BENCHMARKING BIODIVERSITY IN AN ERA OF RAPID CHANGE

EDITED BY: W. Douglas Robinson, Patrick Jansen and Carlos A. Peres
PUBLISHED IN: Frontiers in Ecology and Evolution

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88974-115-1
DOI 10.3389/978-2-88974-115-1

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BENCHMARKING BIODIVERSITY IN AN ERA OF RAPID CHANGE

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Citation: Robinson, W. D., Jansen, P., Peres, C. A., eds. (2022). Benchmarking Biodiversity in an Era of Rapid Change. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88974-115-1

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frontiers in Ecology and Evolution

EDITORIAL
published: 02 December 2021
doi: 10.3389/fevo.2021.810287

Editorial: Benchmarking Biodiversity in an Era of Rapid Change

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Keywords: biodiversity baselines, benchmarking biodiversity, environmental change, monitoring wildlife, conservation

Editorial on the Research Topic

Benchmarking Biodiversity in an Era of Rapid Change

Human activities are amplifying the dynamic nature of Earth's climate and reshaping its landscapes and ecosystems (Ellis et al., 2020), justifying a preeminent need to characterize, identify, quantify, map, and archive data on all forms of terrestrial and aquatic biodiversity (Magurran et al., 2010). Several global efforts have indexed the status of biodiversity but typically at coarse levels of spatial resolution (e.g., the IUCN Red List). Highly endangered species are sometimes understood in great detail, but their roles in contemporary ecosystems are usually comparatively minor. For the vast majority of Earth's biodiversity, even basic taxonomy is poorly resolved (Winfree et al., 2015). Meanwhile, patterns of distribution, abundance and shifting community composition remain poorly quantified even for many of the best-known organisms (Magurran et al., 2018). How shall we truly understand biodiversity responses to environmental change without the anchor of adequate baselines?

In this special issue we introduce different perspectives on benchmarking biodiversity. Benchmarking is the creation of baseline measurements of distribution, abundance, genetic characteristics, and ecological roles of biodiversity. Beyond monitoring studies and one-off characterizations of baselines, benchmarking intentionally uses precisely repeatable methods and archives detailed data to maximize alignment with future replication, thereby promoting rigorous quantification of change through time (Robinson and Curtis, 2020). Widespread use of highly repeatable survey and counting methods can have the obvious benefit of unequivocally demonstrating how biodiversity responds to climate and other forms of inevitable change (Robinson et al., 2020). Given rapidly improving information on taxonomy, the rise of collaborative efforts with citizen scientists, massive public online databases, and GPS-based mapping, we live in an era when reliably benchmarking Earth's biodiversity is not only more feasible than ever but should be one of society's top priorities.

Aside from providing opportunities for future generations to rigorously quantify change, benchmarking biodiversity also creates opportunities for human improvement. It rewards skilled naturalists for their expertise (Tewksbury et al., 2014), improves training of new generations of scientists and the public to improve their understanding of the ecological roles and importance of diverse organisms (Theobald et al., 2015), facilitates current academic investigation of theoretical and empirical ideas (Dornelas et al., 2014; Gotelli et al., 2017), helps society improve their temporal perspective on natural and human-facilitated environmental change (Willis and Birks, 2006), and informs data-driven policy decisions affecting management and societal priorities (Santamaría and Méndez, 2012).

Although this special issue focuses on distribution, abundance, and genetics, present-day knowledge acquisition on a variety of aspects of biodiversity is sorely needed. Knowledge gaps

Edited and reviewed by:
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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *06 November 2021*
Accepted: *16 November 2021*
Published: *02 December 2021*

Citation:
Robinson WD and Peres CA (2021) Editorial: Benchmarking Biodiversity in an Era of Rapid Change. Front. Ecol. Evol. 9:810287. doi: 10.3389/fevo.2021.810287

have been categorized into eight groups: (i) Linnean (taxonomic discrepancies), (ii) Wallacean (species distributions), (iii) Prestonian (population ecology), (iv) Darwinian (species evolution), (v) Raunkiaeran (species traits), (vi) Hutchinsonian (abiotic tolerances), (vii) Eltonian (species interactions) (Hortal et al., 2015), and (viii) Parkerian shortfalls (Lees et al., 2020). These shortfalls in knowledge, when addressed adequately through careful collection of basic natural history knowledge combined with detailed evaluation of population, genomic and physiological information, may be remedied with systematic spatially-explicit species inventories and abundance information (Hortal et al., 2015).

The eight papers in this special issue inform topics tied directly to benchmarking biodiversity. Major gaps in our understanding of even some of the most charismatic, popular and most widely observed organisms, such as birds, are demonstrated by the near total absence of rigorous local abundance data for the most diverse continent, South America (Robinson, Errichetti et al.). A call for expanding a currently small ($N = 6$) network of large survey plots (100 ha or larger) to make precisely repeatable community inventories and abundance measurements is a feasible plan. Likewise, contemporary society has been put on alert recently that insect populations may be declining globally, yet we have few long-term benchmarking data available. Debates about trends and quantities of change are best settled with data. Standardizing reliable methods is a critical initial step. Montgomery et al. establish such standards for benchmarking insect populations. Beyond whole organisms, responses of genes and genomes to change are rarely evaluated in the context of short-term dynamics, yet we now have the technical means to analyze genetic samples from the distant past, now and to preserve them for future evaluation as technical knowledge improves (García and Robinson). Benchmarking biodiversity is an enormous task that may be enhanced with widespread public collaboration as internet-based opportunities expand interactive data archival resources. Contributions by untrained observers raise concerns, especially with abundance estimation, as demonstrated in a rare comparison of bird abundance data gathered by professionals and amateur birders (Robinson, Hallman et al.). Likewise, new technologies may facilitate better data collection and assessment of errors, as well as push measurements toward estimates of density and even population sizes, as demonstrated with camera traps (Green et al.). Moving forward, benchmark data allow assessments of geographic range shifts (Wilson et al.) and comparisons of biodiversity change as a function of disturbances, from smaller scale events such as mining (Lynggaard et al.) to extensive ones such as wildfires (Catullo et al.).

Time is ripe for appreciating the value of carefully collected, vetted biodiversity data gathered with precisely repeatable methods to allow humanity the best chance to understand how life responds to change on our dynamic planet. The longer we postpone the political will to undertake this task at a meaningful scale, the more incomplete our best baselines will become, and the more expensive measures to restore wild nature will become.

AUTHOR CONTRIBUTIONS

WR and CP wrote the manuscript, edited the manuscript, and administered the Research Topic collection. Both authors contributed to the article and approved the submitted version.

FUNDING

WR was supported by the College of Agricultural Sciences and the Mace Professorship at Oregon State University.

ACKNOWLEDGMENTS

We thank all authors and reviewers that contributed to this Research Topic and the staff at Frontiers in Ecology and Evolution for their administrative support throughout this process.

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frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 01 December 2020
doi: 10.3389/fevo.2020.590976

DNA-Based Arthropod Diversity Assessment in Amazonian Iron Mine Lands Show Ecological Succession Towards Undisturbed Reference Sites

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *03 August 2020*
Accepted: *10 November 2020*
Published: *01 December 2020*

Citation:
Lynggaard C, Yu DW, Oliveira G, Caldeira CF, Ramos SJ, Ellegaard MR, Gilbert MTP, Gastauer M and Bohmann K (2020) DNA-Based Arthropod Diversity Assessment in Amazonian Iron Mine Lands Show Ecological Succession Towards Undisturbed Reference Sites. Front. Ecol. Evol. 8:590976. doi: 10.3389/fevo.2020.590976

Human activities change natural landscapes, and in doing so endanger biodiversity and associated ecosystem services. To reduce the net impacts of these activities, such as mining, disturbed areas are rehabilitated and restored. During this process, monitoring is important to ensure that desired trajectories are maintained. In the Carajás region of the Brazilian Amazon, exploration for iron ores has transformed the original ecosystem; natural forest and a savanna formation with lateritic iron duricrust outcrops named *canga*. Here, native vegetation is logged and topsoil removed and deposited in waste piles along with mine waste. During rehabilitation, these waste piles are hydroseeded with non-native plant species to achieve rapid revegetation. Further, seeds of native *canga* and forest plant species are planted to point ecological succession towards natural ecosystems. In this study, we investigate diversity and composition of the arthropod community along a post-mining rehabilitation and restoration gradient, taking seasonality and primer bias into account. We use DNA metabarcoding of bulk arthropod samples collected in both the dry and rainy seasons from waste-pile benches at various stages of revegetation: non-revegetated exposed soils, initial stage with one-to-three-year-old stands, intermediate stage with four-to-five-year-old stands, and advanced stage with six-to-seven-year-old stands. We use samples from undisturbed *cangas* and forests as reference sites. In addition, we vegetation diversity and structure were measured to investigate relations between arthropod community and vegetation structure. Our results show that, over time, the arthropod community composition of the waste piles becomes more similar to the reference forests, but not to the reference *cangas*. Nevertheless, even the communities in the advanced-stage waste piles are different from the reference forests, and full restoration in these highly diverse ecosystems is not achieved, even after 6 to 7 years. Finally, our results show seasonal variation in arthropod communities and primer bias.

Keywords: Amazon, arthropods, metabarcoding, mining, rehabilitation

INTRODUCTION

To minimise the negative impact of mining, and similar forms of disturbance, on biodiversity and ecosystem functioning, the mitigation hierarchy (avoidance, minimisation, rehabilitation or restoration, and offsets) sets guidelines to prioritise the actions that should be taken (Rio Tinto, 2004; Bergès et al., 2020). In fact, many countries have a statutory requirement to restore disturbed areas to their original states (SER, 2004) or to rehabilitate them [i.e., restitution of ecosystem structure and functioning, but with a different set of species than the initial ones (SER, 2004; Aronson et al., 2011)]. In order to measure whether biodiversity and/or ecosystem functioning are indeed converging on designated reference (original-state) sites or are moving towards novel assemblages and/or sets of functions (Hobbs et al., 2009), areas under restoration and rehabilitation thus require monitoring (Derhé et al., 2016; McDonald et al., 2016).

Despite the need for monitoring of these areas, no consensus has been reached about which environmental variables are the best indicators for measuring ecosystem state and change (Gastauer et al., 2018, 2020a). However, measurements of vegetation structure such as canopy openness, tree density, vegetation cover and soil organic carbon are commonly used (Wortley et al., 2013; Lorenz et al., 2019). Specifically, basal area, tree density, tree species richness and leaf area index have previously been shown to be important when assessing environmental rehabilitation status (Ruiz-Jaen and Mitchell Aide, 2005; Gastauer et al., 2020a). Alternatively, arthropods can be used, as they directly make up a large proportion of terrestrial biodiversity and because arthropod species diversity and composition closely follow the diversity and composition of plant species (Basset et al., 2012; Zhang et al., 2016), providing a convenient way to measure both sets of taxa.

Arthropods occur in high abundance and are easily sampled in so-called 'bulk samples' (Rosenberg et al., 1986). However, morphological taxonomic identification of individual arthropods in such samples requires taxonomic expertise across multiple groups and a significant time investment (Basset et al., 2012). As a result, molecular methods, such as DNA metabarcoding, are increasingly being applied to identify taxa within bulk arthropod samples (Yu et al., 2012; Morinière et al., 2016; Liu et al., 2018). DNA metabarcoding is a synonym for parallel and targeted sequencing of taxonomically informative marker regions (Taberlet et al., 2012). Here, taxonomically informative gene sequences are targeted with a metabarcoding primer set and PCR amplified. The resulting sequences can then be used to generate sample-by-species tables and afterwards as input into standard community-ecology analyses. However, the choice of metabarcoding primer set is an important decision, as PCR amplification bias can occur when having mismatches between the primer and the target sequences (Krehenwinkel et al., 2017). Metabarcoding of bulk arthropod samples has been used to quantify the biological impacts of logging and planting oil palm (Edwards et al., 2014); to characterise the diversity of insect samples in montane landscapes in tropical southern China (Zhang et al., 2016); to monitor temporal changes in arthropod communities in different forest types (Brandon-Mong et al., 2018); to measure biodiversity response to subtle differences in forest environmental condition (Barsoum et al., 2019); to follow changes in an invertebrate community in an ecosystem under restoration after sand mining (Fernandes et al., 2019); and to assess reclamation trajectories after mining (Gervan et al., 2020) [i.e., when the area again has a useful function (SER, 2004)]. However, we are far from defining baselines for assessing rehabilitation and from understanding the multiple factors that influence arthropod diversity in post-mining areas under rehabilitation, such as the season in which samples were collected and primer bias.

An example of areas under rehabilitation can be found in the Carajás region in the Eastern Amazon in Brazil. The region is dominated by evergreen and semideciduous submontane forest formations that cover hillsides and lower portions of the landscape. On mountain tops, banded iron formations outcrop, forming a patchy, hyperdiverse, endemic savanna-like ecosystem, locally known as *canga* (Nascimento et al., 2019). Open-cast iron mining has transformed evergreen forests and *cangas* into mine pits and waste piles, which require environmental rehabilitation (Gastauer et al., 2020b). During the mining process, the native vegetation is cut back, the topsoil removed, and together with mine waste, deposited in waste piles (Nascimento et al., 2019). To achieve environmental rehabilitation, the waste-pile benches are hydroseeded with a mix of fertilizers, organic composts, and fast-growing, non-native, non-invasive plant species, to achieve rapid vegetation cover, establish photosynthesis on the site, incorporate biomass into the system, and to attract seed-dispersing fauna. At the same time, seeds of selected native *canga* and forest species are applied, with the longer-term objective of achieving the restoration of the original *canga* and native-forest ecosystems.

In this study, we investigate the trajectories of arthropod communities in post-mining areas under environmental rehabilitation in the Carajás region, by using DNA metabarcoding of bulk arthropod samples collected during the dry and rainy seasons from a temporal gradient of waste-pile sites following iron mining and from untouched *canga* and native-forest reference sites. Specifically, we aim to (i) measure whether the oldest waste piles have or have not achieved restoration of the original assemblages or whether new assemblages are emerging, (ii) assess whether arthropod composition is correlated with local vegetation composition or structure (separately measured) and (iii) assess whether our results are robust irrespective of metabarcoding primer set and season.

MATERIALS AND METHODS

Study Sites

Using Malaise traps, we collected bulk arthropod samples in September 2017 (dry season) and April 2018 (rainy season) in an iron mining area (06°03′31″S 50°10′37″W) in Carajás National Forest, Pará state, Brazil. The traps were left to collect arthropods for 5 days. A total of 32 bulk arthropod samples were collected: 16 for each season. Propylene glycol was used as the collecting liquid. Traps were installed on waste piles representing different stages after the initiation of environmental rehabilitation, as

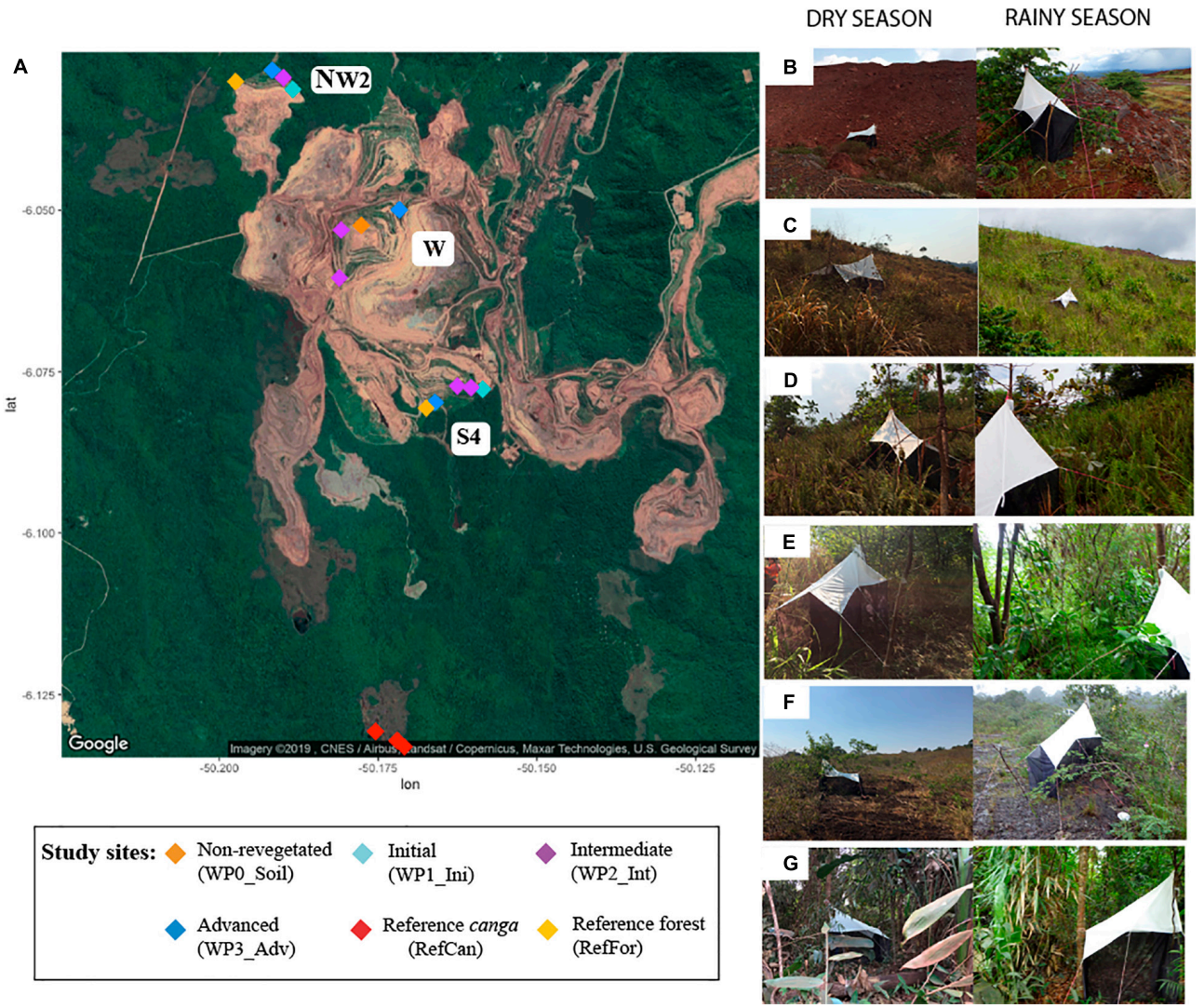

FIGURE 1 | Collection sites in the iron mine in Carajás National Forest, Pará state, Brazil. **(A)** Map of the mine with sample locations: waste piles (W, NW2 and S4) with different rehabilitation stages (non-revegetated, initial, intermediate, and advanced), reference *canga* and reference forest sites close to the waste piles. Pictures of some sites: **(B)** Waste piles with a non-revegetated soil during dry (left) and rainy (right) season showing spontaneous colonization of plants; **(C)** Waste piles with initial status in dry (left) and rainy (right) season; **(D)** Waste pile with intermediate status in dry (left) and rainy (right) season; **(E)** Waste pile with advanced status in dry (left) and rainy (right) season; **(F)** Reference *canga* patch in dry (left) and rainy (right) season; and **(G)** Reference forest in dry (left) and rainy (right) season.

well as within both undisturbed *canga* ecosystems and tropical forest located close to the mine as reference sites (**Figure 1**). Specifically, traps were set in the following locations: three waste piles named West (W), Northwest 2 (NW2) and South 4 (S4), each containing a rehabilitation chronosequence. The different stages of the rehabilitation chronosequence were non-revegetated soils (W), initial stage (one-to-three-year-old stands; NW2, S4), intermediate (four-to-five-year-old stands; W, NW2, S4) and advanced rehabilitation stage (six-to-seven-year-old stands; W, NW2, S4). The two reference forest sites were in NW2 and S4, and the three reference *canga* samples were collected in a *canga* patch near waste piles at S4 (**Figure 1**). Upon collection, samples were transferred to 50 mL Falcon tubes and stored at room temperature until DNA extraction.

To compare arthropod community composition with vegetation structure and diversity, we tagged and identified all trees with stem diameter at breast height $>$ 3 cm within three plots of 10 $\times$ 20 m in each rehabilitation stage from each study site. We estimated tree species richness (S) as the number of species found in each plot, tree density (N) as the number of individual trees in each plot, and basal area (BA) as the cross-sectional area of the tree trunks and stems at breast height. Additionally, the leaf area index (LAI), a surrogate for canopy closure, was measured using a LAI-2200C (LI-COR INC., Lincoln, NE, United States) following the manufacturer's instructions, in which sky conditions were continuously monitored by a sensor in a site free of vegetation. A second sensor was used to capture two below-canopy readings at each

corner and at the centre of each plot, totalling 10 below-canopy readings for each plot. As we retrieved only one bulk arthropod sample per rehabilitation stage per study site, we used the mean values of tree species richness, tree density, leaf area index, and basal area from the three plots from each stage in each waste pile.

DNA Metabarcoding

Prior to DNA extraction, samples were removed from the propylene glycol. DNA was extracted using a non-destructive protocol (Nielsen et al., 2019) modified from Gilbert et al. (2007). To account for possible contamination, a negative extraction control was included every 10 to 20 samples. After DNA extraction, 200 μL of digest were purified using the QiaQuick PCR Purification kit (Qiagen, United Kingdom) following the manufacturer's protocol with minor modifications. Specifically, after the addition of 50 μL elution buffer, samples were incubated at 37°C for 15 min before centrifugation. DNA extracts were stored in LoBind Eppendorf tubes at −18°C until further processing.

Two metabarcoding primer sets were used to PCR amplify arthropod COI markers in DNA extracted from the bulk arthropod samples: (i) ZBJ-ArtF1c (forward 5′-AGATATTGGAACWTTATATTTTATTTTTGG-3′) and ZBJ-ArtR2c (reverse 5′-WACTAATCAATTWCCAAATCCTCC-3′; Zeale et al., 2011) amplifying a ca. 157 bp fragment of the COI gene, hereafter referred to as 'Zeale' and (ii) mlCOIintF (forward 5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′) and jgHCO2198 (reverse 5′-TAIACYTCIGGRTGICCRAARAAYCA-3′; Geller et al., 2013; Leray et al., 2013) amplifying a ca. 313 bp region of the COI gene, hereafter referred to as 'Leray'. Nucleotide tags were added to the 5′ end of primers to allow for parallel sequencing (Binladen et al., 2007). Nucleotide additions consisted of seven to eight nucleotides on both forward and reverse primers, of which six nucleotides were tags and one to two nucleotides were added to increase complexity on the flow cell (De Barba et al., 2014).

Prior to metabarcoding PCR amplification, a subset of the DNA extracts and all negative controls were screened using quantitative PCR (qPCR). This was done to screen for the optimal cycle number, in order to avoid using excessive PCR cycle numbers in the following metabarcoding PCR (Krehenwinkel et al., 2017), to screen for PCR inhibitors by using dilution series (Murray et al., 2015) and to assess contamination [applied in, for example, Bohmann et al. (2018)]. The 20–25 μL qPCR reactions (20 μl for rainy season, 25 μl for dry season) consisted of 1 μL DNA template, 1x Gold PCR Buffer (Applied Biosystems), 2.5 mM $MgCl_2$ (Applied Biosystems), 0.2 mM dNTP mix (Invitrogen), 0.75U AmpliTaq Gold (Applied Biosystems), 0.5 mg/ml Bovine Serum Albumin (BSA; Bio Labs), 0.6 μL of each of the 5′ tagged forward and reverse primer and 1 μl of SYBR Green/ROX solution [one part SYBR Green I nucleic acid gel stain (Invitrogen), four parts ROX Reference Dye (Invitrogen) and 2000 parts high-grade DMSO (Sigma-Aldrich)]. PCR master mixes were set up in a dedicated no-DNA laboratory to minimize contamination risk. The PCR parameters for the Zeale primer were: 95°C for 10 min, 40 cycles of 95°C for 15 s, 52°C for 30 s, then 72°C for 30 s and finally a dissociation curve. For the Leray primer, the PCR parameters were: 95°C for 10 min, 40 cycles of 95°C for 15 s and 51°C for 30 s, then 72°C for 60 s and finally a dissociation curve.

