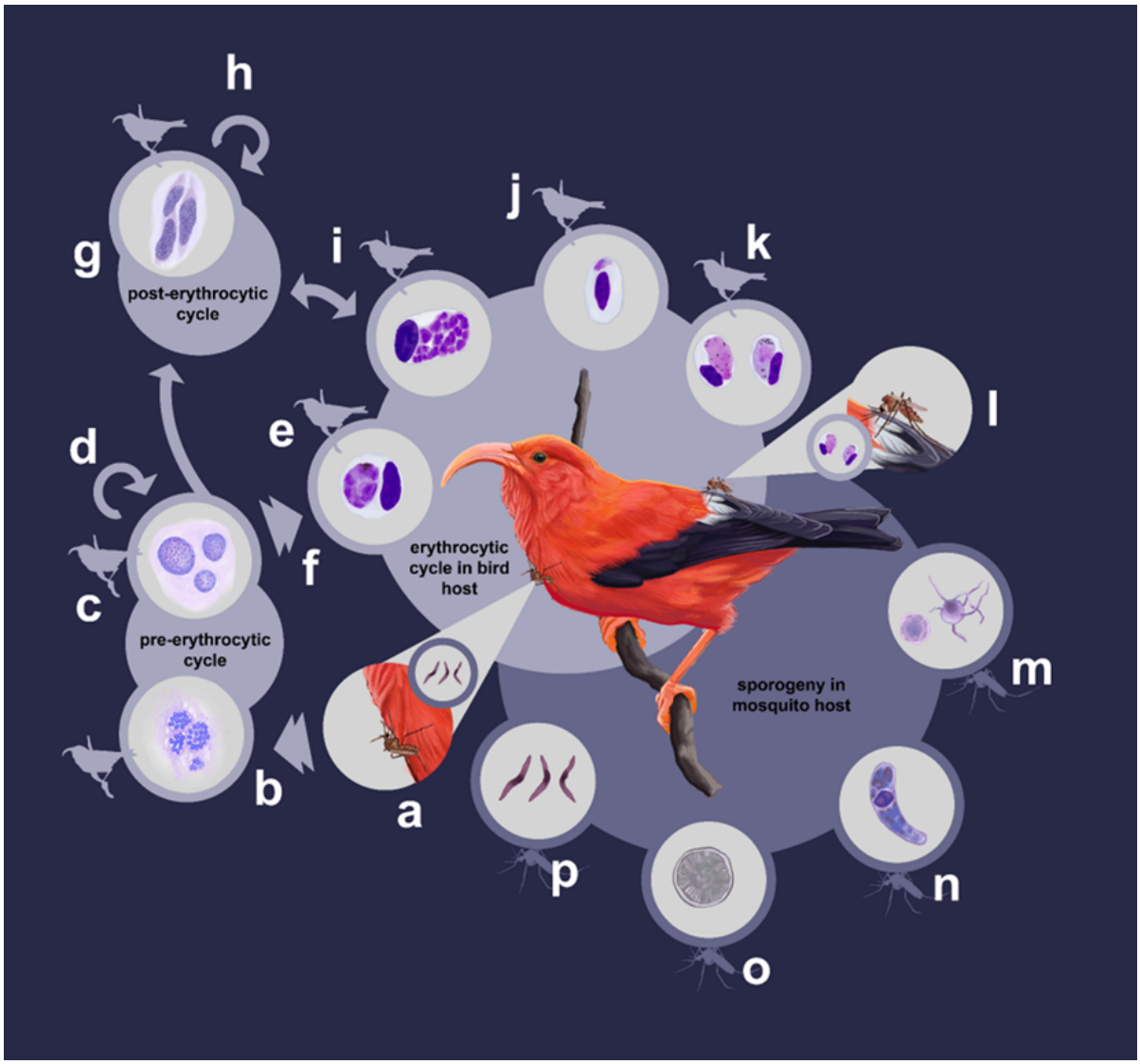

Figure 8. The avian malaria life cycle starts when (**a**) a feeding mosquito infects an avian host with *Plasmodium* sporozoites; (**b**) sporozoites then develop into exo-erythrocytic meronts (i.e. cryptozoites) in reticuloendothelial cells (e.g. spleen, liver, bone) throughout the body (**c**) followed by the development of merozoites into the second pre-erythrocytic exo-erythrytic stage and producing metacryptozoites. **d** Following development into metacryptozoites, further generations of this stage can be formed from previous generations. **e** Alternatively, merozoites from metacrytpzoites can enter the bloodstream, infect erythrocytes and (**f**) become meronts to continue into the erythritic cycle. **g** Merozoites can also develop into the next exo-erythrocytic form, the post-erythritic phanerozoites, which can (**h**) also develop further generations of phanerozoites or (**i**) develop into merozoites. Merozoites can be formed from either phanerozoites or erythrocytic meronts. From (**j**) merozoites, the erythrocytic cycle continues with the development of (**k**) male and female micro/macrogametocytes. These gametocytes are