For DNA metabarcoding, tagged PCR was carried out with three PCR replicates for each sample extract, negative extraction control, and positive control. Two positive controls were used: one consisting of a mix of DNA from *Locusta migratoria* (order: Orthoptera), *Tenebrio molitor* (order: Coleoptera) and *Galleria mellonella* (order: Lepidoptera; used with samples from the dry season) and one consisting a mix of DNA from *L. migratoria, T. molitor* and *Blaptica dubia* (Blattodea; used with samples from the rainy season). PCR negative controls were added for every seventh PCR product. PCR amplifications were performed using matching nucleotide tags (e.g., forward primer tag 1 – reverse primer tag 1, forward tag 2 – reverse tag 2, etc.) to account for potential tag-jumps and to avoid false assignment of sequences to samples (Schnell et al., 2015). The three PCR replicates made for each sample DNA extract, negative extraction control, and positive control were carried out with unique tag combinations. PCR reactions were prepared as the qPCRs, except for the omission of SYBR Green/ROX solution and, in the PCR parameters, the replacement of the dissociation curve with a final extension time of 72°C for 7 min. As the qPCR screening showed no PCR inhibition when using undiluted DNA extracts, 1 μl of undiluted DNA extract was used in all metabarcoding PCRs. Further, based on the qPCR screening the following cycle numbers were used: for the Leray primer, 35 and 32 cycles were carried out for the dry and wet season, respectively, while for the Zeale primer, 32 and 28 cycles were carried out for the dry and rainy season, respectively.

Following PCR amplification, PCR products were visualized on 2% agarose gel using GelRed against a 50 bp ladder. All negative controls appeared negative. Prior to library build, PCR products were pooled. Only samples that showed amplification in at least two PCR replicates were pooled. If a sample only had two replicates showing amplification, only those two replicates were added to the pool. PCR products from positive controls and from negative extraction controls were included in the pooling. Amplicon pools were purified using MagBio HighPrep beads (LabLife) and a 1.6x bead-to-amplicon pool ratio and eluted in 35 μl EB buffer (Qiagen). Purified amplicon pools were built into libraries using the TagSteady protocol (Carøe and Bohmann, 2020). Libraries were purified using MagBio HighPrep beads (LabLife) and a 0.8x bead-to-amplicon pool ratio, eluted in 30 μl EB buffer (Qiagen) and quantified using the NEBNext Library Quant Kit for Illumina (New England Biolabs Inc.). Amplicon pools were pooled at equimolar ratio and sequenced 250 bp PE on an Illumina MiSeq v2 sequencing platform at the National High-throughput DNA Sequencing Centre at the University of Copenhagen aiming at 25,000 paired reads per PCR replicate.

Data Analysis

Bioinformatic Processing

Sequence data from each primer set and for each season was processed separately. AdapterRemoval v2.2.2 (Schubert et al., 2016) was used to remove Illumina adapters and low quality

reads, and to merge the paired reads. The Begum package[1] was used to demultiplex sequences based on primer and tag sequences within each amplicon library. For this, two mismatches to primer sequences and no mismatches to tag sequences were allowed. Begum was then used to filter sequences across each of the two to three PCR replicates per sample. Thresholds for filtering sequences followed a restrictive approach as determined by the sequenced negative and positive controls (Alberdi et al., 2018a). Specifically, we retained only sequences that were present in at least two of the two to three PCR replicates per sample, with a minimum copy number of 10 sequences per PCR replicate. To determine the best clustering values, clustering parameters were assessed for each dataset using SUMATRA (Mercier et al., 2013). Based on these results, SUMACLUST was used to cluster sequences into operational taxonomic units (OTUs) with a similarity score of 97% and create a sample-OTU table. To detect and delete erroneous OTUs from the sample-OTU table, curation of the sequences was done using the LULU algorithm with default settings (Frøslev et al., 2017). No OTUs were assigned to the negative extraction controls, and none of the OTUs found in the positive controls were detected in the bulk arthropod samples, indicating no cross-contamination.

Taxonomy was assigned to the OTU sequences using BLASTn against the NCBI Genbank database[2], and the output imported into MEGAN Community Edition v6.12.7 (Huson et al., 2016) using a weighted LCA algorithm with 80% coverage, top percent of 10, and a min. score of 150. Taxonomic order, family and genus information was complemented with data retrieved using the GBIF sequence ID function[3]. OTUs that could not be taxonomically identified as arthropods were discarded from further analyses. Taxonomic names were verified with information retrieved from the Integrated Taxonomic Information System (ITIS)[4]. The OTU table was converted into a presence/absence dataset, as the number of sequences per OTU is not a reliable measure of absolute abundance (Yu et al., 2012; Nichols et al., 2018). Finally, taxonomic diversity was visualised using Krona charts (Ondov et al., 2011).

Community Analysis

Analyses were performed using R 3.5.1 (R Core Team, 2018). To visualise the community composition between samples, we ran a constrained principal coordinate analysis (PCoA) ordination using *cmdscale* from *stats* v.3.5 package, with Jaccard dissimilarity matrices computed using *vegdist* from *vegan* 2.5–6 (Oksanen et al., 2019). To test for differences in composition between rehabilitation stages and reference sites, we used *mvabund* v.4.0.1 (Wang et al., 2012). This was done using the *summary.manyglm* function in *mvabund* and *p*-values we had corrected for multiple tests using the *p.adjust* (method = fdr) function implemented in base R. Differences in arthropod communities between rehabilitation stages (across sites) and between sites (across rehabilitation stages) in the mine were further visualised with an intercept diagram using *UpSetR* 1.4.0 (Conway et al., 2017). Moreover, to partition beta diversity into turnover and nestedness for waste piles in each location, we used *betapart* 1.5.1 (Baselga and Orme, 2012) using Jaccard dissimilarities. Vegetation data such as basal area (BA), tree density (N), tree species richness (S), and leaf area index (LAI) were fitted on this ordination using the function *envfit* from *vegan*. A smooth response surface was fitted using the *ordisurf* function, as a linear relationship cannot be assumed. As LAI data are not available for the *canga*, community data from these sites were removed before fitting that vegetation data. To identify the taxa driving the community differences between the study sites (the waste piles and the reference sites) in each season, taxonomic heat trees were plotted using *metacoder* 0.3.3 (Foster et al., 2017) using the combined information from both primer sets for a more complete overview, and using presence and absence data.

To investigate the estimated OTU richness (Chao2, q of 0) and Shannon diversities (q of 1), we used the function specpool in *vegan* and iNEXT 2.0.19 (Hsieh et al., 2016). In addition, to compare the estimated species richness between rehabilitation stages, we performed a Welch's *t*-test with a Bonferroni correction. Although clustering parameters were assessed during bioinformatic processing, it is possible that OTUs were oversplit, which would make alpha diversity estimates based on phylogenetic diversity more robust (Wang et al., 2019). To obtain information about the phylogenetic diversity of arthropods in each site, curated OTU sequences for each primer set were aligned using *muscle* v3.8.31 (Edgar, 2004). To build an ultrametric phylogenetic tree, a Bayesian phylogenetic inference was performed using a Markov chain Monte Carlo (MCMC) with 10,000,000 steps using BEAST 2.5 (Bouckaert et al., 2019), outputting trees every 1,000 steps. The resulting 10,000 trees were analysed with Tracer v1.7.1 (Rambaut et al., 2018) and burned using the burntrees.pl script (available at https://github.com/nylander/Burntrees/blob/master/README.md), leaving only the last 5%. These 500 trees were summarised on a maximum clade credibility tree produced with TreeAnnotator v2.5.2 (Drummond and Rambaut, 2007; Bouckaert et al., 2019) with node represented by median heights. This ultrametric tree was used in iNextPD v.0.3.2 (Hsieh and Chao, 2017) to visualise differences in arthropod phylogenetic coverage between the sites. Finally, to perform multiplicative diversity partitioning (i.e., partition diversity into independent alpha and beta diversity components) for each location in the mine, we used the *multipart* function in *vegan* using 999 simulations.

RESULTS

After the filtering steps, the number of OTUs detected per season and per primer set were as follows: for samples collected during the dry season, 327 OTUs for the Leray primer set and 205 OTUs with Zeale, and for samples collected during the rainy season, 234 OTUs using the Leray primer and 252 OTUs with the Zeale primer. The number of reads after each filtering step can be found in **Supplementary Table 1**. The detection of arthropod taxonomic groups, especially within insects, differed between the

[1]https://github.com/shyamsg/Begum

[2]www.ncbi.nlm.nih.gov/

[3]https://www.gbif.org/

[4]http://www.itis.gov

two metabarcoding primer sets. The Leray primer detected more insect orders (14 in dry season and 12 in rainy season), with the orders Lepidoptera, Diptera, Hemiptera, and Hymenoptera having the most taxa. In contrast, the Zeale primer set detected arthropods within fewer orders (9 in both seasons), mainly within the orders Diptera and Lepidoptera, with the detection of taxa within other orders occurring at lower frequencies (**Supplementary Figure 1**). As more OTUs and arthropod taxa were detected with the Leray primer and some of the community composition analyses show similar results, we report here the Leray primer results only. The results from the Zeale primer set can be found in **Supplementary Information**.

Arthropod Community Composition

A constrained principal coordinate analysis (PCoA) ordination was carried out to visualise the community composition between samples. The PCoA ordinations show that the arthropod community composition in the reference sites, and especially *canga*, was different compared to the waste piles. In spite of having few sampling points, in both seasons waste piles under rehabilitation for a longer time (intermediate and advanced) had community compositions that were more similar to the reference forests (**Figure 2** and **Supplementary Figure 2**). In contrast, the community composition of waste piles with a non-revegetated soil, was the most dissimilar to the arthropod community of the reference sites. To test for differences in the community composition between waste piles and the reference sites, a contrast test was done. Although with low statistical power (**Supplementary Tables 2**, **3**), waste piles with non-revegetated soil had community compositions that were significantly different from the rest of the waste piles and the reference sites (**Table 1**). In contrast, the older waste piles (intermediate and advanced) were not significantly different from the reference sites.

An UpSetR diagram was used to supplement the PCoA ordinations and visualise shared arthropod OTUs between rehabilitation stages. These diagrams show that, for both seasons, all study sites had a high number of unique OTUs, with the exception of the waste piles with non-revegetated soil and an initial stage (**Figure 2** and **Supplementary Figure 2**). Waste piles with an intermediate and advanced rehabilitation stage shared the greatest number of arthropod OTUs with reference sites. Moreover, waste piles shared more OTUs during the rainy season than during the dry season. This shows that, although most of the detected arthropods can fly, the interchange of taxa between sites is hindered, especially in the dry season.

The *betapart* analysis was carried out to determine whether the changes in the community composition were due to turnover or nestedness. We found that the observed changes are mainly due to successional turnover in the following sites: waste piles located in S4 for samples collected during the dry season (p-value < 0.01), and waste piles located in S4 and W for the rainy season ($p = 0.001$ and $p < 0.01$, respectively). These results relate to the high number of unique OTU in each site.

Next, we investigated the influence of the waste piles' spatial distribution within the mine. Given the high number of unique OTUs, diversity was partitioned into independent alpha and beta diversity components. The results of this analysis show that, for both seasons, alpha diversity (Alpha. 1) was higher than expected within the samples, assuming a completely random taxa distribution (**Supplementary Table 4**). However, this analysis showed different results for the beta diversity. On the one hand, species turnover (Beta. 1) in both seasons was lower between rehabilitation stages than would be expected by chance. On the other hand, although spatial turnover between locations (Beta. 2) was also lower than would be expected by chance during dry season, during rainy season it met random expectations. This shows that the spatial distribution of the waste piles had an impact in the arthropod community composition.

Arthropod Community and Vegetation Structure

To investigate relations between arthropod community and vegetation structure in the study sites, we measured vegetation diversity and structure and compared it to arthropod community composition. Values for basal area (BA) and leaf area index (LAI) increased in line with the age of the waste pile; non-revegetated waste piles had the lowest values (0 m^2 BA and 0.467 LAI), whereas advanced waste piles had the highest (825.913 m^2 BA and 3.441 LAI). However, tree density (N) and tree species richness (S) had overlapping values between intermediate and advanced waste piles (**Table 2**). Correlations between arthropod composition and vegetation structure were found for BA and S during the dry season and for BA, N, S and LAI during the rainy season (**Supplementary Figure 3**). Different correlations were found with the Zeale primer, as there was a correlation with all vegetation data, except for N during the dry season. This illustrates the impact of primer bias.

Taxonomic Composition of the Chronosequences and Reference Sites

When combining the community data of both primer sets, a total of 17 orders and 76 families were detected in samples collected during the dry season and 15 orders and 75 families in the rainy season (**Figure 3**). The detected arthropods include predators (e.g., centipedes from the family Scutigeridae), disease vectors (e.g., dipterans from the family Tabanidae), pollinators (e.g., lepidopterans from the families Noctuidae and Geometridae), plant parasitoids (e.g., coleopterans from the family Curculionidae) and animal parasitoides (e.g., dipterans from the family Tachinidae).

The trees built with *metacoder* show differences between rehabilitation stages of waste piles. During the dry season, samples from waste piles without revegetated soils had higher numbers of OTUs within the families Lygaeidae (Hemiptera), Dolichopodida (Diptera), and Pyralidae (Lepidoptera) than samples collected in the rest of the sites. In contrast, the families Coenagrionidae (Odonata), Kaloternbidae (Blattodea), Pentatomidae (Hemiptera), Noctuidae (Lepidoptera) and collembolans (Entomophryomorpha) were more abundant in initial stages than in the rest of the sites. Intermediate stages had higher abundance of the order Blattodea and the family Torticidae (Lepidoptera), whereas the families Formicidae (Hymenoptera), Aleyrodidae (Hemiptera), Syrphidae (Diptera)

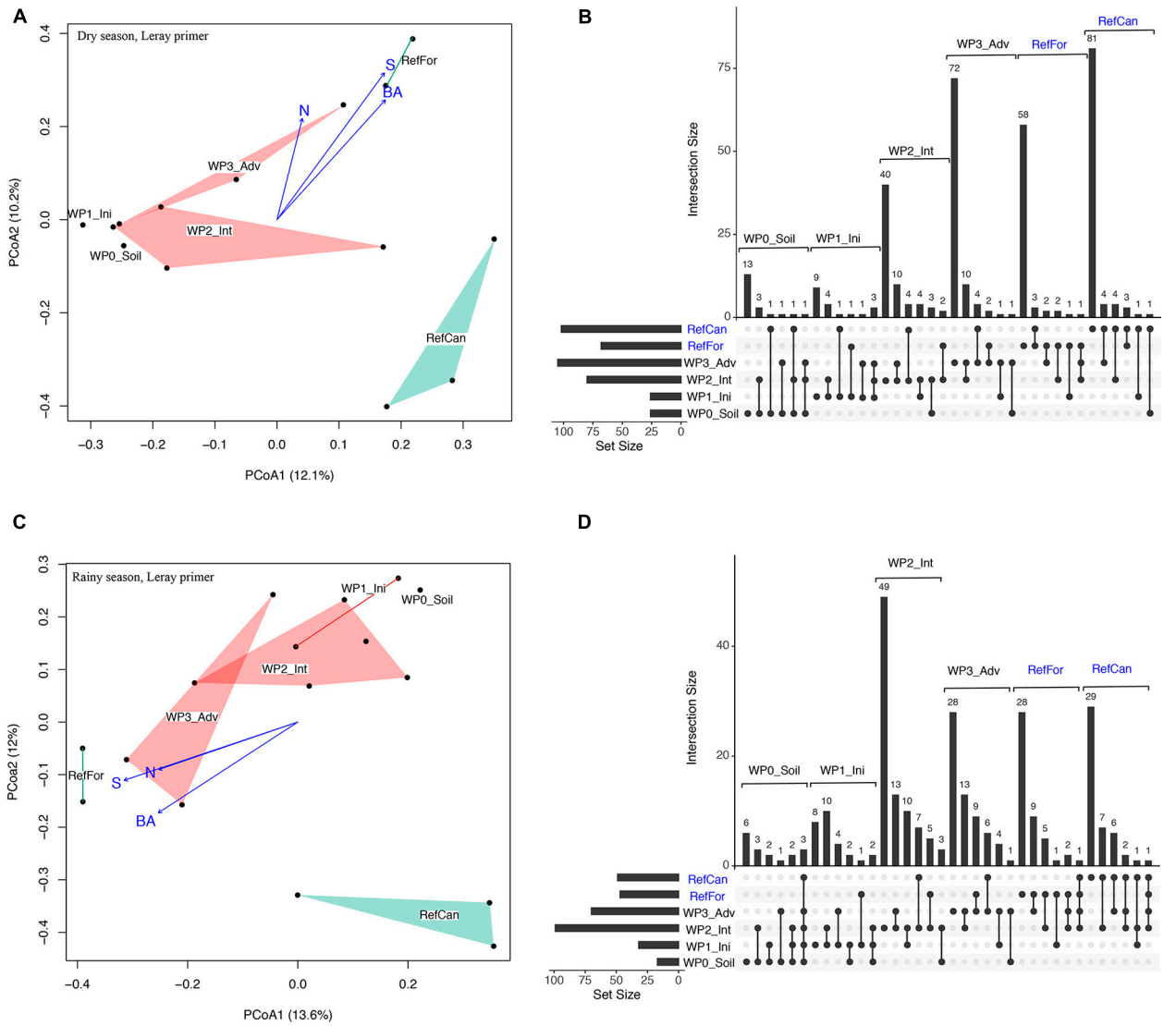

FIGURE 2 | Arthropod community composition for waste piles and reference sites in the dry and rainy season. Dry season: **(A)** Principal coordinate analysis (PCoA) ordination of the Jaccard dissimilarity of the arthropod community from waste piles (red) and reference sites (green). Fitted vegetation information such as basal area (BA), tree density (N) and tree species richness (S). Points represent samples, polygons added using the *ordihull* function. **(B)** UpSetR intersection diagram of unique and shared OTUs in the study sites. Horizontal lines on the left indicate the total number of OTUs in each site. Reference sites are marked in blue. Rainy season: **(C)** Principal coordinate analysis (PCoA) ordination of the Jaccard dissimilarity of the arthropod community from waste piles (red) and reference sites (green), **(D)** UpSetR intersection diagram of unique and shared OTUs in the study sites. Code for study sites as in **Figure 1**. All data is from the 'Leray' dataset.

TABLE 1 | Results from the corrected *p*-values (method = fdr) of the *mvabund* analyses to test differences between the waste piles for samples collected during the dry and the rainy season from the 'Leray' dataset.

	WP1_Ini		WP2_Int		WP3_Adv		RefFor		RefCan	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
WP0_Soil	0.001*	0.002*	0.002*	0.006*	0.003*	0.006*	0.002*	0.002*	0.003*	0.006*
WP1_Ini			0.001*	0.036*	0.001*	0.194	0.001*	0.002*	0.001*	0.194
WP2_Int					0.270	0.209	0.032*	0.024*	0.097	0.280
WP3_Adv							0.373	0.193	0.373	0.193
RefFor									0.347	0.252

Asterisk indicates significance in the p-value. Code for study sites as in ***Figure 1****.*

TABLE 2 | Vegetation data from waste piles under rehabilitation and reference sites where bulk arthropod samples were collected.

Stage	Location	Mean BA (m^2)	Mean N	Mean S	Mean LAI
WP0_Soil	W	0	0	0	0.467
WP1_Ini	S4	1.654	0.333	0.333	0.335
WP1_Ini	NW2	26.890	1.666	0.666	0.457
WP2_Int	W	177.960	7.666	1.666	0.996
WP2_Int	W	123.183	11	5	2.438
WP2_Int	NW2	131.365	16.666	6.666	2.601
WP2_Int	S4	94.579	18.666	5.666	0.858
WP2_Int	S4	265.290	34.333	4	2.845
WP3_Adv	S4	680.388	18	3.666	3.438
WP3_Adv	NW2	480.921	31	8	3.441
WP3_Adv	W	825.913	57.333	5.333	3.373
RefFor	S4	1742.614	25.333	20	5.744
RefFor	NW2	2058.940	34.1667	20.5	5.624
RefCan	N4P4	0	0	0	NA
RefCan	N4P2	791.390	16	5	NA
RefCan	N4P1	540.827	10	3	NA

Mean values of basal area (BA), tree density (N), tree species richness (S) and leaf area index (LAI). Codes for study sites as in ***Figure 1****.*

and Erebidae (Lepidopetera) were more abundant in the advanced stages. Differences between waste piles and reference sites were also observed in the dry season. In general, lepidopterans were more abundant in waste piles, whereas reference forest had more arachnids, coleopterans from the family Curculionidae, hymenopterans from the families Formicidae, Vespidae and Ichneumonidae, orthopterans of the family Gryllidae and the lepidopteran family Nymphalidae. In the same season, arthropods from the orders Coleoptera, Embioptera, Psocodea, Hymenoptera and the lepidopteran families Anthelidae, Mimallonidae and Lecithoceridae were more abundant in the reference *cangas* compared to the waste piles. From all the waste piles, those at an advanced rehabilitation stage also had a higher number of hymenopterans from the family Formicidae, as in the reference forest (**Figure 3**).

The *metacoder* trees for samples collected during the rainy season, show that samples from waste piles with non-revegetated soil had had higher numbers of OTUs within insect families such as Sciaride (Diptera), Ichneumonidae (Hymenoptera) and Geometridae (Lepidoptera). In the initial stages, coleopterans, blattodeans, arachnids, collembolans and the families Libeluliidae (Odonata), Eumeridae (Hymenoptera), Micropeziidae (Diptera) and Noctuidae (Lepidoptera) were more abundant than in the rest of the sites. Families such as Crambidae (Lepidoptera) and Muscidae (Diptera) were more abundant in intermediate stages, and Formicidae (Hymenoptera), Cicadeliidae (Hemiptera) and Chironomidae (Diptear) in advanced stages. Further, the arthropod communities in waste piles had more dipterans and lepidopterans from the families Geometridae and Erebidae, compared to the reference forest. Nevertheless, in the reference forest we detected more collembolans, especially from the family Entomobryomorpha, coleopterans (e.g., from the families Chrysomelidae, Carabidae, Staphylinidae and Curculionidae), arachnids and hymenopterans from the family Formicidae. The waste pile benches that had fewer lepidopterans and more hymenopterans from the family Formicidae, were benches in the advanced rehabilitation stage. For the reference *cangas*, the arthropod community differed from the waste piles due to a higher number of lepidopterans from the family Crambidae and dipterans from the family Chironomidae and a lower number of hymenopterans. Advanced waste piles also presented a higher number of Chironomidae dipterans than the rest of the waste piles (**Figure 3**). Overall, there were more similarities in the arthropod taxa between advanced waste piles and reference forests than with reference *cangas*. The dissimilarities in these arthropod taxa drove the beta diversity results visualized with the PCoA ordination and UpSetR diagram (**Figure 2** and **Supplementary Figure 2**).