then (**l**) capable of infecting another mosquito to begin the process of sporogony in this next host. Once inside the mosquito, the gametocytes develop into (**m**) gametes in the midgut. These gametes come together to form (**n**) a zygote, which then develops into ookinetes that travel from the midgut to the epithelium, (**o**) followed by development into oocysts. Within the oocysts, (**p**) infective haploid sporozoites form and burst through the oocyst wall once reaching maturity. These haploid sporozoites then invade mosquito salivary glands, where they can be transferred to another bird host when the mosquito feeds.

Distribution and spread of avian malaria

The native ranges of *Plasmodium* parasites are distributed worldwide, in diverse habitats (e.g. Nearctic, Palearctic, Oriental, Neotropical and Australian ecozones) [2], and co-vary with similarly wide-ranging ornithophilic mosquito vectors such as *Culex quinquefasciatus* [6]. In addition to this wide historic distribution, *Plasmodium* parasites have been introduced to new regions where highly virulent species have led to substantial population declines and extirpations of endemic birds [7]. For example, native avian populations in the Hawaiian Islands and New Zealand have undergone widespread population declines associated with the introduction and spread of avian malaria. Understanding how these introductions have occurred is important to predict how new diseases and vectors of conservation concern will spread and alter other ecosystems and species.

Introduced parasites require all components of their life cycle to thrive in the new ecosystem [8]. For *Plasmodium* to invade a new ecosystem, susceptible bird hosts and a competent mosquito vector are required. There are at least two possible ways avian malaria may have invaded ecosystems: (i) introduction from infected migrating birds and (ii) the release of infected, non-native passerines into the ecosystems. For example, the Bobolink (*Dolichonyx oryzivorus*) in the Galapagos Islands is a *Plasmodium*-carrying passerine that breeds across North America and migrates to central South America, and it is the only passerine with migratory stopovers in the

Galapagos [9]. Bobolinks harbor a high diversity of haemosporidian parasites, and analysis of avian malaria parasite lineages across the Galapagos suggests that Bobolinks may have contributed to the spread of avian malaria to these islands because of their migratory behavior [10]. Similarly, in the Hawaiian Islands, shorebirds and other waterfowl are known avian malaria carriers and may have brought these parasites to these islands [11]. Alternatively, avian *Plasmodium* may have arrived in Hawai'i via introduced bird species that carried these parasites and were released into the wild [11]. In the early nineteenth century, several species of nonnative passerine birds were introduced into the Hawaiian ecosystem, many of which were competent carriers of avian malaria parasites. As these introduced species spread, these birds may have also spread the parasites they carried into the wild avian populations [11].

In addition to the introduction of the parasite, the establishment of *Plasmodium* species in new areas requires a competent mosquito vector to sustain the transmission cycle. In Hawai'i specifically, lack of a competent vector likely limited the spread of the *Plasmodium* parasite; although migrating shorebirds and waterfowl likely brought *Plasmodium* species to the islands for thousands of generations, the parasite populations could not have sustained themselves because there were no competent mosquito vectors. However, this was no longer a limiting factor after 1826 when the mosquito *Cx. quinquefasciatus* was unintentionally introduced to the Hawaiian islands along with marine cargo, providing the necessary vector to establish avian malaria *Plasmodium* transmission in the Hawaiian Islands [11].