OTU Richness and Phylogenetic Diversity

To investigate differences in the alpha diversity between study sites (the waste piles and the reference sites) and seasons, observed and estimated species richness were calculated and plotted. In both seasons, and for both primer sets, there were different numbers of observed species in waste piles within the same rehabilitation stage in the different locations (**Figure 4**). Nevertheless, the Chao2 estimators indicate no significant differences in estimated species richness between rehabilitation stages (**Supplementary Table 5**). The iNEXT analyses reveal that waste piles at an intermediate and advanced stage present the highest richness (**Figure 4**). Seasonal differences are especially apparent in the intermediate stages, which during the dry season present lower asymptotic estimated species richness and Shannon than in the advanced stages, although during the rainy season they are the same. Interestingly, in both seasons, the species present in these sites are relatively evenly distributed, as the diversity decreases very slightly when increasing the order of diversity q of 0 (species richness) to q of 1 (Shannon diversity).

Differences in the distribution of OTUs in relation to the total phylogenetic diversity detected in the sites can be observed between the waste piles. In both seasons, non-revegetated and initial waste piles lack entire clades that are found in the reference sites and in the intermediate and advanced waste piles (**Figure 5** and **Supplementary Figure 4**). Moreover, waste piles at an advanced stage present the highest phylogenetic coverage during the dry season, and the intermediate stages during the rainy season. These results highlight the impact of the season in the arthropod diversity.

DISCUSSION

Changes in Community Composition

The focus of biodiversity studies should be on the changes in community composition, instead of the species richness (Magurran et al., 2015, 2018; Aggemyr et al., 2018; Wang et al., 2019). In concordance with this, we find that species richness seems to recover rapidly after revegetation and we therefore focus on changes in the community composition. Our results

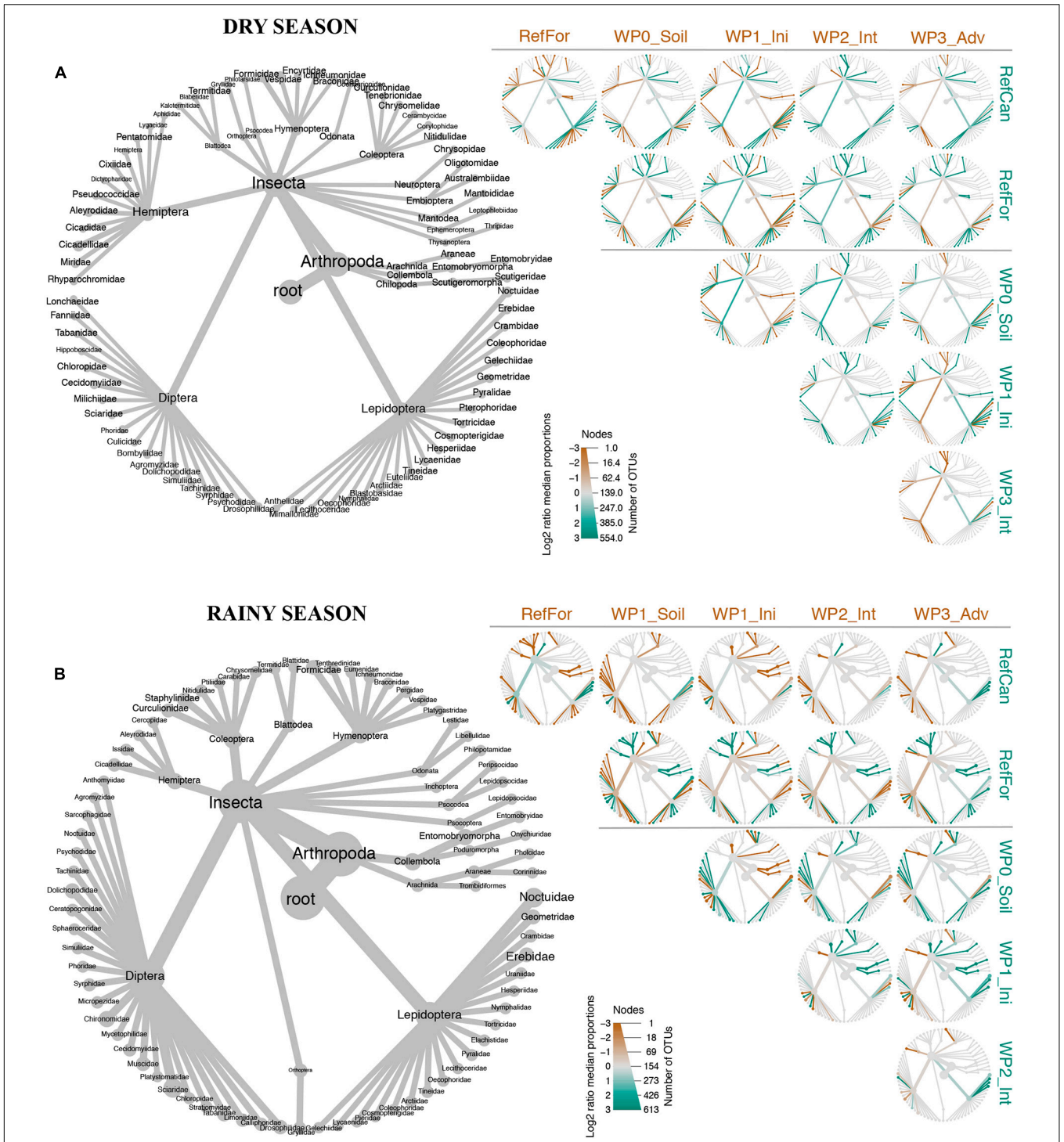

FIGURE 3 | Heat-tree built with *metacoder* showing pairwise taxonomic comparisons between study sites. Combined information from both 'Leray' and 'Zeale' datasets. **(A)** Dry season. **(B)** Rainy season. Brown branches represent taxa that are more abundant (times that OTU was detected) in sites in the top of the columns. Green branches represent taxa from the sites in the row to the right. Node size is related to the number of OTUs belonging to that rank. Code for study sites as in **Figure 1**.

show that the community of the reference forests and *cangas* are compositionally different from the communities undergoing rehabilitation in the waste piles. Nevertheless, waste pile benches under rehabilitation for a longer time (i.e., intermediate and advanced stages) had more similar communities to the reference areas, especially to reference forests, than the waste piles

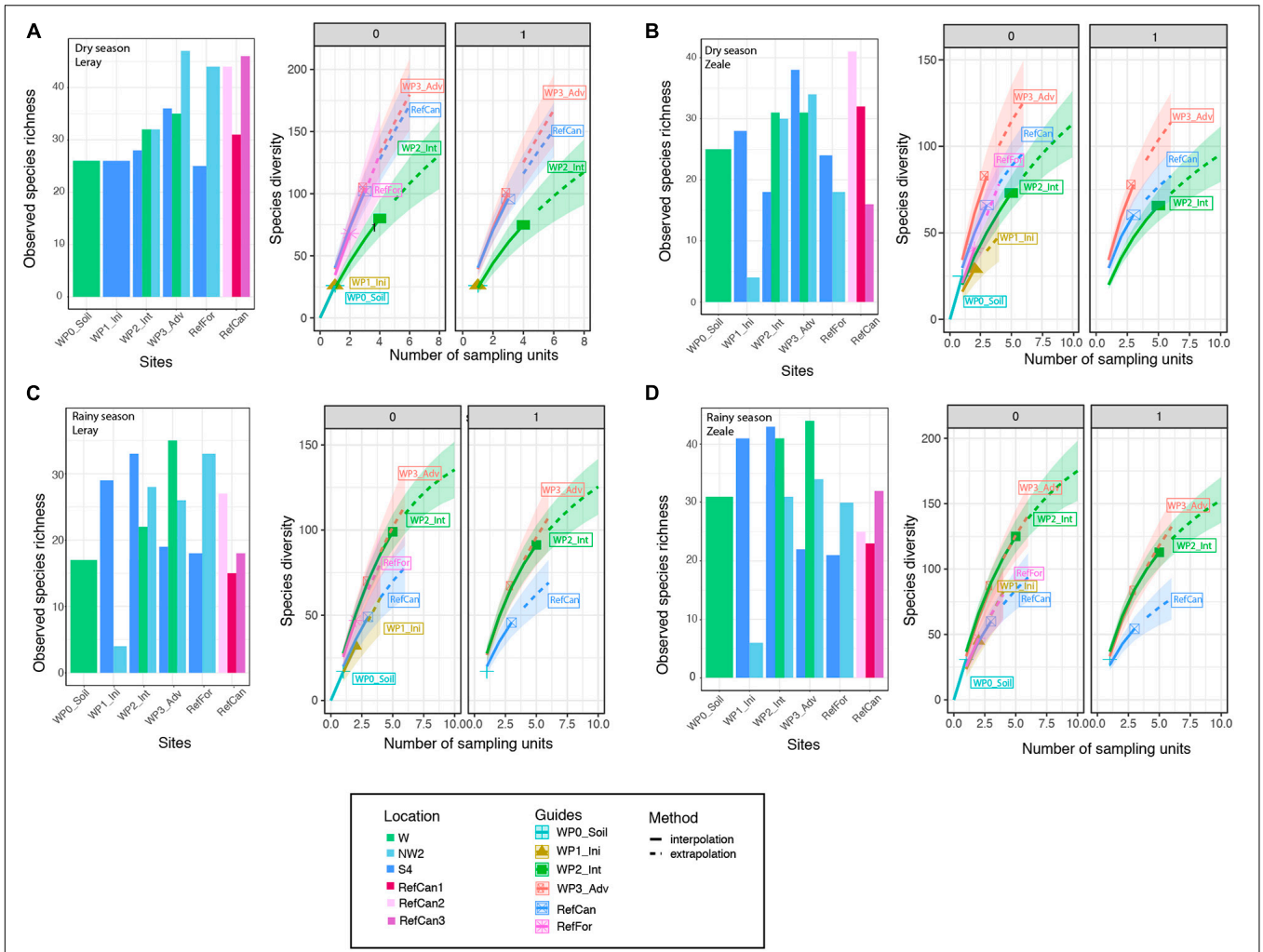

FIGURE 4 | Observed arthropod species richness and iNEXT estimates of total species richness of order of diversity 0 (species richness), 1 (Shannon diversity) of bulk arthropod samples collected at different sites in the mine. Dry season: **(A)** 'Leray' dataset, **(B)** 'Zeale' dataset. Rainy season: **(C)** 'Leray' dataset, **(D)** 'Zeale' dataset. Code for study sites as in **Figure 1**.

with non-revegetated soil (**Figure 2**). Although we found successional turnover to drive the community composition along rehabilitation chronosequences in few locations in the mine (S4 in both seasons and W in the rainy season), the lack of significance for the remaining locations may result from insufficient data. Successional turnover is supported by the results of the UpSetR diagrams, as the communities in these sites consist of unique arthropod OTUs (**Figure 2**). Successional turnover in arthropod communities has also been found in restored sites (Pais and Varanda, 2010). However, in our study, it is important to note that rehabilitation stage is not the only variable shaping the community composition: dispersal limitations and landscape patterns also influence arthropod composition. Therefore, the location of the waste pile within the mine plays an important role for species turnover between sites, as spatial turnover has an impact during the rainy season.

Vegetation structure such as basal area, tree density, tree species richness and leaf area index have previously been shown to be important when assessing environmental rehabilitation status (Ruiz-Jaen and Mitchell Aide, 2005; Gastauer et al., 2020a). As arthropod diversity and composition are known to be correlated with vegetation structure (Basset et al., 2012; Zhang et al., 2016), we fitted vegetation data with arthropod community and found that the basal area and tree species richness were correlated with the detected arthropod community. However, tree density and leaf area index showed different correlations, depending on the season (**Supplementary Figure 3**). Previous studies have also found correlation between vegetation cover and arthropod communities with differences between seasons (Salman and Blaustein, 2018). In addition, in the present study tree density and leaf area index also showed different correlations, depending on primer set used (**Supplementary Figure 3**). As mentioned previously, primer bias can cause DNA amplification of certain arthropod taxa that may be more or less correlated to these vegetation parameters and therefore result in differences between primers used.

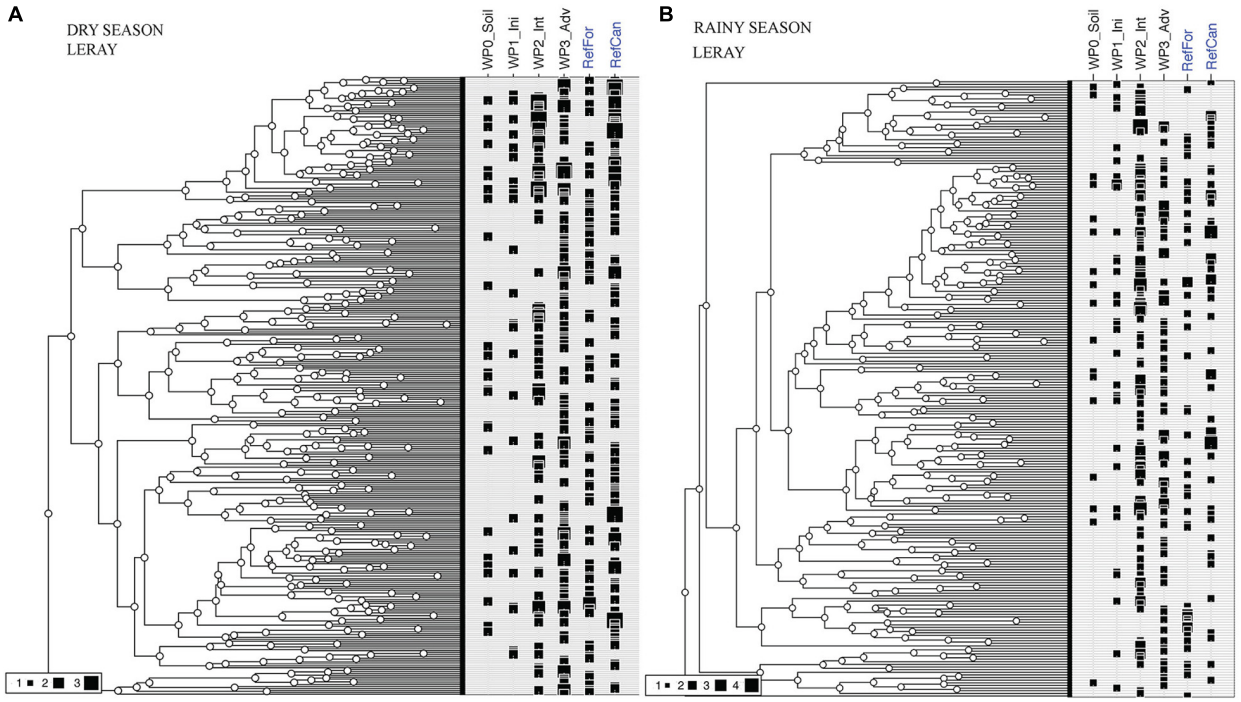

FIGURE 5 | Phylogenetic diversity of the total OTUs detected in bulk arthropod samples collected using Malaise traps. **(A)** Dry season. **(B)** Rainy season. Figure created using an ultrametric tree and iNextPD. Reference sites marked in blue. Size of black squares in each site indicates the incidence frequency of that OTU. Code for study sites as in **Figure 1**. All data is from the 'Leray' dataset.

Apart from the presence or absence of certain taxa, for a complete assessment of environmental rehabilitation status, functional diversity should also be taken into account (Gastauer et al., 2020a). We found the compositional differences in arthropod communities in the waste piles to be driven by the presence or absence of certain groups of arthropods. This in turn depends on different environmental requirements. For example, collembolans (Entomobryidae) are known to play an important role in soil rehabilitation (Rusek, 1998; Langmaack et al., 2001) and the large presence of these in the initial stages is an important contribution to the return of soil functions. Termites (Blattodea) found in the young stages (initial and intermediate) are also important for soil development due to their foraging activities (Whitford and Eldridge, 2013). Although ants (Formicidae) are present in all rehabilitation stages, their great abundance in advance stages indicates that an important factor for their establishment, such as soil cover (da Veiga et al., 2015), is available in those waste piles. In addition, an abundance of ants has been correlated with microbial activity in rehabilitated mine sites (Andersen and Sparling, 2008). Interestingly, even though plant seeds from both reference sites (forest and *canga*) are applied to achieve environmental rehabilitation in these waste piles, the arthropod community in advanced waste piles converged gradually to the reference forests and not to reference *cangas*. The reason for this can be that arthropod communities depend on the establishment of a tree cover, which is not present in the *cangas*, as indicated by the correlations found with vegetation structure. However, it is important to have in mind that the closest reference site to the waste piles is the reference forest, whereas the reference *cangas* is further away. Differences in arthropod community between older waste piles and the reference forests indicate that pre-disturbance communities have not been achieved after 6 to 7 years. This is probably due to the high diversity present in this area (Neves et al., 2020), making rehabilitation more time demanding. Although desired rehabilitation trajectories are being achieved in these waste piles, and no evidence for the emergence of novel ecosystems has been found, the possibility cannot be dismissed and samples from longer chronosequences should also be analysed.

Differences in Species Richness

Although the results are not statistically significant, the alpha diversity analyses show that waste piles with an intermediate and advanced rehabilitation status present the highest estimated OTU richness. However, of these waste piles, only the ones with an advanced status show a high richness in both seasons, in contrast to the intermediate waste piles in which the richness decreases in the dry season. Similarly, the intermediate and advanced waste piles have arthropod OTUs with a high phylogenetic coverage during both seasons. This indicates that certain arthropod groups require specific environmental factors, e.g., vegetation structure, that are not present in the young stages. The data could indicate that the waste piles with an advanced rehabilitation status have a more stable community. This lack of statistical significance can be due to low sample size, but could also be

due to ecological factors, as other studies have recorded no changes in alpha diversity after ecological disturbances (Dornelas et al., 2014; Magurran et al., 2015). However, as mentioned above, there is a low sample size and the asymptotes in the iNext plots are far from being reached, indicating that higher sample size is needed to achieve sampling sufficiency for each rehabilitation status.

Importance of Seasonality and Primer Sets

Although it is known that arthropods have seasonal variation (Liu et al., 2013; Wardhaugh, 2014; Zhang et al., 2016; Barsoum et al., 2019) and that the use of just one metabarcoding primer set can produce biased results when working with arthropods (Clarke et al., 2014; Alberdi et al., 2018b), these issues have not yet been addressed in studies describing arthropod communities in post-mining areas. In our study, seasonal differences were found in community composition, correlating to vegetation data, species richness and phylogenetic diversity. Based on our results, and together with other studies that have found differences in the structure of arthropod communities between seasons (Boulter et al., 2011; Santorufo et al., 2014), we recommend the sampling of bulk arthropod samples in different seasons for a better understanding of the community, especially in tropical and subtropical areas.

In addition, in this study we used two metabarcoding primer sets, which both amplified a marker within the traditional COI barcode region (Zeale et al., 2011; Geller et al., 2013; Leray et al., 2013). When comparing the results from the two datasets, we found the taxonomic identifications between the two primer sets to be different, and when comparing the arthropod community with the vegetation data, we detected different correlations. Differences in arthropod taxa detections between these two primer sets has been discussed previously (see Alberdi et al., 2018b), showing the co-detection of only certain taxa. We therefore recommend the use of more than one set of primers. Collecting samples in only one season or amplifying DNA with one primer set can cause biased results, and not taking this into account can impact the characterisation of the arthropod community.

Final Remarks and Future Studies

Monitoring of areas under environmental rehabilitation is resource demanding, and characterising communities in areas under rehabilitation can be challenging, as hybrid ecosystems that still provide important ecosystem services can arise (Gastauer et al., 2019). Moreover, the high biodiversity present in the study areas and the lack of a complete reference database, which makes taxonomic assignment to the OTUs difficult, makes this type of study challenging. Nevertheless, DNA metabarcoding of bulk arthropod samples has proven to be an efficient method to study changes in ecosystems (Edwards et al., 2014; Zhang et al., 2016; Barsoum et al., 2019; Fernandes et al., 2019; Gervan et al., 2020). Although DNA metabarcoding of bulk arthropod samples has been used for environmental monitoring purposes and has been recently applied to study arthropod communities after mining (Fernandes et al., 2019; Gervan et al., 2020), we are far from defining baselines and understanding the multiple factors that influence arthropod diversity detected within bulk arthropod samples.

Based on our results, we suggest the following considerations to make monitoring of arthropod communities in post-mining areas more effective. Regarding sample collection, bulk arthropod samples should be collected in both main seasons to account for seasonal variation. Further, at least three to four sites at the same rehabilitation stages should be sampled, to increase statistical power. In addition, as spatial turnover plays a role in species distribution, sampling sites should be selected as far away as possible from each other. As vegetation structure is sampled more easily, data collection can be done more regularly, whereas bulk arthropod samples can be collected every 3 to 5 years, for example. Regarding molecular and bioinformatic analyses, researchers should follow robust pipelines. DNA extracted from bulk arthropod samples, especially in tropical regions, will most often consists of DNA from many arthropod taxa. Therefore, the probability of having mismatches between the used primer and the target sequences is higher. The use of two metabarcoding primers targeting arthropod DNA makes it possible to reduce primer bias. With regards to community analyses, studies should focus on beta diversity, as we found that changes in alpha diversity are bad candidates for environmental indicators for areas under rehabilitation. We therefore agree with other authors (Dornelas et al., 2014), that changes in alpha diversity may not be noticeable due to substitution of taxa and therefore the composition of the community is a better indicator of biodiversity changes.

This study is the first to use DNA metabarcoding to characterise arthropod communities in areas under rehabilitation after mining in a megadiverse ecosystem such as the Amazonian forest. Studies such as this are important to benchmark methods with which changes in biodiversity can be objectively studied, leading to a better understanding of the impact of rehabilitation efforts in highly biodiverse areas.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://erda.ku.dk/public/archives/1aad025cea2257ad3a725f33a0236142/published-archive.html, 1aad025cea2257ad3a725f33a0236142; https://github.com/lynggaardc/Arthropods_Brazil.git, no accession number.

AUTHOR CONTRIBUTIONS

CC, CL, KB, MG, MTG and SR conceived the study. CL, MG and ME carried out sample collection. CL and ME generated the data with inputs from KB. CL and MG analysed the data with important contributions from DY. CL wrote the original manuscript and all authors contributed to revisions.

FUNDING

Collection permits were granted by the Brazilian Ministry of Environment (SISBIO 67580-1 and SISGEN R04409B). Collection of samples received support from Vale (Canga Plant and Soil and Biodiversity Genomics), and CNPq to GO (444227/2018-0, 402756/2018-5 and 307479/2016-1), CAPES to GO (88887.130628/2016-00), and the CABANA project [RCUK(BB/P027849/1)]. CL was supported by the National Council of Science and Technology in Mexico (CONACyT) Grant CVU 582667 and the Danish Council for Independent Research, DFF grant 7079-00029B. KB was supported by the Independent Research Fund Denmark, DFF grant 5051-00140.

ACKNOWLEDGMENTS

We thank Wesley de Melo Rangel, Joyce Reis and Gilliana Almeida da Rosa for field assistance and the staff at the Danish National High-Throughput DNA Sequencing Centre for sequencing. Special thanks to Xiaoyang Wang for developing the original R script for the community analyses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.590976/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
published: 18 December 2020
doi: 10.3389/fevo.2020.563477

Spatially Explicit Capture-Recapture Through Camera Trapping: A Review of Benchmark Analyses for Wildlife Density Estimation

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Edited by:
W. Douglas Robinson, Oregon State University, United States

Reviewed by:
Roland Kays, North Carolina State University, United States
Adam Duarte, USDA Forest Service, United States

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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *18 May 2020*
Accepted: *01 December 2020*
Published: *18 December 2020*

Citation:
Green AM, Chynoweth MW and Şekercioğlu ÇH (2020) Spatially Explicit Capture-Recapture Through Camera Trapping: A Review of Benchmark Analyses for Wildlife Density Estimation. Front. Ecol. Evol. 8:563477. doi: 10.3389/fevo.2020.563477

Camera traps have become an important research tool for both conservation biologists and wildlife managers. Recent advances in spatially explicit capture-recapture (SECR) methods have increasingly put camera traps at the forefront of population monitoring programs. These methods allow for benchmark analysis of species density without the need for invasive fieldwork techniques. We conducted a review of SECR studies using camera traps to summarize the current focus of these investigations, as well as provide recommendations for future studies and identify areas in need of future investigation. Our analysis shows a strong bias in species preference, with a large proportion of studies focusing on large felids, many of which provide the only baseline estimates of population density for these species. Furthermore, we found that a majority of studies produced density estimates that may not be precise enough for long-term population monitoring. We recommend simulation and power analysis be conducted before initiating any particular study design and provide examples using readily available software. Furthermore, we show that precision can be increased by including a larger study area that will subsequently increase the number of individuals photo-captured. As many current studies lack the resources or manpower to accomplish such an increase in effort, we recommend that researchers incorporate new technologies such as machine-learning, web-based data entry, and online deployment management into their study design. We also cautiously recommend the potential of citizen science to help address these study design concerns. In addition, modifications in SECR model development to include species that have only a subset of individuals available for individual identification (often called mark-resight models), can extend the process of explicit density estimation through camera trapping to species not individually identifiable.

Keywords: citizen science, conservation biology, biodiversity monitoring, mammals, Carnivora, wildlife ecology, density estimation

INTRODUCTION

Camera Traps and Benchmarking Biodiversity

Human-induced changes to both terrestrial and marine ecosystems are intensifying, especially in areas of the world with historically high levels of biodiversity (Venter et al., 2016). Human activities have a direct effect on biodiversity, altering ecosystems around the globe (Cardinale et al., 2006; Estes et al., 2011; Hooper et al., 2012). During this period of rapid change, and in order to better understand the effects of human activity on biodiversity, it has become increasingly important to provide baseline measurements of species distributions and population sizes, especially for rare, elusive, and difficult-to-monitor species like carnivores, which play particularly important roles in regulating ecosystems (Beschta and Ripple, 2009; Laundre et al., 2010; Ripple et al., 2014). Providing these benchmark analyses, and establishing the methodology and analysis framework to compare changes over time, is essential to understanding and quantifying the ways in which these species are both affected by rapid change and how they, in turn, affect human well-being.

Camera traps have been used in animal ecology studies for decades, and are particularly suitable for studying large carnivores, which can be difficult to study with other methods (Griffiths and Van Schaik, 1993; Rowcliffe and Carbone, 2008; Trolliet et al., 2014; Burton et al., 2015). Cameras provide researchers with a non-invasive survey tool to sample wildlife communities and usually require less intensive labor commitment than standard trapping and marking techniques (Meek et al., 2014). Consequently, camera traps have become powerful research tools for scientists and wildlife managers investigating a wide variety of ecological questions, management situations, and conservation strategies (Karanth and Nichols, 1998; and Glen and Dickman, 2003; Hirakawa, 2008; O'Connell et al., 2011; Meek et al., 2014).

Measuring Biodiversity: Density Estimation

In order to measure how species respond to rapid change, and to establish proper avenues for comparative studies, researchers must first establish a reference or baseline population size. In biodiversity studies, density estimation is often considered the gold-standard of population assessment and for species conservation, wildlife management planning, and long-term population monitoring (O'Connell et al., 2011; Tobler and Powell, 2013; Royle et al., 2014). Wildlife density has long been estimated through capture-recapture methods (Otis et al., 1978). Karanth (1995) and Karanth and Nichols (1998) pioneered the use of camera traps in a photographic capture-recapture framework to estimate population size of tigers *Panthera tigris* in Nagarhole, India. The authors used camera trap images, which come with an accompanying GPS coordinate (and date and time stamp), as individual "captures." They then used the photographs from these individual captures to build a dataset of multiple individual tigers. From there, they could create separate capture histories for each one. Since this work, multiple independent investigations have adopted camera traps and this analysis framework to estimate the densities of tigers in other areas of the world (O'Brien et al., 2003; Linkie et al., 2006; Harihar et al., 2009; Gopal et al., 2011), as well other individually identifiable animals (Kelly et al., 2008; Paviolo et al., 2008).

Many of these early investigations relied on closed model capture-recapture methods (Otis et al., 1978; White et al., 1982). This method requires compiling individual-specific capture histories across a defined study area where the boundaries of an individual animal's movement may not be well-known. The detection histories contain information about individual capture probability, and can thus be used for estimating population abundance. However, these models provide little information on the movement patterns of each individual, as well as the spatial distribution of the traps themselves. Therefore, under this framework, density is estimated according to an arbitrarily set area, usually defined as the camera trap polygon plus a buffer with radius equal to either the maximum distance moved by an individual across the trap array or half the distance moved (O'Connell et al., 2011). As density requires both an abundance and an area, arbitrary designation of area is an obvious hindrance to closed model capture-recapture methods. Consequently, this method is often considered to measure density *implicitly* (Royle et al., 2014). That is, density is estimated without explicitly measuring all of its elements. The population size is functionally unrelated to an explicitly monitored area, which can make it impossible to compare across studies or even different models (Royle et al., 2014). Furthermore, research has shown this method to consistently overestimate density by underestimating the distances moved by individual animals (Obbard et al., 2010; Noss et al., 2012; Pesenti and Zimmermann, 2013).

Spatially Explicit Capture-Recapture

Spatially explicit capture-recapture (SECR) density estimation was developed independently by Borchers and Efford (2008) and Royle and Young (2008) (see also Efford, 2004, 2011; Efford et al., 2009; Royle et al., 2009). What separates SECR density estimation from closed capture-recapture models is the incorporation of an explicit spatial component to each individual's detection history, as well as a defined state-space over which density is estimated (Efford and Fewster, 2013; Royle et al., 2014). Therefore, SECR analysis represents an *explicit* way of measuring density (i.e., both components of density are estimated without *ad hoc* calculations). However, because of the additional parameters to estimate, SECR models can be more data hungry than their implicit counterparts (Royle et al., 2014).

A detailed breakdown of SECR analysis is beyond the scope of this paper. Efford et al. (2009) offer a thorough introduction and explanation of SECR analysis through Maximum Likelihood-based methods, and Royle et al. (2014) provide a thorough introduction and explanation of SECR analysis through Bayesian techniques incorporating data augmentation. Here, instead, we provide a brief summary based on the work of Royle and Young (2008) and Borchers and Efford (2008).

SECR models are *hierarchical*, where the full model is described by multiple component models (Royle and Dorazio, 2008). The first of these components describes the distribution of activity centers $\boldsymbol{s}$, or home range centers, of individual animals. In this characterization, $\boldsymbol{s}_i$ represents the geographic point where individual i's movement is centered (the movement around the point $\boldsymbol{s}_i$ is then described according to a specific probability

function), and $\mathbf{s}_i$; $i = 1, 2, \ldots, N$ represents the activity centers of every individual within a defined state-space $\boldsymbol{S}$, the region over which density is estimated (Royle and Young, 2008). This model is a spatial point process, capable of measuring density as either constant across the state-space or with spatial variation (Efford et al., 2009; Royle et al., 2014). $\boldsymbol{S}$ is typically described by specifying coordinates of a polygon that is substantially larger than the area sampled, allowing some individuals to have $\boldsymbol{s}_i$ outside of the sampled area. As mentioned above, individuals are assumed to move around the state-space randomly as specified by some probability distribution. Finally, the sum of activity centers, $\boldsymbol{N}$, over the state-space $\boldsymbol{S}$, specified $\boldsymbol{u}$, represents the estimated population density.

Another component, the observation model, describes $\boldsymbol{y}$, or how the observed data occur based on the locations of $\boldsymbol{N}$ individuals (Efford et al., 2009; Royle et al., 2014), as it is assumed that individuals are sampled imperfectly due to detection probability being <1. The observed data are binary observations during a specific sample that state whether an individual was captured or not (Royle and Young, 2008). These observations are used to create encounter histories for each individual. In addition, each encounter comes with a pair of coordinates that specify where each encounter occurred. These encounters are defined by at least two parameters, $\boldsymbol{p}$ and $\boldsymbol{\sigma}$, which describe the probability of capturing or detecting an individual at a given location by using the distance between each individual's activity center and a given encounter location. In this formulation, when individuals are marked, $\boldsymbol{p}_{ij}$ is the probability of capturing individual $\boldsymbol{i}$ at trap location $\boldsymbol{j}$, and $\boldsymbol{\sigma}$ is the spatial scale parameter that defines how capture probability declines with distance (Efford et al., 2009; Royle et al., 2014).

The most basic SECR models come with the following major assumptions: (1) within the population of interest, and during the period of study, there exists both demographic and geographic closure; (2) individual activity centers are randomly distributed and do not change; (3) the probability of detection at a given location is a function of distance to an individual's activity center; and (4) there is independence in individual encounters among individuals and within the same individual.

The first assumption means that basic SECR models assume no exit or entry into the population through either recruitment or mortality or permanent emigration or immigration from the area of study. However, the model does allow for "temporary" variability to encounter around the state-space (Royle et al., 2014). Violations of closure can result in detection probability estimates that are too low or the effective trap area being considered too small, resulting in positive bias in resulting density estimates (Dillon and Kelly, 2008; Obbard et al., 2010). Typically, practitioners are encouraged to either (a) keep their survey period as short as possible or (b) use an open population model (e.g., Gardner et al., 2010a; Ergon and Gardner, 2014; Schaub and Royle, 2014) to avoid violating this assumption. The second assumption deals with the distribution of individual activity centers across the state space. This is often referred to as the "uniformity assumption," (Royle et al., 2014) modeled as,

$$\mathbf{s}_i \sim \text{Uniform}(\mathbf{S})$$

This creates what is known as a *homogenous point process model;* however, inclusion of site-specific covariates can make it possible to estimate density as a function of state-space heterogeneity (Royle et al., 2018). Accompanying this assumption is that individual home range centers are spatially stationary for the duration of study. However, this assumption may be relaxed by modeling $\boldsymbol{s}_i$ with some type of latent movement model. Thus, the activity centers of all or some of the individuals within a population are allowed to drift (Royle et al., 2016).

The third assumption states that each animal has an activity center and the probability of capture decreases with distance to that activity center. Typically, a half-normal detection function is applied to describe how detection probability decreases with distance, but a variety of functions are available. In this formulation, the detection function is described by the detection probability and the scale parameter, which denote the probability of detection when the distance between an individual and their activity center is 0 and how that probability declines in response to distance, respectively. The most basic models assume that these parameters do not change across individuals, but this assumption can be relaxed to vary across time, individuals, and covariates (Royle et al., 2018). Finally, the assumption of independence of encounters states that the encounter of one individual does not affect the encounter of another individual at the same trap, and encounter of an individual at one trap location is independent of encounter at any other trap location. It is natural to think that species may have a behavioral response to certain areas, making them more or less likely to visit specific trapping locations. Recent model developments allow for this behavioral response to be explicitly accounted for (Gardner et al., 2010b; Royle et al., 2011).

Camera Traps and SECR Analysis

Camera trapping lends itself well to measuring density through SECR analysis. SECR analysis requires marking a sample of individuals and monitoring their presence across multiple surveys and study sites (Borchers and Efford, 2008; Efford et al., 2009; Royle et al., 2014). Traditionally, monitoring requires setting up live-trapping stations, using natural marks or marking individuals caught in each trap, and repeating the process over a given time-frame. This results in multiple visits (usually daily) to each trap station, individual processing of animals caught in the traps, and consistent maintenance of traps to ensure that each is capable of capturing animals, resulting in a time and effort-intensive process that hinders the number of traps that can be deployed during a particular investigation (Jimenez et al., 2017; Loock et al., 2018; Whittington et al., 2018; Petersen et al., 2019). This is problematic for species with low detection or capture rates due to natural rarity or large individual home ranges. To compensate, researchers are required to increase the duration of time each trap is active during a season, which can lead to violations of the closure assumption.

However, camera traps are non-invasive, remote sensing devices that can monitor animal populations over a wide-geographic area (Kelly et al., 2008; Linden et al., 2017; Luskin et al., 2017). They are relatively cost and time-effective monitoring tools, requiring no intensive and individually-invasive capturing techniques, and they can be paired with other methodological approaches that bolster the predictive

power of population monitoring investigations (O'Connell et al., 2006; Lyra-Jorge et al., 2008; De Bondi et al., 2010; Roberts, 2011; Welbourne et al., 2016). Camera surveys require little maintenance once initially setup, and they offer the unique ability for researchers to mark individual animals without having to maintain the traps they were caught in or process the individuals captured. Furthermore, since SECR analysis requires that density is estimated over an explicitly determined state-space, and that a state-space is typically defined as the polygon surrounding the outermost traps of a particular array (aka the minimum convex trap polygon), using camera traps instead of other trapping methods allows researchers to explicitly adjust the size of their study area. Finally, the ease of setup and relatively low maintenance requirements for camera traps allows researchers to establish a higher density of traps within their camera array compared to more traditional methods, with more than one trap within the average home range size of the species studied, another requirement of SECR analysis (Borchers and Efford, 2008; Royle et al., 2009, 2018).

In this review, we aim to explain the current extent of camera trap SECR analysis, identifying whether benchmark density estimates have been precise enough to monitor change over time, especially for species where no other estimates exist. Our goals were to (1) summarize the current efforts of SECR analysis through camera trap surveys and (2) analyze study design criteria to identify important predictors of density precision and suggest recommendations to improve density precision in future studies. Our review provides an accurate picture of the current direction of the science. We document the publication outlets, species studied, and geographic extent of these efforts. As a guide for future research, we highlight the analysis software used, the study designs adopted, and both the amount of effort and number of detections recorded. Finally, we report on the study design factors that lead to increases in density estimation precision and how incorporation of new analysis techniques, online technologies, and citizen science may offer ways to increase these factors for future investigators, as well as pave the way for new developments.

MATERIALS AND METHODS

Literature Review

Our literature review took place between 24 April and 21 October 2019. We searched the Web of Science™ for papers using the following title and topic search terms: "spatial capture-recapture" AND "spatially explicit capture-recapture" AND "spatial mark-recapture" AND "spatially explicit mark-recapture" AND "spatial mark-resight" AND "spatially explicit mark-resight" AND "spatially explicit density estimation." We reviewed the resulting dataset of 309 papers and included only those that used camera traps. The resultant dataset included 88 scientific articles. We then expanded this dataset by searching through all studies citing Royle et al. (2014), which resulted in an additional 7 studies. The final dataset included 95 papers (**Supplementary Table 1**).

Categorical variables were extracted from each study. We recorded the title, author(s), journal, year, pagination, class and species studied, and continent and country of focus for each study. If more than one species was included in a single study, a separate record was produced for each. This resulted in a dataset with 110 species-specific records. Each study's objective was classified as either single-species, two-species, or multi-species density estimation. Spatially-explicit capture-recapture (SECR) analysis is typically done using freely available data analysis software and can be implemented in either a maximum likelihood or Bayesian framework, so we recorded the method of analysis as either MLE (for maximum likelihood), Bayesian, or both, and the statistical program used to implement the analysis was also included in the database. We recorded whether or not each study used site-specific covariates within their analysis framework. For studies that paired non-covariate spatially explicit density estimation with diet, movement, or occupancy analyses that included site-specific covariates ($n = 8$), the study was classified as using covariates and the discrepancy was noted on a separate column in the dataset. We recorded any methods (simulations, occupancy analysis, live trapping, etc.), besides spatially explicit density estimation through camera trapping, implemented during the course of each study. Furthermore, if a study made any comparisons between SECR and another density estimation framework ($n = 23$), the specific models compared and the results of these comparisons were recorded. Finally, we recorded if each study included baited camera trap stations and whether or not community engagement or citizen science was implemented during any stage of the project.

We extracted a number of numerical variables from each study. The number of camera stations was recorded as the average number of stations implemented per year of study. We recorded the length of each study in years. We included, when recorded, the minimum convex polygon of the camera station array. If this camera polygon was not reported in the manuscript ($n = 5$), the state-space of the study was used instead (see section Measuring Biodiversity: Density Estimation above). We recorded the average camera spacing in meters. When the average spacing was not explicitly reported, we recorded the average of the reported camera spacing range ($n = 13$), the minimum distance between stations ($n = 1$), or the maximum distance between stations ($n = 1$). The number of trap days was recorded as the total accumulated effort for all camera stations across all years of survey. This total was then averaged across years for analysis. The total number of photo-captured target species was recorded, as was the total number of individuals tracked throughout the study. The scaling parameter, σ, was recorded for each study as the average across years per species using either the author-specified top model or the author-reported model average. If the best model was not specified ($n = 9$), σ was extracted as the average across all models reported. When more than one area was surveyed during a particular study and no average was recorded ($n = 7$), the scaling parameter was recorded as the weighted average of estimates based on the size of each area's assessed state-space. Furthermore, if the scaling parameter was reported to vary based on sex ($n = 8$), the estimate was averaged using an assumed 1:1 sex ratio ($n = 7$) or the specified sex ratio provided ($n = 1$). Density was recorded as the number of individuals reported per 100 km^2 on a per species basis. Estimates were averaged

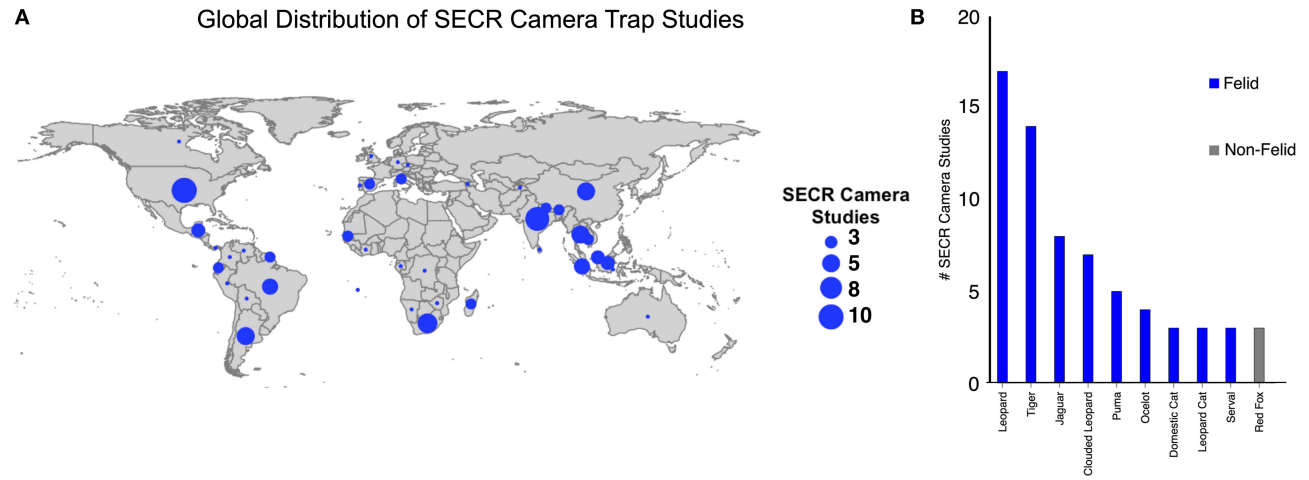

FIGURE 1 | Camera trap SECR study spatial distribution and species focus. **(A)** Global spatial distribution of camera trap SECR studies by country. **(B)** Number of studies focused on the top 10 focal species. The top 10 species listed accounted for 61% of all SECR camera trap studies. Most (75%) SECR camera trap studies focused on felids.

across year using either the top model reported or the author-reported model average. As with the scaling parameter, when the best model was not specified ($n = 9$), density was extracted as the average across all models reported. When more than one area was surveyed during a particular study and no average was recorded ($n = 7$), density was recorded as the weighted average of estimates based on the size of each area's assessed state-space. One study did not report the specific state-space of each area surveyed, so the density estimate for this study was calculated without area-specific weights. Lastly, to assess the precision of density estimates, the coefficient of variation (CV) was calculated on a per species basis across studies. When the standard deviation of the maximum likelihood estimator or the posterior standard deviation of density were not explicitly reported, the standard error was used to calculate CV ($n = 12$). One study provided only a 95% confidence interval, and the standard deviation for this study was calculated as the range of the confidence interval divided by 3.92 (assuming a normally distributed density estimate).

Data Analysis

In an effort to identify important study design parameters for increases in density precision, we modeled each study's coefficient of variation against study design parameters. However, all predictor variables were correlated with at least one other variable (Pearson's $r > 0.5$). Therefore, we conducted Principal Component Analysis (PCA) on study design factors and modeled density precision as a function of the first three principal components (PC1, PC2, and PC3), which collectively accounted for 72.6% of the variation in study design factors. Since each predictor was on a different scale, predictor variables were standardized to have a mean = 0 and a standard deviation = 1 before running the PCA. We then used PC1, PC2, and PC3 as covariates in modeling density precision to study design components using a Gaussian linear model. We determined significant associations between precision and principal components at $\alpha = 0.05$. Predictors included in the PCA were: density, target captures, individuals monitored, camera stations, camera days, and study area.

RESULTS

Dataset Summary

SECR analysis through camera trapping has focused on multiple species across a wide geographic range. The results from our dataset were published in 37 different journals. Five journals accounted for 42.1% ($n = 40$) of publications (PLoS One = 13, Oryx = 12, Biological Conservation = 7, Ecology and Evolution = 4, and Nature = 4). Publication rate has steadily increased since 2010 (the earliest publication year included in our dataset), with 67.3% ($n = 64$) published between 2015 and 2019 (**Supplementary Table 1**). All studies focused on mammals. Of the 110 species density estimates, 60.9% ($n = 67$) were of 10 different species: leopard (*Panthera pardus*) = 17, tiger (*Panthera tigris*) = 14, jaguar (*Panthera once*) = 8, clouded leopard (*Neofelis nebulosi*) and (*Neofelis diardi*) = 7, cougar (*Puma concolor*) = 5, ocelot (*Leopardus pardalis*) = 4, domestic cat (*Felis catus*) = 3, leopard cat (*Prionailurus bengalensis*) = 3, red fox (*Vulpes vulpes*) = 3, serval (*Leptailurus serval*) = 3. All other species were included in fewer than three occasions (**Supplementary Table 1**, **Figure 1**). 90.9% ($n = 100$) of estimates were of carnivores, and of those 82% ($n = 82$) were of felids. 91.6% of studies focused on only one species ($n = 87$), 5.3% on two species ($n = 5$), and 3.1% on more than two species ($n = 3$). SECR studies using camera traps were conducted on six continents, with Asia and South America representing 58.9% (Asia = 38, South America = 18) of all studies (**Supplementary Table 1**, **Figure 1**).

SECR models incorporated both maximum likelihood and Bayesian analysis methodologies. Researchers estimated density

using exclusively maximum likelihood estimation 46.3% (n = 44) of the time, with 72.7% (n = 32) of these studies using the R package *secr* (Efford, 2010) for analysis; Bayesian inference was used exclusively in 35.8% (n = 34) of studies, where the program *SPACECAP* (Gopalaswamy et al., 2012) was used for analysis in 40.6% (n = 13) of these studies; and both methods were incorporated in the remaining 17.9% (n = 17) of studies, with *secr* or *SPACECAP* used in 88.2% (n = 15) of these studies.