Recent advances in understanding avian malaria evolutionary ecology

Interaction of avian malaria and ecosystem features

Understanding the mechanisms and rates of *Plasmodium* and vector introduction and spread into new ecosystems is critical to prevent population declines and extinctions in immunologically naive bird populations. For example, *Plasmodium* has restricted the range of Hawaiian Honeycreepers. This shrinking distribution is due to *Plasmodium* transmission being limited at higher altitudes where lower temperatures reduce mosquito reproduction and *Plasmodium* development, effectively pushing surviving Honeycreeper populations to higher and higher altitudes [12]. Because of this relationship between mosquito and *Plasmodium* development with temperature, global climate change is likely to further restrict the range of *Plasmodium*-free habitats for many bird species, putting further pressure on these ecosystems. For example, Loiseau et al. [13] demonstrated that *Plasmodium* is transmitted in northern Alaska and also predicted continued range expansion for *Plasmodium* as the area continues to warm. Through these changes, additional populations are expected to become infected with avian malaria and spread *Plasmodium* parasites. Similarly, mechanistic models focused on Hawaiian populations predicted that climate-driven environment and disease patterns will continue to substantially reduce available habitat for native bird populations [14]. Because many Hawaiian passerines are already at high extinction risks due to habitat loss and the introduction of non-native predators, the additional pressure of avian malaria in this and similar systems (e.g. New Zealand and Galápagos Islands) is of high conservation concern [6]. Understanding how to predict the effects of avian malaria and how to limit new vector pathways into additional ecosystems is critical to ongoing conservation action to prevent extinction in these high-risk avian species [14].

Immune response to avian malaria infection

The survival of avian hosts infected with *Plasmodium* parasites is dependent on the host immune response and its efficiency in detecting and removing *Plasmodium*. One important aspect of adaptive avian immunity is the major histocompatibility complex (MHC). The MHC is essential for survival as it governs the host's ability to detect pathogens, affecting its susceptibility to infections and diseases [15]. The occurrence of specific MHC variants correlates with parasite burden in many Aves species [16], suggesting host genes can influence host fitness by conferring tolerance. In Great Tits (*Parus major*), tolerance to malaria is conferred by two MHC supertypes; individuals with these supertype MHC variants have greater tolerance to malaria compared to individuals without these variants. Importantly, each MHC variant confers tolerance to malaria from a different parasite source (*P. circumfexum* and *P. relictum*) and does so by limiting the physiological effects of infection, not by preventing infection outright [16]. This suggests that susceptibility to avian malaria by the host is dependent on both the virulence of the parasite species and the host's immunity to the parasite. Immune responses are also influenced by previous exposures to parasites and diseases. In Canaries (*Serinus canaria*), for example, mortality decreases after reinfection with *Plasmodium* compared to the proportion of individuals that succumb to the first *Plasmodium* infection [17]. Importantly, these effects are not limited to recovery from infection of malaria: *Plasmodium*-infected canaries subjected to a secondary immune challenge are not as effective at eliminating *Plasmodium* compared to those that did not have a secondary challenge. This suggests that there is a tradeoff between control of chronic malaria infection and reaction to new host infections and that this tradeoff may manifest in substantial lifelong effects.

Some immunologically naive populations can evolve resistance and tolerance to avian malaria through natural selection. However, remote island species with reduced genetic variation, such as the Hawaiian species ‘I’iwi (*Drepanis coccinea*) and other species of conservation concern, may lack the genetic diversity or variation in disease response to support adaptation (i.e. no individuals survive infection) [18]. To overcome this, some researchers suggest turning to new gene-editing technologies to bolster the immune system against avian malaria, ultimately helping at-risk populations to recover demographically [18]. For ‘I’iwi, simulated release scenarios suggested that releasing gene-edited ‘I’iwi at midelevation forests would substantially reduce extinction risk in the long term [18]. Although this approach has the potential to be feasible and successful, the cultural and ecological implications surrounding modifications of this nature require careful considerations prior to the release of any gene-edited individuals [18]; more research is needed to understand these gene-controlling mechanisms, how introduction of genes can affect wild populations and how these genes will be introduced to wild populations efficiently.

Incompatible insect technique

Mosquitoes are an essential part of the transmission cycle of avian malaria, as they are the link between infected and uninfected birds. Therefore, one potential way to reduce transmission of avian malaria is to control mosquito populations via chemical means. For example, in New Zealand, the use of insecticides targeting two invasive mosquito species (*Ae. camptorhynchus* and *Cx. sitiens*) led to eradication of these species on the treated island [6]. In contrast, some African populations of *Cx. quinquefasciatus* have evolved resistance to commonly used insecticides, substantially hindering the ability to control mosquito populations [12–21]. The observed concern over the effectiveness of an insecticide-based vector control program has led to

an interest in shifting to control by sterile insect techniques (SITs) [22]. Such methods have been successfully used in many insect pest species (e.g. New World screwworm fly, *Cochliomyia hominivorax*; tsetse, *Glossina spp.*) [22]. Males of the target species that are sterilized by radiation or chemicals, genetically modified with lethal genes or harbor incompatible endosymbionts are released into the wild to mate with females, resulting in infertile eggs [23]. Future research to manage and mitigate avian malaria can focus on these and related mechanisms as they provide potential revolutionary mosquito control.