Camera trapping methodology varied in both spatial scale and temporal extent, resulting in highly variable numbers of target captures and individuals monitored. Most studies lasted for 1 year or less (71.6%, n = 68, mean = 1.9), and a median of 57.5 camera stations were deployed per study per year (mean = 100.1, min = 12, max = 849). Surveys lasted for a median of 3,124 camera-days per year (mean = 7,762, min = 478, max = 114,854). The minimum convex camera trap polygons covered a median area of 306 km^2, with large-scale, multi-year studies having a major effect on the mean (mean = 2,646, min = 4, max = 70,096). Camera stations were placed, on average, 1,962 m apart (median = 2,000, min = 100, max = 8,740), and bait was used in 24.2% (n = 23) of studies. SECR studies recorded a median of 129.5 detections of their target species (mean = 340.1, min = 21, max = 3,163) and resulted in a median of 27 individual animals tracked (mean = 60.8, min = 4, max = 1,240). The median scaling parameter varied across species and dietary preferences (**Supplementary Table 2**). Density was lowest for large carnivores and varied across species and geographic locations (**Supplementary Table 2**).

Camera trapping studies deployed for SECR density estimation incorporated a number of supplemental methodologies and compared the effectiveness across these methods, as well as across data analysis approaches and modeling schemes. Twenty-two (23.2%) studies incorporated site-specific covariates into their analysis. As noted in section 2.1, 36.4% (n = 8) of these studies used the information from site-specific covariates in analysis separate of density estimation through SECR analysis. Slightly under half of studies (46.3%, n = 44) incorporated methodologies in addition to camera trapping. Of these methodologies, GPS tracking, telemetry, and live trapping were used most frequently (27.3%, n = 12), followed by simulations (22.7%, n = 10). Nineteen studies (20.0%) compared the results of SECR analysis with closed-population capture-recapture analysis (n = 16), Random Encounter Model analysis (n = 1), distance sampling analysis (n = 1), and Royle-Nichols occupancy analysis (n = 1). Authors self-reported that SECR analysis either outperformed closed-population capture-recapture or they recommended SECR analysis 93.8% of the time (n = 15). One study self-reported that closed-population capture-recapture analysis outperformed SECR analysis. Twenty-six (27.3%) studies surveyed across multiple years or seasons.

Density Precision Predictors

The precision of density estimates, as measured through the coefficient of variation (CV), was reported or extracted as explained in section 2.1 for 90 species-specific density estimates. The median CV was 30% (mean = 31.1%). 75.6% (n = 68) of studies reported a CV of ≤40%, but only 21% (n = 19) of studies reported a CV ≤20%.

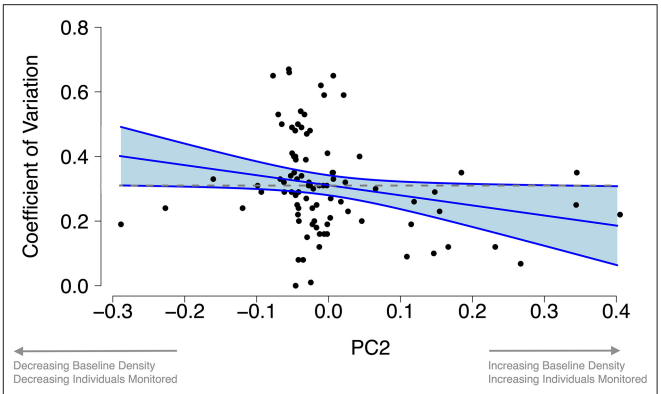

FIGURE 2 | Study design characteristics predicting increases in density precision. Density precision increased with increasing values of PC2 (describing axes of increasing density and increasing individuals monitored). Data points are species-specific values of the Coefficient of Variation. Larger values mean lower precision. Blue line and shaded area represent the slope and 95% Confidence Intervals from our linear model. Dashed gray line represents the mean Coefficient of Variation in our review.

The first three principle components of our PCA, which accounted for 72.6% of the variation in study design characteristics, described axes of increasing camera stations and camera days (PC1), increasing density and individuals monitored (PC2), and increasing density and decreasing individuals monitored (PC3; **Supplementary Tables 3, 4**). Density precision did not differ significantly across PC1 and PC3 (p = 0.131 and p = 0.919, respectively; **Supplementary Table 5**). However, density precision increased significantly with higher values of PC2 (increases in density and individuals captured; p = 0.038; **Figure 2**).

DISCUSSION

In this review, we summarized the current publication extent, geographic coverage, and species focus; study design specifics; and available analysis pipelines of SECR camera trap studies. Our review highlights the flexibility of SECR analysis through camera trapping, which makes this methodology a tool for providing benchmark analysis of previously understudied species. Our review also sheds light on the current geographic and species bias toward areas with rare, elusive, and individually-identifiable species, particularly large felids. We also found that many studies produced relatively imprecise density estimates (see below for details), and that precision could be increased with increases in the number of individuals captured, which can be accomplished with a larger study area.

Benchmarking Rare and Elusive Species

Our review highlights the importance of camera trapping for studying rare, elusive, and human-intolerant large carnivores (Ripple et al., 2014). These are species that are both exceptionally important to ecosystems throughout the world and difficult to

study through other means. For many of the species in our review, the density estimates calculated were the first reported population estimates for them, highlighting the ability of camera traps to monitor previously understudied species, providing benchmark estimates that can be compared over space and time. As the world continues to change at an increasingly rapid rate, benchmarking and archiving density estimates for these species will be critical for tracking the effects of rapid global change.

SECR density estimation through camera trapping is currently focused on rare, elusive, large-ranging, and individually identifiable carnivores, specifically large felids, and this methodology represents one of the best ways to study these species. More than a third of all studies included in this review focused on one of three species: leopard, tiger, and jaguar (35.5%, $n = 39$). This explains the subsequent bias in geographic focus of camera trap SECR studies (Results section; **Figure 1**). Focusing on species that are capable of individual identification through photographic analysis alone is the obvious reason for this bias, as it represents the simplest avenue to robust density estimation without the need to employ more-intensive methodology (e.g., live trapping; scat, environmental, and/or hair sampling for DNA analysis, etc.). Large felids tend to be wide-ranging, naturally rare, and heavily affected by human influence (Seidensticker and McDougal, 1993; Turner, 1997). Many of these species are currently threatened or endangered with extinction, so information about their population densities and trends through space and time, especially in relation to human influence and climatic change, is needed for their continued conservation (Ripple et al., 2014). Since these species require large areas of undisturbed habitat, they tend to be excellent indicators of general ecosystem health and conservation of these umbrellas species is thought to affect the conservation of other species at lower trophic levels (Dalerum et al., 2008a,b; Estes et al., 2011).

Addressing Imprecise Density Estimates

Although the goal of many studies in this review was to assess the current population size of a particular species and/or lay the framework for a long-term monitoring project, multiple density estimates from studies included in this review may not be precise enough to monitor population trends through time. The mean reported or derived coefficient of variation (CV) was relatively high (31.1 %). In fact, less than a quarter of studies reported high precision in their density estimates (CV $\leq$20%). Conducting a power analysis before implementing a specific study protocol can reduce "… [wasting] time and effort on a program that is unlikely to yield useful information" (Gerrodette, 1987). This power analysis can be conducted for multiple fieldwork scenarios using the readily available software TRENDS and the R package *emon* (Gerrodette, 1993; Barry and Maxwell, 2017). For example, using *emon* and the average density and standard deviation of tigers in our dataset (CV = 0.31), assuming a normal distribution for random values and that density is measured twice per year, the likelihood of detecting a 50% linear decline in tiger density over 10 years is only 32.7%. This likelihood increases to 68.0% with a CV = 0.20 and to 89.2% with a CV = 0.15. This simple exercise shows that a majority of camera trap monitoring programs designed around species where precise density estimates are needed to assess population change through time may be inadequate. Furthermore, pairing simulation with SECR density estimation through camera trapping has great potential. Only 10.5 % ($n = 10$) of studies performed any type of simulation before implementing their field protocol. Conducting simulations before implementing field protocol can help elucidate the effects particular study designs could have on density estimation, and recent developments in SECR simulation and design (see Efford, 2019a,b; Efford and Boulanger, 2019) make it relatively straightforward to evaluate study designs using prior information. Given that the majority of studies were conducted on species where prior information on home range size and density were available (over 60% of studies were conducted on only 10 species), including this information into simulation models could help structure studies where a certain measure of precision is needed to monitor population trends. For example, Efford (2019a) designed the R package *secrdesign* and the accompanying web-based application "SECRDESIGNAPP" (Efford, 2019b) for researchers of all levels of statistical proficiency. Using the average study design characteristics for tiger SECR studies in this review (**Supplementary Table 2**), as well as the accompanying average density in the above power analysis, assuming a grid-based design with a half-normal detection function, Poisson distribution for n, and three temporal replicates per site (a common camera trap study design used in SECR analysis), the program recommends that this design proceed with caution. SECRDESIGNAPP makes this recommendation based on the power to detect a trend in population density exceeding 80% only in cases of a net density decrease of $\geq$64.1% or a net increase of $\geq$94.9%. With all of the other study design criteria held equal, a similar study would need to deploy 240 camera stations (nearly 100 more than average) to achieve a design that meets the app's recommendations for statistical power. Moving forward, we strongly recommend future studies conduct these simulation exercises before following through with a potentially unsatisfactory field protocol.

Increasing Density Precision

Density precision increased with increasing values of baseline density and the number of individuals captured. As the former cannot be controlled by researchers beforehand, the best way to increase precision from a study design perspective would seem to be through increasing the number of individuals captured. This can be done naturally by increasing the survey area, thus exposing a greater number of individuals to sampling. However, increasing survey area is not always feasible in many typical research situations. Investigators are hindered by the amount of resources available to them, and any one study's scale can be limited by labor, money, time, political boundaries, and other factors. In order to increase the efficacy of SECR density estimation through camera trapping, especially in the context of long-term population monitoring, researchers must adopt new techniques and technologies [e.g., automatic detection through artificial intelligence (Norouzzadeh et al., 2018), online data entry and

verification platforms (eMammal:: https://emammal.si.edu/)] to increase the scale of their investigations and improve the precision of density estimates.

Future Research Using Camera Traps and SECR Analysis

There are exciting avenues through which research using SECR analysis and camera traps could be expanded. The incorporation of community science (aka citizen science) into SECR camera trapping studies can increase the scale of their investigations. Community science has expanded recently due to changing views of science and because of its scientific and societal benefits (Silverton, 2009; Adler et al., 2020). One of the hallmarks of community science is its ability to increase the spatial scale and temporal extent of investigations (Devictor et al., 2010; Abolafya et al., 2013; Jarvis et al., 2015; Adler et al., 2020). Specifically, community science has been shown to be effective in gathering baseline population and habitat usage data tracked through both space and time (Conrad and Hilchey, 2011; Sullivan et al., 2017; Horns et al., 2018; Neate-Clegg et al., 2020). Community science allows for the effective tracking of species distributions, as it allows projects to cover much greater areas than through more traditional methods (Gallo and Waitt, 2011; Hawthorne et al., 2015; Chandler et al., 2017). With camera traps, volunteers can setup cameras, maintain them in the field, and even upload and tag images to an online database. Furthermore, employing volunteers to help setup camera traps may even be a way for researchers to access land not previously available (e.g., private land, farmland, etc.). Finally, online camera trap databases (e.g. eMammal: https://emammal.si.edu/; Smithsonian Wild: http://siwild.si.edu; Wildlife Insights: https://www.wildlifeinsights.org/home; and the Urban Wildlife Information Network: https://urbanwildlifeinfo.org) make it possible for online data entry, data upload, project management, and expert review, each of which is critical to the operation and maintenance of a community science project, and these above-mentioned programs have already initiated multiple successful citizen science initiatives.

It is important, however, to note the potential drawbacks and limitations of citizen science camera trapping projects. A consistent and critical challenge to citizen science is maintaining data quality and consistency (Hecker et al., 2018). For example, qualitative analysis of citizen science data quality showed that only 62% of citizen science data meets scientifically accepted precision parameter thresholds (Aceves-Bueno et al., 2017; Adler et al., 2020). Citizen science data quality can be improved with close communication between project leads and volunteers and rigorous citizen science training, but this requires both extensive time and resources (Dickinson et al., 2010; Vann-Sander et al., 2016; Alexandrino et al., 2019). Additionally, collaboration with citizen science projects and online programs such as eMammal (https://emammal.si.edu/) make it possible for experts to review each citizen science classification. Another potential limitation of any citizen science camera trapping project is the ability to retain volunteers (Sauermann and Franzoni, 2015; Seymour and Haklay, 2017; Alexandrino et al., 2019). In one study, Wald et al. (2016) found that only a few participants complete large portions of work. The authors suggest that providing project-based benefits to return participants, sharing data with participants, and consistent communication between scientists and participants could address these low levels of retention. Furthermore, scientists must understand and empathize with the motivations of both new and return participants, especially with how these motivations change as volunteers progress through the project (Rotman et al., 2012).

Finally, modifications to spatially explicit density estimation are worth noting. Spatially explicit mark-resight models (Kelly et al., 2008; McClintock et al., 2009, 2012) incorporate information about both marked and unmarked individuals to estimate density. By using both marked and unmarked animals in density analysis, they have the ability to potentially expand the number of species that can be studied using camera traps by including species where not all individuals are identifiable. Gilbert et al. (2020) recently reviewed the methods for estimating the abundance of unmarked animals using camera traps, as well as their potential shortcomings, assumptions, and recommended uses. Although the authors show that mark-resight methods are not consistently used to estimate abundance or density of unmarked animals (appearing in $< 5\%$ of included studies) throughout the camera trap community and that relative abundance across study covariates remains the most common methodology, the method holds promise and is becoming increasingly more common.

CONCLUSIONS

Camera traps have been used for population monitoring for decades. Spatially-explicit mark recapture (SECR) methods make it possible to accurately estimate density over a given area, eliminating the need for *ad hoc* approaches like estimating individual movement through the maximum distance traveled across camera stations or applying an arbitrary buffer around the camera trap array. Currently, SECR analyses have focused on large-ranging, rare and elusive, and easily identifiable carnivores, specifically felids. These analyses have answered previously unknown questions about how these species are distributed across particular landscapes. However, a bias toward spotted, striped, or individually-identifiable animals has left much of the world's species out of the conversation when it comes to camera trap SECR benchmark studies. Furthermore, this review shows that some density estimates may not be precise enough to monitor population trends over space and time, and we offer some recommendations for increasing density precision in future studies. Conducting power analysis or simulations using readily available software should help future researchers and managers design SECR studies that meet their desired ability to monitor trends through space and time. We recommend that studies focus on increasing the total number of individuals monitored throughout a study area, which can be done by increasing the area of the camera trap array. As many studies lack the resources or labor to accomplish such an increase in

effort, we recommend that researchers think about ways to incorporate new technology, such as machine-learning, web-based data entry and deployment management, and citizen science into their study design, while recognizing that the latter comes with associated drawbacks and limitations. Lastly, SECR model development to include species that have only a subset of individuals available for individual identification (often called mark-resight models), which incorporate information from both these individuals and individuals captured without individual markings, hold promise in extending the process of explicit density estimation through camera trapping to species not individually identifiable.

SECR density estimation through camera trapping is a powerful tool in the conservation biologist's or land manager's toolbox. If executed effectively, these models can be used to monitor populations of rare, elusive, large-ranging, and individually recognizable species, making it one of the best ways to benchmark the current standing of species with recognizable individual markings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AG developed the idea for the paper, conducted the literature review, analyzed the data, and wrote the manuscript. MC helped develop the idea for the paper and reviewed the manuscript. ÇŞ helped develop the idea for the paper and wrote the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

AG would like to thank the Global Change and Sustainability Center at the University of Utah for supporting this work. ÇŞ thanks Hamit Batubay Özkan and Barbara J. Watkins for their generous support. The authors would like to thank Roland Kays, Adam Duarte, a reviewer and the handling editor for their helpful comments. Their revisions greatly improved the quality of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.563477/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 15 January 2021
doi: 10.3389/fevo.2020.579193

Standards and Best Practices for Monitoring and Benchmarking Insects

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Benchmark studies of insect populations are increasingly relevant and needed amid accelerating concern about insect trends in the Anthropocene. The growing recognition that insect populations may be in decline has given rise to a renewed call for insect population monitoring by scientists, and a desire from the broader public to participate in insect surveys. However, due to the immense diversity of insects and a vast assortment of data collection methods, there is a general lack of standardization in insect monitoring methods, such that a sudden and unplanned expansion of data collection may fail to meet its ecological potential or conservation needs without a coordinated focus on standards and best practices. To begin to address this problem, we provide simple guidelines for maximizing return on proven inventory methods that will provide insect benchmarking data suitable for a variety of ecological responses, including occurrence and distribution, phenology, abundance and biomass, and diversity and species composition. To track these responses, we present seven primary insect sampling methods—malaise trapping, light trapping, pan trapping, pitfall trappings, beating sheets, acoustic monitoring, and active visual surveys—and recommend standards while highlighting examples of model programs. For each method, we discuss key topics such as recommended spatial and temporal scales of sampling, important metadata to track, and degree of replication needed to produce rigorous estimates of ecological responses. We additionally suggest protocols for scalable insect monitoring, from backyards to national parks. Overall, we aim to compile a resource that can be used by diverse individuals and organizations seeking to initiate or improve insect monitoring programs in this era of rapid change.

Keywords: survey, methodology, metadata, entomology, insect decline

OPEN ACCESS

Edited by:
W. Douglas Robinson, Oregon State University, United States

Reviewed by:
Ek Del-Val, National Autonomous University of Mexico, Mexico
Katherine LeVan, National Ecological Observatory Network, United States

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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *01 July 2020*
Accepted: *18 December 2020*
Published: *15 January 2021*

Citation:
Montgomery GA, Belitz MW, Guralnick RP and Tingley MW (2021) Standards and Best Practices for Monitoring and Benchmarking Insects. Front. Ecol. Evol. 8:579193. doi: 10.3389/fevo.2020.579193

INTRODUCTION

"The best time to plant a tree is 20 years ago. The second best time is now." -Unattributed proverb

The threat of widespread insect declines, supported by accumulating evidence across the globe (Conrad et al., 2006; Forister et al., 2011; Hallmann et al., 2017; van Klink et al., 2020), has sparked broad and outspoken concern. But even this general pattern of insect decline is heterogeneous in

time and space, and drivers of declines in particular taxa and locations remain unclear, though they are likely myriad (Fox, 2013; Wagner, 2020). To better understand insect declines in the face of data gaps and other challenges (Didham et al., 2020), researchers need more systematic and long-term monitoring of insect abundance and diversity. Though many monitoring schemes already exist, relatively few have been operating long enough to draw robust, independent conclusions about insect populations and diversity over time (e.g., Shortall et al., 2009), and these monitoring schemes are necessarily limited in their geographic and taxonomic coverage. As scientists, we can and should lament our severely limited data on insect declines—long-term monitoring efforts should have been underway long before now, but were prevented for many reasons, including a lack of funding, motivation, and organization. Given increased societal interest in insects, there is the potential for widespread, long-term monitoring at the scale necessary to benchmark and track insect trends moving forward.

Many new monitoring efforts have been recently initiated, motivated by reports of insect declines. Researchers, managers, and community scientists are currently increasing efforts to document insects, whether photographing insects at porch lights, counting pollinators on transects, or establishing structured malaise trap programs. These efforts are a crucial first step toward broadly tracking trends in insect abundance and diversity. To maximize the information gain from these largely independent efforts, we recommend integration with established insect monitoring methods to coordinate sharing data that are accessible and interoperable. As much as possible, new monitoring efforts should align methods, metadata, and data access with those that already exist to increase explanatory power, streamline analysis, and facilitate the development of a global insect monitoring network. This network is already beginning to form through the efforts of organizations like PollardBase (Taron and Ries, 2015), the National Moth Recording Scheme (Fox et al., 2011), the Global Malaise Program (Geiger et al., 2016), as well as regional efforts, taxon-specific programs (e.g., for monarch butterflies and lady beetles), and even groups of Twitter users organizing nights to check their porch lights for insects[1]. Recently, Woodard et al. (2020) took the important step of proposing a national bee monitoring network in the United States. However, the urgency of insect declines requires even more rapid development and integration in an era of purported "insect apocalypse."

To reach the goal of a long-term monitoring network on a global scale, we will need data that, through standardization and well-defined metadata, can be integrated across monitoring efforts. Without standardized data and metadata collection, researchers will assemble datasets that are difficult or impossible to integrate, hindering synthesis. In other words, the efforts of thousands working independently are most valuable when those efforts can be assembled into a collective whole.

To meet this challenge, our aim here is to inform new monitoring projects with standardized data collection and metadata collection practices, facilitating future integration. We present a standardized toolbox for monitoring methods and metadata practices, aimed as a starting point for non-specialists and a reference point for specialists. Specifically, we provide: (1) overviews of common insect monitoring methods, including malaise, light, pan, and pitfall trapping, beating sheet, and audio and active visual surveys; (2) specific recommendations for how to carry out each method in the field; (3) an overview of metadata considerations; (4) recommendations for standardized metadata collection for each method (**Table 1**); and (5) a forecast of emerging methods that can complement and extend existing methods.

Our audience is anyone interested in insect monitoring, from community members motivated to contribute to science, to entomological specialists who want to make their data more broadly useful. We especially hope these recommendations will aid those interested in insect monitoring but are not sure where to start. Workers can choose monitoring methods from the toolbox we present, then modify as needed for their goals and systems. We make these recommendations with scalability in mind—the methods we discuss are generally low cost, field-tested, and can be performed by a single individual. We generally organize these methods by following the framework presented by Ferro and Summerlin (2019), while our summaries and recommendations are especially influenced by Southwood and Henderson (2000), Samways et al. (2010), and previous efforts to advocate for sampling alignment for bees (Droege et al., 2017) and birds (Ralph et al., 1993). Along these lines, we do not advocate for existing monitoring networks to change their methods even if they are not easy to integrate with other efforts. Though methodological standardization is ultimately a goal, we pragmatically advocate as much for *alignment* with and among existing monitoring efforts as we do for standardization.

In the following sections, we outline recommended methods for sampling a given set of insect taxa for monitoring purposes. These methods are generally suitable for a variety of key benchmarking goals, including the measurement of occurrence and distribution, phenology, abundance and biomass, and diversity and species composition (**Box 1**). This is not a guide for conducting exhaustive species inventories (e.g., BioBlitzes or site lists), which often emphasize maximizing species counts, nor is it a guide for maximizing insect catches. This guide also does not aim to eliminate bias—no method is free of bias, but if methods and metadata are documented carefully and are consistent over time, then bias can largely be estimated and controlled. Additionally, monitoring insects in sites with rare and endangered species also requires unique considerations that must be site- and species-specific, and we do not cover the complexities of those considerations here, nor do we go into details of how to analyze data once collected. Finally, we do not recommend these sampling methods for entomologists with highly specific taxonomic goals; the class Insecta is simply too diverse in its niches and behaviors to be comprehensively assessed by one or even a few sampling methods. Here, we attempt to create a balance between being too general or too specific, by presenting a variety of methods for sampling broad taxa and guilds, each with its own strengths and weaknesses (**Figure 1**). If you find yourself wanting to monitor more specific insect groups, your sampling

[1]https://www.anecdata.org/projects/view/738

TABLE 1 | Recommended metadata for each of seven methods of insect monitoring.

	Required metadata	Malaise trapping	Light trapping	Pan trapping	Pitfall trapping	Beating sheet	Acoustic monitoring	Active visual surveys
Locality	GPS coordinates of sampling location(s)	X	X	X	X	X	X	X
-	Location description	X	X	X	X	X	X	X
-	Photo of trap *in situ*	X	X	X	X	X	X	X
Site description	Habitat description	X	X	X	X	X	X	X
-	Photos in four cardinal directions showing habitat	X	X	X	X	X	X	X
-	Description of plant phenology (e.g., leaf-out, flowering, senescence)					X		X
-	Amount of light pollution		X					
-	Sampling substrate (e.g., plant species)					X	X	
-	Substrate size (including number of leaves)					X		
-	Substrate condition (e.g., wetness)					X		
Temporal	Date trap or monitoring established	X	X	X	X	X	X	X
-	Date of data collection	X	X	X	X	X	X	X
-	Time beginning data collection	X	X	X	X	X	X	X
-	Duration of data collection	X	X	X	X	X	X	X
-	Time of detection							X
Environmental	Wind during sampling (Beaufort scale)	X	X	X		X	X	X
-	Temperature during sampling	X	X	X	X	X	X	X
-	Precipitation during sampling		X	X	X	X	X	X
-	Humidity during sampling		X			X		
-	Cloud cover during sampling		X			X	X	X
-	Lunar phase during sampling		X				X	
Sampling description and placement	Trap or sampling equipment type	X	X	X	X	X	X	
-	Trap or sampling equipment photo	X	X	X	X	X	X	

(Continued)

TABLE 1 | Continued

	Required metadata	Malaise trapping	Light trapping	Pan trapping	Pitfall trapping	Beating sheet	Acoustic monitoring	Active visual surveys
-	Trap or sampling equipment manufacturer and model	X	X	X	X	X	X	
-	Trap or sampling equipment dimensions (e.g., size of capture area)	X	X	X	X	X		
-	Mesh hole size, density, and shape	X						
-	Killing agent	X	X	X	X			
-	Use of scent or bait			X	X			
-	Trap orientation	X						
-	Height from ground	X	X	X		X		
-	Bulb type, wavelength, power, and brightness		X					
-	Amount of liquid evaporation during sampling			X				
-	Trap, pan, or sheet color	X		X	X	X		
-	Collecting method (e.g., aspiration)					X		
-	Number of hits per substrate					X		
-	Object dimensions and weight used for hitting					X		
-	Detection distance							X

In order for systematic insect monitoring data to be fully used and integrated by future researchers, scientists need information on the methods and conditions underlying data from insect monitoring (i.e., metadata). Metadata fall into a number of general classes, documenting details on: locality, site, temporal, and environmental conditions, and sampling methods. Some metadata will need to be collected every time insect data are collected, while other metadata will only need to be collected once, for example, when traps are first set up. Metadata should be stored alongside insect data (both on paper and digitally) and efforts should be made to include all metadata when contributing survey data to data aggregation projects.

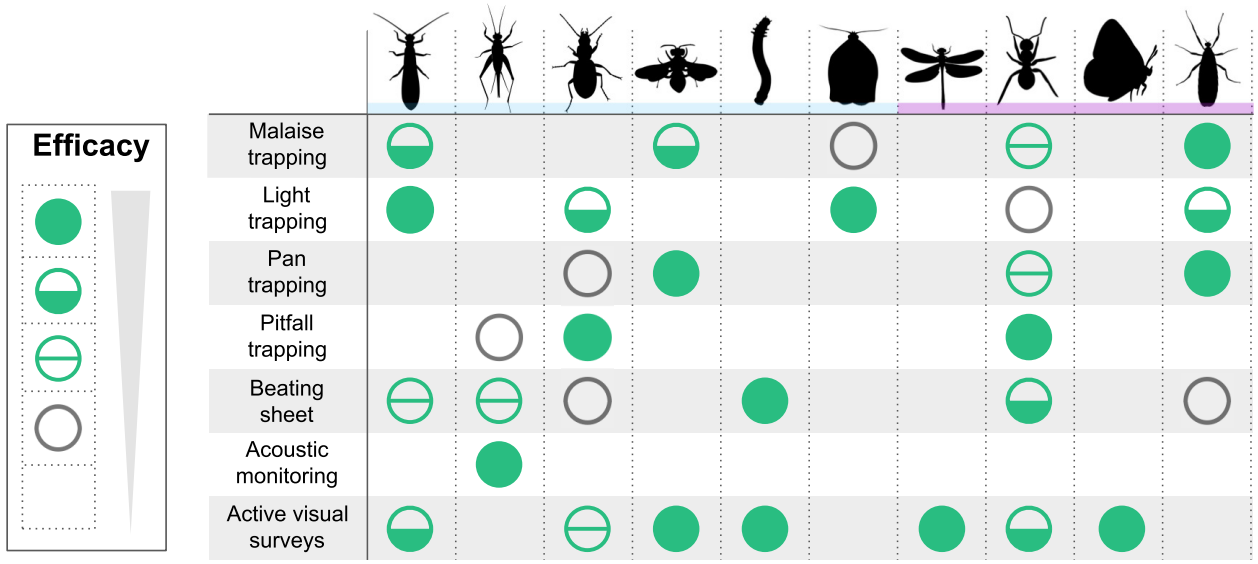

FIGURE 1 | Commonly-monitored insect guilds and taxa and the efficacy for each of seven benchmarking methods. Efficacy of each method for a given insect group is scored as follows: filled green circles indicate optimal suitability; half-filled circles indicate possible suitability; divided, unfilled circles indicate marginal suitability; unfilled gray circles indicate bycatch only; and no circle indicates general unsuitability. Insect groupings are defined by ecological traits (blue bar) or taxonomic clades (purple bar). In order by column, insect groupings are: adult semi-aquatic insects (Plecoptera, Ephemeroptera, and Trichoptera); singing insects (Orthoptera & Hemiptera: Cicadoidea); ground-dwelling beetles (Coleoptera: Carabidae and Staphylinidae); non-lepidopteran pollinators (Hymenoptera, Diptera, Coleoptera); leaf-chewing larvae (Lepidoptera and Hymenoptera: Symphyta); night-active moths (Lepidoptera); dragonflies and damselflies (Odonata); ants (Hymenoptera: Formicidae); butterflies (Lepidoptera: Papilionoidea); and flies (Diptera).

methods may need to be modified from those discussed here or may not be covered.

STANDARDIZED MONITORING PRACTICES FOR DIFFERENT TAXA AND METHODS

Malaise Trapping

Overview

Malaise traps (Malaise, 1937) are large tent-like structures made of netting meant to funnel insects to a common area (**Figure 2A**). In essence, an insect flies into a vertical wall of netting, responds by flying upwards, then is gradually funneled by sloped netting into a collecting vial. This vial is then checked and emptied periodically over days or weeks. The Townes-type malaise trap is the most common style used, but at least four other types—Gressitt malaise, Schact malaise, Sea, land, and air malaise (SLAM), and Cornell malaise—are also in use (Matthews and Matthews, 1983; van Achterberg, 2009). For detailed accounts of history and methodology, see van Achterberg (2009). Exemplar malaise trapping programs include the School Malaise Trap Program in Canada (Steinke et al., 2017) and the Swedish Malaise Trap Program (Karlsson et al., 2020). For those interested in joining an existing network, the Global Malaise Trap Program/BIOSCAN (Geiger et al., 2016) is accepting new members.

Taxonomic Considerations

Malaise trapping is only appropriate for monitoring flying insects (**Figure 1**). Many flies (Diptera) and some wasps, flying ants, bees (Hymenoptera), bugs (Hemiptera), moths (Lepidoptera), and semi-aquatic taxa are effectively sampled by malaise traps (Matthews and Matthews, 1970; Noyes, 1989; Campbell and Hanula, 2007; Fraser et al., 2008; Mazon and Bordera, 2008; Diserud et al., 2013; Schmidt et al., 2019). Within these groups, malaise trapping is especially appropriate for Tenthredinidae, Ichneumonoidea, Scelionidae, Mymaridae, and other hymenopterans with similar life histories, as well as Cicadellidae and Cercopidae (Hemiptera), microlepidopterans (Lepidoptera), and the semi-aquatic orders Plecoptera, Ephemeroptera, and Trichoptera—if traps are placed alongside aquatic habitats. It is important to note that malaise trap efficacy for a taxon can depend on habitat. For example, bees (Apoidea) are sampled well in some habitats, like tallgrass prairie (Geroff et al., 2014), but pan-trapping is generally more effective for sampling this superfamily (Campbell and Hanula, 2007). The narrower the taxon of interest, the more necessary it is to customize these recommendations to your own system.

Methodological Considerations

Location

Spatial placement is extremely important for malaise trapping; it is critical to document the trap's exact position and microhabitat by photograph, written description (particularly for habitat), and precise coordinates (see **Table 1** for additional metadata needed).

BOX 1 | Measurement goals of benchmarking studies.

The goals of benchmarking and monitoring studies typically aim to measure change in at least one of the following: (1) occurrence and distribution, (2) phenology, (3) abundance and biomass, or (4) diversity and species composition. The seven monitoring methods we highlight can be used with the goal of measuring changes in as many of these responses as desired. Each of these four categories of response is important for different reasons, requires a different minimum spatiotemporal scale of sampling, and is currently used to study how insect populations and communities change over time.

Occurrence and distribution: Changes in occurrence and distribution are important indicators of how shifts in underlying processes affect organisms. Occurrence can also sometimes serve as proxies for abundance (Royle and Nichols, 2003). Estimating occurrence and distribution requires, at minimum, the formal identification of a taxon at a location (i.e., a presence), but the addition of data on what taxa were not present (i.e., an absence), allows for a more powerful analysis of occurrence. For insects, occurrence and distribution monitoring is perhaps the most widespread benchmarking method (e.g., Chen et al., 2009; Boyes et al., 2019; Outhwaite et al., 2019), especially for invasive species and in the context of shifting ranges due to climate change.

Phenology: Shifts in phenology can indicate changes in the factors governing the timing of insect life cycles, from temperature and precipitation patterns, to flowering periods in plants. Shifts in insect phenology can cause mismatches with other taxa in their communities, from plants to birds, and can have demographic consequences for those taxa (Visser and Gienapp, 2019). Estimating phenology typically requires, at minimum, presence data for a taxon at a location repeatedly over a short time span (i.e., a "season"), but presence and absence data together allow for stronger inference. Changes in insect phenology are poorly documented in most taxa, but recent interest in the effects of climate change has spurred a larger focus on insect phenology in monitoring efforts (Gimesi, 2012).

Abundance and biomass: Changes in abundance and biomass, both measures of ecosystem function, are important for understanding the health of the ecosystem as well as for conservation and management. Estimating abundance and biomass typically requires presence and absence data in addition to accurate counts of individuals of each taxa. This form of monitoring is historically rare but has been perhaps the most influential in spurring recent interest in insect declines (Hallmann et al., 2017; Wepprich et al., 2019).

Diversity and species composition: Changes in measures of biodiversity, such as species diversity and composition, indicate how communities respond to environmental change. Estimating diversity and species composition typically requires presence and absence data for multiple taxa in a community, with some expectation that sampling is equally likely across taxa. Monitoring insect diversity and composition is relatively common compared to abundance, biomass, and phenological monitoring (e.g., Brooks et al., 2012; Valtonen et al., 2017).

Choice of placement will vary based on the particular taxon of interest, but malaise traps should generally be placed along natural flight corridors (e.g., streams or gaps between bushes) to maximize catch. Amount of wind exposure should also be recorded, as wind can limit efficacy. The vertical panel of netting (i.e., the interception area) that insects hit should be oriented to be perpendicular to the expected movement corridor.

Design

We recommend Townes-type malaise traps due to their broad efficacy and already widespread use. Multiple sizes are available, with a 165 × 110 cm interception area being most common. The vertical wall of netting is commonly black to reduce visibility, while the dome of the tent is white to increase insects' propensity to fly upwards (i.e., toward "the sky"), which increases catch. We recommend 95% ethanol (as is used by BIOSCAN) as a killing agent that preserves DNA, but lower concentrations (down to 80%) can be used if evaporation or cost prohibits use of 95%. Alternative approaches to collection include cyanide (hazardous to humans), ethyl acetate (destroys DNA), and live collecting (needs to be checked daily, and specimens are often damaged). Ethanol, however, does remove scales from taxa like lepidopterans. Mesh size and shape are also important to note: holes that are too wide are less effective at sampling small flies, wasps, and other microfauna, but large mesh sizes can better sample groups like stinging hymenopterans (Darling and Packer, 1988). Some insects, such as many beetles, drop after hitting the mesh screen, rather than trying to escape by flying higher. To take advantage of this, pans with collecting liquid can be placed underneath the mesh wall, creating what is called a flight-intercept trap (traditional malaise traps are a specialized flight-intercept trap that only samples upward-moving insects). If this is done, pan color should be recorded.

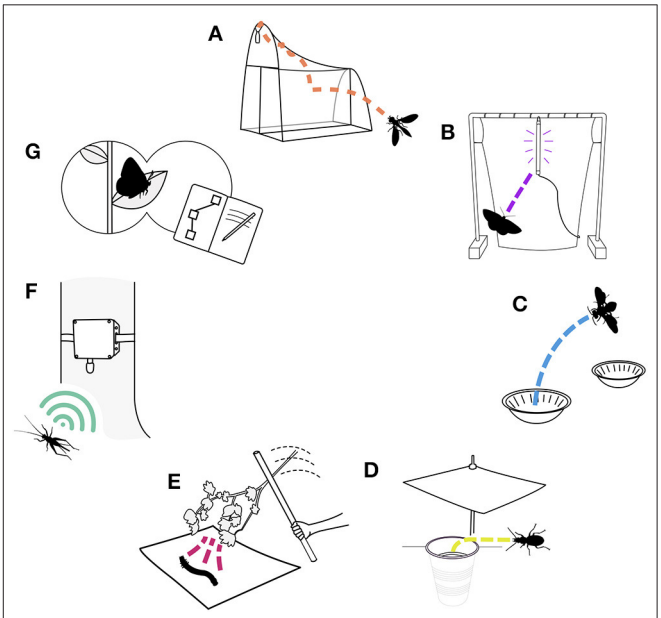

FIGURE 2 | A visual overview of the seven insect benchmarking methods summarized here: **(A)** malaise trapping, **(B)** light trapping, **(C)** pan trapping, **(D)** pitfall trapping, **(E)** beating sheet, **(F)** acoustic monitoring, and **(G)** active visual surveys.

Scalability

Commercial malaise traps can be expensive (usually > US $230). Costs can be reduced by constructing home-made malaise traps (e.g., Blackmon, 2010). Mosquito netting can be used for the interception area (Lamarre et al., 2012), but mesh size

and shape should be considered. Setup effort is generally low (up to 1 h the first time). Sampling effort is minimal once a malaise trap is set up (10 min per week once at the site): the sample vial simply needs to be emptied into a storage vial. Post-sampling effort can be high, however, since malaise trapping can yield large numbers of insects (e.g., up to 10,000 specimens per week depending on the site). DNA metabarcoding of the sample is a faster post-sampling identification method for measuring diversity (though abundance is lost), and non-destructive methods to sample from fixative fluids show promise (Marquina et al., 2019; Nielsen et al., 2019; Zizka et al., 2019). Although metabarcoding lowers the time costs off post-sampling identification, metabarcoding does come with increased costs related to DNA sequencing and additional genetic expertise is needed. See Hausmann et al. (2020) for a recent example of a malaise trap study employing metabarcoding. Finally, because malaise traps are sensitive to microhabitat variation—like most stationary sampling methods—multiple traps at a site are better than a single trap.

Light Trapping

Overview

Light traps are one of the most common and efficient methods for surveying insect that fly at night. At their most basic, light traps simply consist of a light attractant and a viewing surface, often a bedsheet (**Figure 2B**). More structured light traps commonly consist of a funnel, vanes (which deflect insects toward the funnel), and a collection container, which together are used in conjunction with the light source to form a structured trap. In either case, light-attracted insects fly toward the light source, hit a surface or vanes surrounding the light, and can then be observed and recorded or sampled and collected. Common styles of vaned light traps include Robinson traps and Heath traps (Macgregor et al., 2017). Light traps provide an opportunity to gather standardized and comparable data, but many factors influence the abundance and composition of light trapped insects, including trap type, season, time of day, lunar phase, duration of sampling, and light attractant (Jonason et al., 2014). Consequently, these details are all important to track (**Table 1**). Mercury vapor bulbs are the most commonly used attractant and have consistently caught a higher abundance and diversity of insects than other standard bulbs due to the powerful low-wavelength light emitted (Jonason et al., 2014; White et al., 2016). Other commonly used bulb types include UV, metal halide, and LED (Ferro and Summerlin, 2019). Although many commercial light traps are available and can be deployed in remote locations, light trapping can be as simple as documenting the moths that are attracted to your porch light. Individuals interested in joining the Discover Life's Mothing project can join a network of people working to photograph and identify moths that come to their porch light (Pickering, 2015). Exemplar long term light-trapping programs include the Hungarian Light-trap Network (Szentkirályi, 2002) and the Rothamsted Insect Survey (Macgregor et al., 2019), both of which have been surveying phototactic insects for over 50 years.

Taxonomic Considerations

Light trapping is appropriate for monitoring phototactic (i.e., light-attracted) night-flying insects in both terrestrial and aquatic habitats and is used for surveying a wide range of insect taxa (**Figure 1**), including flies (Diptera), true bugs (Hemiptera), beetles (Coleoptera), caddisflies (Trichoptera), parasitic wasps (Hymenoptera, and moths (Lepidoptera), among other groups. Light trapping is especially appropriate for moths (Lepidoptera) (Macgregor et al., 2019), caddisflies (Trichoptera) (Waringer, 2003), and many beetle taxa (Coleoptera) (Liu et al., 2007).

Methodological Considerations

Location

Spatial placement is extremely important for light trapping. Although light traps can attract insects from the surrounding environment, insects are rarely attracted at distances >30 meters (Truxa and Fiedler, 2012). Therefore, the microhabitat of the trap location will influence what organisms are trapped, making it important to describe the trap location in field notes and record the precise coordinates of the trap location (**Table 1**). Light pollution can decrease the flight-to-light behavior of moth populations (Altermatt and Ebert, 2016), so light sensors (low quality light sensors are available as smart phone apps) should be used to note the lumens/m^2 of light pollution at the trap location.

Design

The wavelength and brightness of light attractants differs dramatically among different light bulbs and are important to consider when designing light trapping projects. If the goal is to sample the greatest abundance and diversity of insects, then we recommend using mercury vapor bulbs, as these are consistently found to attract the most moths (Jonason et al., 2014; White et al., 2016). However, mercury vapor traps may not be the optimal tool because of their cost and the logistics required to deploy them (e.g., an outlet or automotive battery is needed). Therefore, low-cost, light-weight, and easy to deploy light traps offer convenient alternatives and facilitate insect trapping at more sites and in more diverse settings (White et al., 2016). If light traps can be checked early in the morning, insects can be trapped alive by having traps lined with egg cartons to provide areas for the insects to hide (Jonason et al., 2014). Live specimens can be photographed in the field or later in the lab, after cooling in a refrigerator and then released the following night (Ford et al., 2020). If observation alone is undesirable, light trap containers can be lined with pest strips (18.6% dichlorvos [2,2-dichlorovinyl dimethyl phosphate]) or filled with ethanol.

Scalability

Commercial light traps can be expensive (between US $75 and US $500). Additionally, commercial light traps often require outlets or car batteries, making carrying them into remote locations challenging. Homemade light traps can be inexpensively constructed and can greatly reduce weight by running efficient LED strips using small 12V batteries (White et al., 2016). Effort required for field sampling can be high if insects are live trapped and identified in the field or transferred to jars to be photographed. If insects are lethally trapped, sampling

effort can be low, with just a few minutes spent setting the light trap each sampling event and a few minutes spent collecting the specimens the following morning. However, post processing costs can be high, as light traps can yield a high diversity and abundance of insects. Technological advances in "smart light traps," where insects attracted to lights are automatically photographed throughout the night, offer great promise to increase the scalability of light trap surveys (Hogeweg et al., 2019).

Pan Trapping

Overview

Pan traps (Moericke, 1951) are trays filled with liquid set out to collect insects. Pan traps often rely on color as an attractant and are effective primarily because insects mistake them for food resources. An insect flies to a pan, attempts to land, then becomes trapped in the liquid solution—often soapy water, propylene glycol, or saline (**Figure 2**). Pan traps can be made from nearly any object that holds liquid—i.e., a disposable plate filled with water and a few drops of dish soap—and this accessibility has made them more popular than more training-intensive methods that may sample more diversity (e.g., standardized sweep-netting; Cane et al., 2000). Like all sampling methods, there is no doubt that pan traps have considerable sampling bias for certain taxa (Portman et al., 2020). For detailed accounts of history and methodology, see Droege et al. (2017), LeBuhn et al. (2003), Vrdoljak and Samways (2012), and Southwood and Henderson (2000). An exemplar pan trap monitoring networks is the UK Pollinator Monitoring Scheme, and a data-recording scheme designed for bee monitoring can be found in LeBuhn et al. (2003).

Taxonomic Considerations

Pan trapping is appropriate for monitoring flying insects (**Figure 1C**). It is effective at sampling aphids (Hemiptera), thrips (Thysanoptera), bees and parasitic wasps (Hymenoptera), flies (Diptera), some beetles (Coleoptera), and even some grasshoppers (Orthoptera) (Evans and Bailey, 1993; Westphal et al., 2008; Vrdoljak and Samways, 2012). Trap efficacy for each taxon varies strongly with pan color (Vrdoljak and Samways, 2012). Yellow is most commonly used, as yellow traps often collect the largest catches and highest total insect diversity, but other common colors include blue, white, red, and green. As with other monitoring methods, habitat and geographic region can affect the trap efficacy for a given group (Vrdoljak and Samways, 2012; Saunders and Luck, 2013). For those with broad taxonomic interests for their monitoring programs, we recommend what has become a common standard: yellow pan traps in conjunction with white and blue pan traps (Vrdoljak and Samways, 2012; Sircom et al., 2018), as is done in the UK Pollinator Monitoring Scheme. If needed, traps can be painted using colors defined by the Bee Inventory Plot program (LeBuhn et al., 2003).

Methodological Considerations

Location

Pan traps are typically placed in open areas where they can be seen by target insects. Traps can be placed together as close as 5 m—the minimum distance at which they do not influence each other (Droege et al., 2017). Although a large diversity of spatial arrangements exist, we recommend one of two methods. The first method, used by the UK Pollinator Monitoring Scheme, places 1 trap per square km, and is suitable for sampling large geographic regions. The second method, from the Bee Inventory Plot (LeBuhn et al., 2003), uses 15 traps in a single array, each separated by 5 m and placed in two perpendicular lines forming an "X." This arrangement is suitable for targeted monitoring, with two arrays (30 traps) demonstrated as being adequate for sampling local bee diversity (Shapiro et al., 2014).

Design

For discussion of pan trap color, see *Taxonomic considerations*. Pan traps can be placed on the ground (most common), elevated above the ground, or placed flush with the substrate (i.e., essentially modified pitfall traps). Elevated pan traps sometimes yield larger numbers of specimens (Tuell and Isaacs, 2009), and pan traps flush with the ground can also attract ground-dwelling species (Ernst et al., 2016). Trap size may not affect catch (Gonzalez et al., 2020), so small pan traps are desirable to minimize costs; circular pans with a 7 cm diameter are common. In arid areas, the trap solution may evaporate too quickly between visits, so larger pan traps can be used, for example, 2-gallon buckets. The amount of liquid in a trap can affect trap efficacy and should be recorded when setting up and checking traps. We recommend premixing the liquid solution recipe of LeBuhn et al. (2003): 1 part dish soap to 750 parts water (approximately 1 teaspoon soap for a gallon of water).

Because pan color is the main attractant, it is important to maintain trap color through frequent cleaning and eventual trap replacement when color fades. Scented water like rose water can be used to increase catches for some taxa (Laubertie et al., 2006) but since maximizing the number of individuals caught is not necessarily a goal of standardized monitoring, we recommend against using scents or baits for benchmarking. Small amounts of preservative chemicals can be added to prevent fungal growth when the time between visits is necessarily long, but chemical safety precautions should be taken.