Future management and mitigation of avian malaria through genetic modifications

Limiting vector populations can occur via several genetic manipulations, including chemosterilization, engineered transgenes, and application of endosymbionts like *Wolbachia* [23–25]. When successful, these modifications are expected to substantially reduce population sizes. However, modifications can also be quickly selected against and removed from the population when the mutations or other genetic changes have large and negative effects on survival [26].

Using a chemosterilization approach

The use and effectiveness of chemosterilization via a sterile insect technique (SIT) in mosquito control has been extensively tested on *Plasmodium* vectors such as *Cx. quinquefasciatus*. One effective example of SIT deployment in this species has occurred on the island of Seahorse Key; when sterilized male mosquitoes were released onto the island, the island's larval *Cx. quinquefasciatus* populations were eliminated [27]. However, other attempts to emulate this approach have been less successful because of immigration of mated females from other populations that bypass the sterile male issue [28]. As a result of this spotty effectiveness, new

techniques for generating sterile insects may be required, including radiation-based efforts that have been trialed in the apple moth (*Teia anartoides*) in New Zealand [29]. In addition, environmental concerns, mating competitiveness and political climate that has limited the widespread use of sterile insect approaches will have to be overcome before these methods can be deployed widely [6].

Using genetic engineering systems

The CRISPR-Cas genetic engineering system, which targets specific sequences and results in functional genetic change, has shown great potential in limiting mosquito vector population sizes. In mosquitos, several genes have been identified that would be a suitable gene target for the CRISPR system. These systems work by identifying a homing region where a cargo gene that reduces carrier fitness can ultimately be inserted [30]. As these engineered genes spread through a population, the population declines because of reduced fitness [6]. Importantly, these genetic changes can be engineered to be self-limiting; in the Oxitech™ system, female offspring resulting from mating between a male with the cargo gene and a wild female do not survive, providing important safeguards to the modified mosquito system [31]. While this genetic engineering system has merit, use of these technologies requires additional development of safety and regulatory measures. For example, the use of genetic control systems can have negative impacts on the endemic New Zealand *Culex* species, limiting the ways the system could be applied in New Zealand [6]. Using this strategy will require advances for limiting unwanted consequences and conducting in situ tests to determine modification efficiency [32].

Using endosymbionts

Endosymbionts like *Wolbachia* can also be used to control certain mosquito populations and reduce the occurrence of avian malaria. In some species, when *Wolbachia*-carrying male mosquitoes were released and mated with wild females, the resulting eggs did not hatch and population size was reduced [6]. This approach to limiting mosquito population size has been successful in *Ae. aegypti* in several locations, including the west coast of North America [33]. *Wolbachia* infections have also been deployed as a pathogen-blocking mechanism when infection was not used to prevent egg hatching but instead to block vector parasite infection, diminishing vector competence and disease transmission [34]. However, this approach has its limitations. For example, *Cx. pipens* mosquitos are naturally infected with *Wolbachia* [35], and having a *Wolbachia* infection can increase a mosquito's susceptibility to *Plasmodium*. This suggests that *Cx. pipens* infected with *Wolbachia* can be better vectors of avian malaria [36]. Thus, the decision to use endosymbiont control methods for mosquito populations requires species-specific information and careful monitoring of mosquito populations. In addition, future work is needed to develop methods for moving and releasing millions of modified mosquitoes to remote locations. Finally, understanding the distribution and diversity of *Wolbachia* and their dynamics with mosquito hosts is important for planning future *Wolbachia*-based control programs [22].

Conclusions

Avian malaria has caused the decline and extinction of many bird species globally [6]. Since birds are intercontinental migrants, and avian malaria-causing parasites are found worldwide, addressing transmission in the avian malaria system is exceedingly complicated [37]. However, many individuals and species exhibit some resistance or tolerance, suggesting persistence of

these species is possible. Targeting the mosquito vector populations may improve health outcomes for many species with mosquito-borne diseases, including humans. However, these vector managing strategies are not without their own risks as each species of mosquito and parasite differs in their relationship to control molecules and their effects on disease spread. Future work is needed in this innovative space on vector control approaches that consider parasite-host eco-evolutionary processes, and research that could help control mosquito vectors of disease relevant to conservation and public health.

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