Scalability

Pan traps are low cost but require frequent (often daily) trap visits and maintenance. For an estimated time budget for a 24-pan transect, see Droege et al. (2017). Specimen processing times can be high depending on target taxon abundance. A typical pan in a field could yield only a few specimens over 24 h, but even with low abundances, numbers can rise quickly if using multiple traps over long time periods. Sieving with nylon mesh (e.g., an aquarium net) is a common practice that speeds up specimen processing, but can damage small, fragile taxa such as aphids.

Pitfall Trapping

Overview

Pitfall traps (Hertz, 1927) are containers placed flush with ground level to capture ground-dwelling (epigeic) insects. In essence, an insect walks to the trap edge, loses balance, and falls in (**Figure 2D**). The container is then checked, the catch collected or documented, and reset. Several recent reviews have discussed

pitfall trapping (Skvarla et al., 2014; Brown and Matthews, 2016; Hohbein and Conway, 2018), and standardized traps have been proposed by Brown and Matthews (2016). Like any insect sampling method, pitfall traps produce taxonomically biased samples (Topping and Sunderland, 1992; Lang, 2000), but are inexpensive and popular for monitoring. For detailed methodological accounts, see Southwood and Henderson (2000), Brown and Matthews (2016), and Hoekman et al. (2017). Existing pitfall trap monitoring networks include the US National Ecological Observatory Network (NEON; Hoekman et al., 2017), and the UK Environmental Change Network (Brooks et al., 2012).

Taxonomic Considerations

Pitfall traps are most appropriate for sampling ground-dwelling beetles (Coleoptera)—especially Carabidae and Staphylinidae—and ants (Hymenoptera: Formicidae) (Baars, 1979; Skvarla et al., 2014). They may incidentally collect flying taxa, especially if the trap is roofless and white or yellow (Buchholz et al., 2013), but are not an effective sampling method for most other groups (**Figure 1**).

Methodological Considerations

Location

Pitfall traps can be placed nearly anywhere with suitable substrate for digging. There is some controversy over how far apart traps should be placed; some studies have found that traps provide independent samples even when only 1 m apart (Ward et al., 2001), while others recommend 10 m (Hohbein and Conway, 2018). NEON, which conducts standardized trapping across North American sites, separates traps by at least 25 m. Digweed et al. (1995) found that population depletion occurs when traps are separated by 10 m or less, but not if separated by distances of <25 m. Until there is more consensus, a 25 m distance between traps should be adequate to ensure independence of samples.

Design

Pitfall traps can be made of glass, plastic, or metal, but disposable plastic cups have become perhaps the most widespread trap container. The container should be placed with its lip flush with the soil surface. As might be expected, the diameter of the trap affects catch (Abensperg-Traun and Steven, 1995). Collecting fluid should generally be used to avoid damage to specimens from other trapped insects and to prevent escapes, but the type of collecting fluid used can affect the taxa attracted (Skvarla et al., 2014). Ethylene glycol has been traditionally used but is toxic for wildlife if consumed and can be easily substituted with propylene glycol, which we recommend for most uses. Propylene glycol evaporates more slowly than ethanol and adequately preserves most DNA, at least over the short-term (Nakamura et al., 2020). Traps with baits will be more readily disturbed by vertebrates (Vandenberghe, 1992), and should be avoided. Fences, or guidance barriers that direct insects toward the trap, can increase catch (Boetzl et al., 2018) but require more effort to set up. Pitfall color affects taxonomic composition of the catch (Buchholz et al., 2013) and should be recorded; we recommend using transparent containers as described in Brown and Matthews (2016). Using funnels increases catch efficiency while simultaneously reducing vertebrate bycatch (Radawiec and Aleksandrowicz, 2013), although low roofs also reduce vertebrate bycatch (Hoekman et al., 2017). Roofs also prevent rain from diluting the collecting fluid and appear to not influence the composition or magnitude of insect catch (Buchholz and Hannig, 2013). Containers should be nested to allow fast and easy removal of samples. Disturbance from trap placement can affect catch, so a latent period of 1–2 weeks before trap monitoring begins should be observed if possible (Greenslade, 1973).

Calls for pitfall trapping standardization have a longer history than other monitoring methods (Brown and Matthews, 2016), and we recommend alignment with existing programs. Given the broad extent of NEON, we recommend that new monitoring programs (at least those in North America) adopt the NEON pitfall trapping protocol when practical (Hoekman et al., 2017). This protocol involves nested clear plastic cups (diameter: 11 cm, depth: 7 cm, volume: 473 mL) and a roof made of hard plastic raised 1.5 cm above the trap entrance. Each trap is filled with 150 mL of an equal ratio of propylene glycol to distilled water. Traps are placed in arrays of 4, arranged in a square with sides 25 m long.

Scalability

Pitfall trapping using disposable plastic cups is relatively cheap and easily scalable. Set-up can be labor intensive depending on the design (and the inclusion of fences), but under the NEON protocol is limited to simply digging an appropriately sized hole and placing the trap and roof. Checking traps is also a low time commitment, especially when using a nested cup design, which allows for easy removal.

Beating Sheets

Overview

A beating or beat sheet is a piece of fabric supported by a frame, which is placed below a substrate of interest (e.g., a tree branch). An insect rests or feeds on the substrate, which is then shaken or hit ("beat"), dislodging the insect so that it falls on the sheet where it can be collected or recorded (**Figure 2E**). This active sampling method is often used in conjunction with an aspirator to suck up fast-moving taxa. Currently, the most common design is two pieces of wood or PVC pipe forming an "X," with a piece of white fabric (e.g., bedsheet) stretched behind. Alternative designs include simply placing a sheet on the ground, or even using an umbrella. Beating sheets are cheap, easy to build, and straightforward to use. Insects can be recorded visually or collected for further identification depending on project goals. The Caterpillars Count! citizen science program (Hurlbert et al., 2019) is an example of a beating sheet monitoring network in North America.

Taxonomic Considerations

Beating sheets are appropriate for sampling tree and shrub dwelling insects, such as caterpillars (Lepidoptera), some true bugs (e.g., aphids and scale insects; Hemiptera), some beetles (Coleoptera), and other plant-feeding insects (**Figure 1**). It is not

a good method for sampling flying insects; they will often fly away when the branch is hit, or hit the sheet, then quickly escape.

Methodological Considerations

Location

Beating sheets can be used anywhere vegetation is found for beating: typically shrubs and trees, but also groundcover in some cases.

Design

The standard size and shape for a beating sheet is a square with sides of about 90 cm (3 feet), using two pieces of PVC or wood 1.3 m (51 inches) long for crossbars. A cloth can then be stapled or glued to the crossbars. White cloth should be used to maximize visibility of insects that fall on the sheet. The object used for hitting the substrate can vary, but dimensions should be recorded; a stick of about 2.5 cm (1 inch) diameter and 60 cm (2 feet) long works well. The surveyor should strongly hit the branch 10 times, but not so strongly that the plant is damaged. Because many insect species have some degree of host specificity, substrate type (e.g., tree species) strongly predicts insect species diversity and abundance. This will affect sampling decisions depending on your goals; sampling plants of only one species or of multiple species are both reasonable. Either way, we strongly recommend always recording the plant species (**Table 1**). If collecting specimens, using an aspirator helps capture fast-moving or flying insects.

Scalability

A simple beating sheet can be constructed in <15 min using materials that cost <US $10[2]. As an active sampling method, using beating sheets can be more time consuming than passive methods, but usually not prohibitively so. Sampling a substrate and collecting the specimens can take <5 min. Visual surveys can be substituted for specimen collection to reduce time spent on post-sampling identification, but at the likely cost of taxonomic resolution. Only one beating sheet is needed per person sampling. Some research suggests that three plants of the same species is the minimum necessary to accurately estimate insect abundance (Harris et al., 1972).

Acoustic Monitoring

Overview

Acoustic monitors provide a passive, non-destructive method to detect and identify insects (Ganchev et al., 2007; Mankin et al., 2011). Insects may generate bioacoustic signals as a means of communication (Penone et al., 2013), or as a by-product of locomotion (Kawakita and Ichikawa, 2019). These bioacoustic signals may be captured as sounds with microphones or as vibrations with contact sensors (**Figure 2F**). Although contact and ultrasonic sensors have been successful in detecting insect pests that live inside agricultural products (Mankin et al., 2011), we focus here on acoustic recording units and their use for surveying the relative abundance and diversity of insects. Many factors influence the efficacy of acoustic devices in identifying and estimating density of insects, including the frequency range, substrate (air or water), type of sensor, the size and behavior of the insect, and the distance between the insects and the sensors (Gibb et al., 2019). These factors should be considered and noted when conducting surveys using acoustic monitoring. Large-scale acoustic monitoring has been successfully coordinated by the French National Museum of Natural History to assess the impacts of anthropogenic stressors on Orthoptera communities (Penone et al., 2013; Jeliazkov et al., 2016).

Taxonomic Considerations

Acoustic monitoring is an appropriate method for monitoring insects that use sounds or vibrations in communications. Some of these noises, such as cicada and cricket songs can be detected over long distances. If the bioacoustic signal produced by insects follows a consistent species-specific pattern, it can be extracted from background noise for identification purposes (Ganchev et al., 2007). Therefore, passive acoustic monitoring is particularly well-suited for loud terrestrial insects such as Orthoptera or Cicadoidea because they produce species-specific mating calls (Penone et al., 2013) (**Figure 1**), but may also be useful for a variety of other insects including bees (Galen et al., 2019; Kawakita and Ichikawa, 2019) and aquatic Hemiptera (Desjonquères et al., 2020; Gottesman et al., 2020).

Methodological Considerations

Location

The distance at which acoustic signals can be detectable above ambient noise varies depending on the sound's amplitude and frequency, landscape heterogeneity such as topography and vegetation, and weather (Gibb et al., 2019). Additionally, anthropogenic sounds or sounds from other animals can mask target sounds. The precise GPS coordinate of where static sensors are deployed must be recorded and potential sources of sound pollution should be noted (**Table 1**). Bioacoustic devices can also be used while traveling along transects, but we recommend keeping bioacoustic devices in fixed locations to collect data that is easier to standardize across sites and replicate across visits.

Design

Commercially available acoustic monitors can be flexibly programed to collect acoustic signals and on-board metadata for long intervals across a variety of sampling regimes (Hill et al., 2019). The use of inexpensive components (e.g., microelectromechanical systems microphones) may decrease financial barriers to initiating multisensor surveys but can lower data quality by having lower signal-to-noise ratios and inconsistent frequency response (Gibb et al., 2019). Critical to successful acoustic surveys is the development of efficient pipelines to process sound files and output annotated data. Manually annotating data is time consuming and can be biased by the analyst's knowledge level. Developing automated machine learning pipelines to process individual sound files—which can each include more than 10 min of ambient sound recording—can both increase the efficiency of data processing while also making data processing more reproducible and interoperable.

[2]https://vimeo.com/43932105

Scalability

Recent advances in custom built electronics and the lowering costs of small but usable microphones provide novel opportunities to monitor select insect taxa across greater spatial and temporal scales. Passive acoustic monitors can automatically collect data over long periods (e.g., a month), with minimal maintenance needed to replace batteries and digital memory cards (Hill et al., 2019). Typically, they are programmed to record periodically during a window of interest. Recent developments in customizable acoustic devices have dramatically lowered costs closer to US $50 (Hill et al., 2018). Developing automatic identification pipelines using machine learning algorithms is critical to scaling acoustic monitoring and discussed further in section looking forward.

Active Visual Surveys

Overview

Visual surveys are commonly used to document the abundance and diversity of insects that can easily be visually identified in the field, often with the aid of close-focus binoculars and nets (**Figure 2G**). These surveys typically involve researchers documenting the presence of a species or counting the total number of individuals of each species observed during a standardized survey. The most frequently used methods include (1) transects, (2) point counts, and (3) area counts. Although mark/recapture is another frequently used visual survey technique to document insect population dynamics, we do not consider that a viable benchmarking technique as it takes enormous effort and would not be tractable to do simultaneously for large numbers of insect species.

All three of the commonly used methods have extensive histories of standardized protocols. Transect counts use visual identification while searching along predefined transects with specified search distances. Pollard walks are a commonly used transect method used in butterfly research, in which an observer visualizes a box that extends 5 m ahead and 5 m to the sides as they walk a transect counting butterflies (Pollard, 1977). Point counts, where an observer stands still and identifies and counts the number of individuals of the target taxa around them during a set period of time, provides an alternative to transects in sites that are difficult to walk in or where habitats are fragile or at-risk (Henry et al., 2015). Distance sampling techniques, where observers note their distance from the observed insect, can be implemented with both transects and point counts to estimate densities (Isaac et al., 2011; Henry et al., 2015). By incorporating imperfect detection, distance sampling allows for density and absolute population size to be estimated in closed populations (Buckland, 2001). Area counts, such as the North American Butterfly Association's count circle (Taron and Ries, 2015), consist of surveyors counting each species within a delimited study plot during a certain time period. Insects that are challenging to identify quickly or in flight can be netted and transferred into vials and placed in a cooler to chill until the end of the survey period (Loffland et al., 2017). Photos of chilled individuals can then be taken for later identification before releasing these individuals. Exemplar visual survey programs include multiple butterfly monitoring schemes (BMS) such as the UK BMS, the Dutch BMS (Schmucki et al., 2016), and the Ohio Lepidopterists BMS (Wepprich et al., 2019).

Taxonomic Considerations

Visual surveys are only appropriate for large insects that can be easily detected and identified or photographed in the field. Butterflies (Papilionoidea), dragonflies and damselflies (Odonata), and large bees (Apidae) such as bumblebees are effectively sampled using visual surveys (**Figure 1**). When species cannot be identified, individuals can be netted and then identified or photographed (Loffland et al., 2017; Holtmann et al., 2018). However, not all species can be identified in the field; for some species, microscopic examination of the genitalia and abdominal appendages is necessary for identification. Visual surveys generally focus on generating a complete list of species observed (with or without counts), and therefore, visual surveys must focus on a select target group of insects (e.g., butterflies or bumblebees).

Methodological Considerations

Location

The location of the visual survey is important as many insects are habitat specialists. After selecting sites for visual surveys, multiple transects, points, or areas should be randomly selected to ensure sampling across the heterogeneity of a site. It is important to document the coordinates of the survey location and note the habitat type. Visual surveys occurring in difficult terrain or in at-risk ecosystems may consider choosing point counts to limit trampling or allow for more flexible walking routes.

Design

Care should be taken when selecting the location of visual surveys, as we recommend these locations remain fixed to enable surveys to be compared from year to year. Transect surveys should be at least 1 km in length, although visual survey methods allow for the correction of survey effort by adjusting by the length of the transect or by time of survey (Taron and Ries, 2015). Thus, detailed information must be documented on the length of transects and the start and end times of surveys (**Table 1**). Consistent and repeated surveys are needed to capture the seasonal abundance of individual species and to fully capture the diversity of the community. Therefore, surveys should begin before the first adult individuals of the target group are presumed to be active and terminate after the final adult activity. During this period of activity, visual surveys are recommended to occur weekly when conditions meet the time of day and weather criteria suggested by Pollard and Yates (1993). Surveys should occur between the midday hours of 1,000 and 1,700 when air temperature exceeds 13°C (although this may be reduced to 11°C in polar, upland areas) and there is at least 60% sun or 17°C in any conditions, providing it is not raining and wind speeds are below a six on the Beaufort scale (Pollard and Yates, 1993).

Scalability

Visual surveys can require extensive field effort with the potential of a single survey taking multiple hours complete. Due to the field effort required to complete visual surveys across numerous sites,

many successful visual survey programs rely on the dedication of numerous trained volunteers (Schmucki et al., 2016; Wepprich et al., 2019). Critical to the success of visual surveys is a rigid observer training protocol, as untrained observers tend to have biased distance estimates and observer experience can significantly affect detection functions (Buckland, 2001). Visual surveys generally have limited post-sampling effort, with the main effort being transcribing field notes and data collection sheets. This process can be further enhanced by using GPS handheld tablets to record data in the field (Hackett et al., 2019).

GENERAL METHODOLOGICAL CONSIDERATIONS

Replication

Four types of replication are especially important when sampling: spatial, inter-annual, intra-annual, and within-sample replication. All types of replication are important and broaden the inferential scale of any monitoring program while also expanding the analytical options and flexibility. We caution, however, that when sampling lethally, large-scale replication (particularly, spatially and temporally intense sampling at a local scale) could theoretically lead to abundance declines, especially perhaps in rare taxa (Minteer et al., 2014, but see Gezon et al., 2015). Lethal sampling should only be done when scientifically and ethically justified (Drinkwater et al., 2019).

The required amount of spatial replication for accurate and precise monitoring is still unknown for many insect monitoring methods, and contentious for others. We provide specific discussion in each method section, but in general, the more spatial replication, the more accurate and precise the estimates of measured responses. One way to increase the degree of spatial replication without increasing individual effort is to join an existing monitoring network, thereby increasing the network's degree of spatial replication.

A high degree of inter-annual replication is important for monitoring of most taxa (Wauchope et al., 2019), but is especially important for measuring insect abundance, where large year-to-year fluctuations are common (Didham et al., 2020). Failure to account for high inter-annual variability has led to disagreements over whether some insect populations are truly declining or not (see Willig et al., 2019 in response to Lister and Garcia, 2018).

A high degree of intra-annual replication is also important because insect phenology is complex and variable. For example, the week of peak abundance for a species one year could be different the next year, and even the number of generations produced by a species can vary across years. Even if only interested in studying occurrence or abundance, intra-annual variation in phenology of insect activity and generations can lead to strong biases in these responses if samples are only collected once per year (i.e., the "groundhog effect"; Didham et al., 2020). To account for this, we strongly recommend that monitoring efforts be carried out for the entire season of activity for the taxon of interest, allowing easier comparison across years.

Finally, within-sample replication—that is, multiple samples of a response at a single site during a period when the occurrence and abundance of target taxa are assumed to be constant—can be important for the statistical analysis of trends when trying to account for imperfect detection of individuals and species, such as with occupancy modeling (MacKenzie et al., 2006). Within-sample replication can be achieved either through spatial sub-samples—for example, using "array" designs, as discussed above for pitfall and pan traps—or temporal sub-samples—such as conducting visual or acoustic surveys at the same location multiple days in a row. In general, the ability to collect within-sample replication of monitoring data depends on the design and scalability of a chosen method. Although within-sample replication can substantially increase effort of monitoring, the ability of increased samples to account for sampling noise can be extremely powerful when detection probabilities of target taxa are low. For recent examples of occupancy modeling using some means of sampling replication for insects, see Isaac et al. (2014), Loffland et al. (2017), Outhwaite et al. (2019), Szewczyk and McCain (2019), and Powney et al. (2019).

Curation of Specimens

Many of the monitoring methods discussed require collecting insect specimens for subsequent identification. After identification, it is generally up to the scientist whether specimens should be kept or discarded, although vouchering of representative taxa can be key to the long-term value of datasets, particularly given taxonomic revisions and new technologies. To that end, great care should be taken to ensure specimens are preserved properly, if kept. For an overview of insect specimen storage, see Heraty et al. (2020). It is becoming increasingly common to preserve specimens in 80–95% ethanol to better preserve DNA. Regardless of the preservation method, it is worth developing a plan for deposition and cataloging of specimens prior to beginning a monitoring project. If discussed ahead of time with university and museum insect curators or collection managers, specimens that are properly preserved and curated should be donated. Optimally, archival deposition should include all survey notes, along with specimens, to enhance long-term re-use value.

Curation of Data and Metadata

Just like physical specimens, data are valuable resources for future science. Just as important as data are metadata, that is, the collective information about how the monitoring data were collected. Each method of insect monitoring has its own unique set of critical metadata (**Table 1**). Monitoring programs should strive to meet FAIR data principles to ensure their data and metadata are: findable, accessible, interoperable, and reusable (Wilkinson et al., 2016). Findable and accessible data will require that collected data are digitized and uploaded to websites or online databases that are constructed to hold data about the collecting method. There is much work on this topic that needs to be done, but some resources are developing rapidly.

Interoperable data may be the most challenging aspect of FAIR principles, given that each monitoring program often develops its own reporting standards, even for the same monitoring methods, which ultimately places the burden on downstream users to reintegrate, often with loss of key information. Recent

efforts have called for unified, global monitoring standards, such as the Humboldt Core metadata standard (Guralnick et al., 2018). While it is unlikely that one data standard will fit all insect monitoring, the Humboldt Core provides a typology of different survey and inventorying processes, such as restricted or open searches, along with key definitions of taxonomic, spatial, and temporal scopes, that strongly aid in discovery of monitoring datasets. More specific metadata describing particulars of different monitoring schemes can and should be accommodated (**Table 1**). We argue that rather than assume an improbable utopia of full data integration, monitoring programs should work in federations and take seriously the production of detailed metadata, and, as much as possible, develop standardization for metadata that can be as easily linked as possible into existing frameworks. The end result will be FAIR data that can most easily be integrated into flexible modeling frameworks that allow statistical integration of well-described data to better answer broad-scale ecological questions.

As with specimens, it is critical that a plan for data management be considered prior to beginning a monitoring project. Data standards are a key part of that plan since monitoring metadata is crucial for generating insights from monitoring outcomes. However, other factors are also critical, including developing local data storage solutions, deciding on a longer-term repository for data, assuring appropriate credit models for those involved with data collection, and licensing and use agreements of data products. Each of these issues deserves its own longer contribution and we point readers to Michener (2015) and Hardisty et al. (2019) for further reading. Here we make two broad recommendations. First, we strongly suggest development of a coherent data storage and sharing plan that has community buy-in. A best-case approach is development of an internal content management system that provides tools for data access and curation for program participants along with a broad, coherent, and multipronged, data sharing policy that assures long-term access. One part of this sharing policy should focus on best practices for archiving data in community repositories such as Zenodo and Dryad. A second part should focus on publication to aggregators that specialize in biodiversity data mobilization, such as the Global Biodiversity Information Facility (GBIF). The value of publishing to GBIF is enhanced discoverability, since it acts as a single, global access point to biodiversity data and information. However, it can still be challenging to properly publish all survey metadata given GBIF's reliance on standards that were built for incidental records (Guralnick et al., 2018). Finally, we also encourage monitoring programs to explicitly state data collection and review policies, including how individuals within the programs are credited for the work they do. Such credit models may include attribution for use of data, which can be supported by both licensing mechanisms such as creative commons licenses, and data use policies.

We also encourage development of digital tools to support the capture of field data. Digital tools (e.g., phone apps) can limit transcription errors and are sometimes easier to manage in the field. However, physical data sheets still play an important role in most monitoring programs and are a reliable backup over the very long-term. For these, archival-quality paper and ink should be used to maximize longevity. Though the cost and effort are not trivial, undigitized data can be digitized increasingly easily via scanning and optical character recognition (OCR) capture. Finally, the entoGEM project[3] (Grames et al., 2019) is soliciting unpublished insect abundance and diversity time series for inclusion in a global systematic map and meta-analysis. EntoGEM is a database, not a repository, but can serve as a temporary mechanism for archiving until a suitable repository is found and is a way to increase the utility of your data.

LOOKING FORWARD

Traditional survey methods are limited by being labor and time intensive, but ecological monitoring of animals has recently undergone a dramatic transformation with the development of technologies that expand the spatial, temporal, and taxonomic scales possible to monitor biodiversity (Pimm et al., 2015). These technological advances could facilitate the collection and availability of vast quantities of data by reducing the effort and expense of insect monitoring. No single method will be able to monitor multiple different insect groups across diverse landscapes. However, a combination of emerging technologies in surveying methods, processing, and data sharing pipelines will allow insect trends to be extracted at currently unprecedented scales.

We are entering an era where passive automated monitoring is already augmenting the traditional methods discussed above. Passive acoustic monitoring using arrays of acoustic sensors are already being deployed and tested. Such new methods have enormous promise, but also produce enormous volumes of data. A single acoustic recording unit can easily generate hundreds of gigabytes of data, with much of the data consisting of non-target (i.e., non-insect) sounds. Algorithms to automatically locate and identify target sounds within audio recordings are being developed (Gibb et al., 2019), and machine learning approaches can substantially improve detection and classification accuracies by discriminating spectro-temporal information directly from annotated spectrograms. These algorithms have been demonstrated to outperform alternative detection and classification methods in a variety of settings (Fairbrass et al., 2019). Unfortunately, one of the greatest barriers to detecting and classifying species using passive acoustic monitoring is the limited availability of expert-verified sound databases for reference and training data (Gibb et al., 2019). This problem may be especially exacerbated for insects, given their vast diversity, the paucity of audio libraries, and that only 5% of published terrestrial acoustic monitoring research has been on invertebrates (Sugai et al., 2019).

Camera traps are another emerging surveying tool that are being used to monitor a variety of wildlife (Burton et al., 2015). Networks of many camera traps allow for data to be collected across greater spatial and temporal scales (Kissling et al., 2018). Like with acoustic monitoring, deep learning convolutional neural networks are being developed to automatically count and identify wildlife (Norouzzadeh et al., 2018). The relatively

[3]http://entogem.github.io

small size of insects compared to wildlife typically captured using camera traps provides unique challenges to monitoring insects with cameras. However, recent studies have shown the potential of camera traps to monitor the overall abundance of flying nocturnal insects (Ruczyński et al., 2020). Additionally, a portable computer vision light trap has been developed to attract and identify live moths (Bjerge et al., 2020), and a monitoring network of camera traps that are made with smart image processing has been proposed to monitor light-attracted insects in the Netherlands (Hogeweg et al., 2019). Continued effort into developing camera traps designed to monitor insects has great potential for passive surveying of non-acoustically detected insects at greater spatiotemporal scales.

Environmental metabarcoding is an emerging tool that provides rapid and cost-effective means for taxonomic identification of many organisms in terrestrial and aquatic environments (Piper et al., 2019). These approaches can provide detection/non-detection data for insects collected from a variety of methods, especially if specimens are stored in 80–95% ethanol. Metabarcoding insect feces (e.g., frass) offer another non-lethal surveying tool, as caterpillars have been identified to species by amplifying larval DNA from caterpillar feces (Rytkönen et al., 2019). Unfortunately, most insects have insufficient reference sequences in public archives such as GenBank, making genetically identifying insects challenging. Still, environmental metabarcoding provides a cost-effective and efficient option to identify insects collected in large quantities.

Radar can also create standardized monitoring data for insects at broad spatial and temporal scales (Didham et al., 2020). Filtering insects from meteorological data can provide previously unused datasets to monitor insects through time and have been used to document the decline of burrowing mayflies (*Hexagenia* spp.) across North America (Stepanian et al., 2020). In most cases, species-level identification cannot be accomplished using radar approaches, but specialized entomological radar shows promise in monitoring insects that may migrate in large abundances at heights difficult to monitor using traditional approaches.

New methods do not need to work in isolation nor are they replacements for traditional monitoring methods. Rather, these new approaches are ways to augment existing ones, and to lower costs for onerous activities that may be partially or wholly automated. We envision passive monitoring tools that can be deployed in conjunction with traditional trap or restricted search methods. For example, acoustic monitors and camera-loaded light traps could be controlled by one device and augment, for example, pitfall trapping, to capture a broader spectrum of insects at a single site. If these sensor approaches also have means to easily share data across a network of sensors and people, it may speed up necessary steps to create the most usable data resources for broad-scale insect monitoring.

CONCLUSION

We live in an era of rapid change that affects nearly all life on Earth. We can only understand this change and its effects on insects by pooling effort, integrating projects, and working together. Methods standardization is a relatively simple first step, but what challenges come next? For one, we urgently have to coordinate our use of these tools. Networks of networks need to be built for data collection. This means incentivizing participation, coordinating new and existing projects, and organizing efforts on a trans-national scale. Large networks should communicate with each other to increase complementarity, and smaller networks should seek to fit in with what is already being done while maximizing the utility of their own data. But coordination is likely not the greatest challenge.

The largest bottleneck for insect monitoring is getting from trap to accessible data—we need to accelerate the time-consuming stage spent processing and identifying specimens and build tools for the efficient capture of all data and metadata associated with an observation. Improving identification is an area where we have immense potential for advancement over the near term. Bringing more automation to this stage will result in much shorter lag times between data collection and analysis and increase scalability of new and existing projects. Tools for automating identification include metabarcoding, computer vision, and machine learning. Efficient expansion of these identification tools will not only facilitate the broader participation of individuals in insect monitoring (e.g., those without specific skills in taxonomy), but the digital nature of automated and semi-automated identification will speed up data accessibility and metadata capture.

We also need analytical advances for integrating data collected by multiple sampling methods. Assimilating data collected using the same benchmarking method into a composite database is the first step, but ultimately, integrating data collected by multiple different means will vastly improve our ability to understand the broader insect community. But even if we arrive at the point where all the necessary data are being collected on a global scale, the best data in the world are useless if they are not made available—where availability means in a digital format that adheres to all of the FAIR principles. Consequently, we also need the infrastructure to aggregate, store, and share data widely–existing databases such as GBIF are paving the way—while also recognizing the importance of attributing credit (e.g., Chavan and Penev, 2011) to incentivize participation in the process of infrastructure development and data curation.

Every one of these challenges will require collective action to overcome. In the face of rapid Anthropogenic change, there is an intense urgency to this effort. These are no small tasks, and the timeline for completing them is short. Benchmarking has no point when there is nothing left to benchmark. Appropriate foresight and funding would have developed large-scale insect monitoring long ago, but the second best option is to rapidly build capacity now.

AUTHOR CONTRIBUTIONS

GM and MT conceived the idea for the manuscript and proposed the initial outline. GM and MB led writing of the first draft. All

authors contributed critically to drafts and gave final approval for publication.

FUNDING

This research was supported by the US National Science Foundation (Grants EF 1703048 and 2033263) to MT and RG. Additional support was provided to GM through the UCLA Department of Ecology and Evolutionary Biology and to MB through a University of Florida Biodiversity Institute Fellowship.

ACKNOWLEDGMENTS

We thank David Wagner for providing useful comments that significantly improved the manuscript, as well as the valued input of two reviewers.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 10 February 2021
doi: 10.3389/fevo.2021.568278

Benchmark Bird Surveys Help Quantify Counting Accuracy in a Citizen-Science Database

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The growth of biodiversity data sets generated by citizen scientists continues to accelerate. The availability of such data has greatly expanded the scale of questions researchers can address. Yet, error, bias, and noise continue to be serious concerns for analysts, particularly when data being contributed to these giant online data sets are difficult to verify. Counts of birds contributed to eBird, the world's largest biodiversity online database, present a potentially useful resource for tracking trends over time and space in species' abundances. We quantified counting accuracy in a sample of 1,406 eBird checklists by comparing numbers contributed by birders (N = 246) who visited a popular birding location in Oregon, USA, with numbers generated by a professional ornithologist engaged in a long-term study creating benchmark (reference) measurements of daily bird counts. We focused on waterbirds, which are easily visible at this site. We evaluated potential predictors of count differences, including characteristics of contributed checklists, of each species, and of time of day and year. Count differences were biased toward undercounts, with more than 75% of counts being below the daily benchmark value. Median count discrepancies were −29.1% (range: 0 to −42.8%; N = 20 species). Model sets revealed an important influence of each species' reference count, which varied seasonally as waterbird numbers fluctuated, and of percent of species known to be present each day that were included on each checklist. That is, checklists indicating a more thorough survey of the species richness at the site also had, on average, smaller count differences. However, even on checklists with the most thorough species lists, counts were biased low and exceptionally variable in their accuracy. To improve utility of such bird count data, we suggest three strategies to pursue in the future. (1) Assess additional options for analytically determining how to select checklists that include less biased count data, as well as exploring options for correcting bias during the analysis stage. (2) Add options for users to provide additional information that helps analysts choose checklists, such as an option for users to tag checklists where they focused on obtaining accurate counts. (3) Explore opportunities to effectively calibrate citizen-science bird count data by establishing a formalized network of marquis sites where dedicated observers regularly contribute carefully collected benchmark data.

Keywords: biodiversity benchmarks, birder behavior, citizen science, eBird, statistical bias, statistical error, wildlife counts

OPEN ACCESS

Edited by:
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Hungarian Academy of Science, Hungary

Reviewed by:
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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *31 May 2020*
Accepted: *20 January 2021*
Published: *10 February 2021*

Citation:
Robinson WD, Hallman TA and Hutchinson RA (2021) Benchmark Bird Surveys Help Quantify Counting Accuracy in a Citizen-Science Database. Front. Ecol. Evol. 9:568278. doi: 10.3389/fevo.2021.568278

INTRODUCTION

Contributions of volunteers to scientific databases are increasing as the popularity of citizen science continues to grow (Miller-Rushing et al., 2012; Chandler et al., 2017). Many citizen science projects are open-access and anyone can contribute observations without required training in best data collection practices (Cohn, 2008). eBird is an open online database with more than 560,000 users (eBirders) contributing millions of bird observations annually via checklists (Sullivan et al., 2009). Each checklist contains a list of bird species identified on a particular date and, ideally, counts of each species, as well as information on location visited, basic protocol used while birding (traveling, staying stationary, etc.), and duration of effort (Wood et al., 2011). The huge spatial extent of presence-absence data in eBird has facilitated efforts to model species distributions across continental and global spatial scales once data have been filtered to exclude potentially problematic checklists (Fink et al., 2013). The degree to which the count data may reliably inform scientific and management objectives remains unclear.

Although efforts to quantify issues associated with bird species detection have been studied and continue to be developed, both in citizen science databases and in structured scientific surveys (Buckland et al., 2008; Hutto, 2016; Walker and Taylor, 2017), less is known about potential counting errors and biases leading to noisy data. Counting birds is difficult, even by the most proficient observers (Robbins and Stallcup, 1981; Robinson et al., 2018). Methods to account for detection issues in bird counting studies continue to expand with development of new data collection and analytical methods (Buckland et al., 2008; Barker et al., 2018). Nearly all the methods, however, require a sophisticated sampling protocol that would exclude most volunteer birder contributions and therefore limit the advantages of gathering data at massive geographic scales. Yet, the potential windfall from large quantities of data can quickly be eroded if a lack of structured protocols leads to data quality concerns (Kelling et al., 2019). Given that abundance is one of the fundamental influences on population dynamics, functional roles in ecosystems, and even extinction risk (Brown, 1984), a better understanding of the potential value of count data contributed to massive online databases by untrained volunteers is needed (Greenwood, 2007). For example, species count errors in eBird data could limit our abilities to observe important abundance trends (Horns et al., 2018). Effective processes for evaluating and handling such errors need further development, owing to the potentially huge value of tracking population changes at continental and even global scales during this era of rapid environmental change (Bird et al., 2014; Fink et al., 2020).

Among the primary concerns are errors, bias and noise. Errors, for our purposes here, are differences in counts between a reference (benchmark) value and values included in eBird checklists for the same species on the same date. Errors are comprised of both bias and noise. Bias is the tendency for the errors to be consistently higher or lower than the reference value. Noise is the additional random counting error that increases variance of the counts. All three impede efforts to determine true count values, and are challenges common to many branches of biology (West, 1999; Guillery, 2002). We acknowledge that labeling such count differences as errors risks offending some eBird contributors. Errors, bias and noise all have objective statistical definitions. Our applications of the terms here are intended to improve understanding of the sources of variability in eBird count data. To acknowledge that there are sources of error in all measurements, however, we often refer to such deviations as count differences. We consider the terms "error" and "count differences" to be synonymous.

Robust comparisons of count differences are improved when data are collected in situations where detectability challenges are expected to be low. Such situations are rare but uniquely valuable. We used an extensive data set focused on benchmarking the richness and abundances of birds at a water treatment site in Oregon, USA. We compared count data gathered by a professional ornithologist focused specifically on creating an accurate benchmark measurement of daily fluctuations in waterbird counts with counts submitted by birders to eBird. We quantified the magnitude and directionality of count differences. Our data span 10 years and include 1,406 eBird checklists contributed by 246 observers, as well as 2,038 checklists in the benchmark data. The site is well suited for rigorous comparisons because all waterbirds are in the open, largely tolerant of human activity, and so provide a best-case scenario for detection, identification, and counting of birds. No adjustments for detectability or availability issues should be needed because all parts of the ponds are visible. Thus, discrepancies in counts between a professional observer focused on obtaining accurate numbers and data reported to eBird should be attributable to counting errors instead of availability and detectability issues. While there could be very minor detectability issues, like some diving waterbirds being under water briefly, the vast majority of error in this setting should be attributable to counting error.

We first quantified count differences then sought to understand potential factors explaining the magnitude and directionality of count differences. We hypothesized that count differences would be influenced by traits associated with the species being counted, with an index of observer experience (percent of species detected), and with seasonal changes in numbers of birds present. For example, we expected count differences might be slightly greater for diving ducks, which are sometimes briefly under water while foraging, and lower for dabbling species, which sit in the open continuously. We expected smaller count differences in checklists that included a higher proportion of the species present each day. We also hypothesized that count differences would be greater when overall total number of waterbirds present was high, potentially causing observers to be overwhelmed and therefore more prone to counting errors. Finally, we explored the possibility that, even if count data were biased on individual checklists, the waterbird community might be adequately characterized as a whole by combining count data from multiple observers and checklists. We conclude by proposing additional approaches that may reveal the extent to which citizen-science bird count data may be used to estimate abundances reliably.

MATERIALS AND METHODS

Study Area

Bird count data were gathered from 2010 to 2019 at the Philomath Wastewater Treatment facility, in Philomath, Oregon USA. The site contained two 35-ha ponds until 2011 when two additional 35-ha ponds were added. Each pond is rectangular and enclosed by a berm with a single-lane road. Birders circumnavigate the ponds typically by vehicle, rarely by walking or bicycling; WDR drove. Vegetation does not obscure the view at any pond. All shores are covered by large rocks (riprap). Birders circle all four ponds during a visit, very rarely restricting visits to fewer ponds. We found that the distribution of visit durations was unimodal (median = 60 min; Median Average Deviation (MAD) = 37; skew = 1.161; N = 1,646 checklists) suggesting that birders use similar methods while at the ponds.

Study Species

We included 20 species we refer to as "waterbirds," species that swim in the open while on the ponds and should be easily seen (**Table 1**). The species are primarily ducks and geese, but also include grebes, American Coot (*Fulica americana*), and gulls. These are species birders identify by sight, not by sound. We excluded species that occurred primarily as fly-overs, such as Cackling Goose (*Branta hutchinsi*), species whose counts rarely exceeded two per day, and species whose numbers varied strongly within a day. The number of waterbirds present at the site varied seasonally from a few dozen during mid-summer (June) to 5,000 or more during fall migration (October-November).

Benchmark Counts

All birds of all species were counted during each site visit by WDR. We call these our benchmark counts (R^*) and they serve as the reference values against which all other count data are compared. Waterbird counts were made to plus or minus one individual except for Northern Shoveler (*Spatula clypeata*), which were plus or minus 10 because they forage in constantly moving dense aggregations rendering more precise counts problematic, and Bufflehead (*Bucephala albeola*), which were counted to plus or minus 5 because they dive so frequently while foraging in the early morning period surveyed by WDR that more accurate counts were difficult. Counts were tallied separately for each species on each pond then aggregated later. On average, except for shovelers and coots, the two most numerous species at the site, the number of individuals of the remaining 18 species was less than 15 individuals per pond on 90% of dates. Numbers of several species were greater for 5 weeks in fall but the same method of individually counting birds was employed. In the time frame of the daily counts, movements between ponds were normally minimal. Duration of counting time was recorded separately for each pond.

On some days (N = 84), WDR counted birds more than once. These second-visit data, which we call Ref2 counts, were also complete counts of the study species and averaged 13% shorter in duration. Ref2 counts were used to characterize within-day variability in numbers across the 10-year study period. We consider them to provide a conservative estimate of variability in R^* counting accuracy because they were largely conducted on days with exceptional levels of migratory movements. Thus, they estimate a probable upper bound on the expected amount of within-day variability in waterbird numbers and R^* count accuracy (averaging 0 to −8% across the 20 species). We also used these Ref2 data to evaluate time-of-day effects when comparing WDR counts with data from the ten observers contributing the most study site data to eBird, because eBirders tended to count birds later in the day than did WDR. The times of day eBird checklists were initiated as well as the difference in start times of eBird and benchmark checklists were unimportant in predicting percent error in our across-species and species-specific model sets. Therefore, we concluded that comparisons of count differences between R^* and eBird checklists were appropriate and that possible time-of-day effects could be ignored.

Our R^* counts are from one expert observer. R^* counts are not without error. Aside from comparisons with Ref2 counts made by the same observer, our data lack contributions from other experts as independent quantifications of accuracy and potential error of R^* counts. To estimate the error in R^* counts, we compared counts made in the field with counts in photos taken within 2 min of count completion. Comparisons were made in November and December, 2020, and included a range of count values from 1 to 1,050 for 17 of our 20 waterbird species (2 gulls and the scoter are not normally detected in November and December).

eBird Checklists

We downloaded eBird checklists from the Philomath Sewage Ponds eBird hotspot as well as eBirder personal locations within 1 km from 2010 to 2019. Only data obviously restricted to the ponds were included. No other waterbird sites are present within 4 km of the site. Most eBirders used the pre-established hotspot as the checklist location but some created new personal locations each time. We included eBird checklists following the stationary, traveling, and area protocols. We removed checklists with greater than ten observers or durations of over 5 h. We included only complete checklists with all birds reported and removed any checklists where observers reported no waterbirds. From each complete eBird checklist, we collected data on date, start time, observer, duration of count, identity of waterbird species reported (to allow calculation of percent richness; see below), and count data for our 20 focal species. When species were recorded as present but not counted (X noted instead of a number), those data were excluded because no count difference could be calculated.

Comparisons of Count Data

We restricted our comparisons to dates where WDR counted birds and at least one eBird checklist was contributed on the same day (N = 767 dates). Our questions were about counting differences and not detection rates of rare species, so we further restricted our comparisons to counts of greater than three for each species detected on WDR's first visit (R^*). We calculated the *Count Difference* for each species by subtracting R^* from eBird counts on each checklist. Count differences were positive when eBird checklists reported higher numbers than R^* or negative when eBird checklists reported fewer birds than R^*.

TABLE 1 | Twenty species were included in the study. Scientific names, sequence, and short-hand codes follow American Ornithological Society (http://checklist.aou.org/taxa).

English name	Scientific name	Code	Dabbler (0) or diver (1)	Dispersed (0) or aggregated (1)	Plumage dichromatism
Wood duck	*Aix sponsa*	wodu	0	0	1
Cinnamon teal	*Spatula cyanoptera*	cite	0	0	0
Northern shoveler	*Spatula clypeata*	nsho	0	1	1
Gadwall	*Mareca strepera*	gadw	0	0	1
American wigeon	*Mareca americana*	amwi	0	1	1
Mallard	*Anas platyrhynchos*	mall	0	0	1
Northern pintail	*Anas acuta*	nopi	0	0	1
Green-winged teal	*Anas crecca*	gwte	0	1	1
Canvasback	*Aythya valisineria*	canv	1	0	1
Ring-necked duck	*Aythya collaris*	rndu	1	1	1
Lesser scaup	*Aythya affinis*	lesc	1	0	1
Surf scoter	*Melanitta perspicillata*	susc	1	0	0
Bufflehead	*Bucephala albeola*	buff	1	0	1
Hooded merganser	*Lophodytes cucullatus*	home	1	0	0
Ruddy duck	*Oxyura jamaicensis*	rudu	1	1	0
Pied-billed grebe	*Podilymbus podiceps*	pbgr	1	0	0
Eared grebe	*Podiceps nigricollis*	eagr	1	0	0
American coot	*Fulica americana*	amco	0	1	0
Ring-billed gull	*Larus delawarensis*	rbgu	0	0	0
California gull	*Larus californicus*	cagu	0	0	0

See text for definitions of dabbler vs. diver and dispersed vs. aggregated foragers. Plumage sexual dichromatism was scored based on the period of year in which the species is most numerous at the study site: weak or no dichromatism (0) and moderate to strong dichromatism (1).

Numeric values of count differences spanned three orders of magnitude, so we focus on reporting *Percent Error*, which we calculated by converting each difference to a proportion of R^*.

Hypothesized Predictors of Percent Error

To evaluate factors hypothesized to be associated with percent error, we included variables associated with species, checklists, time of year and observer experience. *Species characteristics* included categorization as dabbler vs. diver, degree to which species form dense aggregations, and the degree of sexual dimorphism. *Checklist characteristics* included start time, duration and number of observers. *Time-of-year characteristics* were associated with daily numbers of waterbirds (R^*, Ref2 and their sums for all 20 species) and waterbird species richness present at the study site [measured as the richness detected by the professional (proRichness) as well as the aggregate of species listed in eBird checklists and proRichness]. Because *observer experience* at the site might also influence counting accuracy, we compared data from the 10 observers who contributed the most checklists with the R^* and Ref2 benchmark data. Additional details on each variable are explained below.

Species Characteristics

To explore patterns of species-specific variability in count data, we created categorical variables for species traits that might impact counts (**Table 1**). We categorized birds as dabblers vs. divers. Dabblers were any species that foraged primarily by swimming on the surface of the water, which included gulls, American Coot, and *Aix*, *Anas*, *Mareca,* and *Spatula* ducks. Divers foraged below water regularly and included scoters, grebes, and *Aythya* and *Bucephala* ducks.

We also included an index of spatial aggregation on the ponds. Some species, for example Northern Shoveler, often forage in densely packed groups, creating challenging circumstances to accurately count birds, while other species forage singly or as spatially-distanced groups where enumeration should be much easier. The aggregation index was simply a subjective binary classification (0 for foraging alone or in loose aggregations vs. 1 for foraging in aggregations that might render counting difficult) based on our years of experience at the site.

The degree of plumage dimorphism and similarity to other species could influence error and bias in counts because of species misidentification. We categorized species as those with weak or no obvious plumage dichromatism during most of the period of time when each species was present (e.g., geese, coots) vs. strong dichromatism (males and females distinctly visually different).

To evaluate the possibility that species identification of similar species might influence count differences, we used another subjective binary category called "Doppelganger;" 1 indicated the species co-occurred with a similar species whereas 0 indicated the species was unique in appearance and unlikely to be confused with other species. The categorization may vary seasonally, especially in late summer when many waterbirds molt to eclipse plumage. Because total waterbird numbers were low during late summer, we utilized one value for each species.

Checklist Characteristics

Daily start time among eBird checklists was highly variable, covering all daylight hours. The mean start time was 4 h

later than the mean start time for WDR visits. Although we only compared counts conducted on the same day, we wanted to evaluate potential effects of time-of-day and temporal lag between the eBird checklist counts and R^*. To do so, we converted checklist start time to minutes since midnight then calculated the difference in start time between eBird checklists and WDR first visits.

Because our Ref2 counts occurred later in the day when more eBird checklists were initiated, we included Ref2 as an "additional observer" in some comparisons to provide an important check on within-day variability in counts as a possible explanation for count differences between R^* and eBird checklists. Because Ref2 counts were generated on days with high levels of migratory movement, we consider the count differences between R^* and Ref2 to represent an upper bound on expected levels of within-day variability in waterbird numbers.

Additional factors associated with each checklist could influence count differences. We reasoned that duration of time spent at the site should be positively related to count accuracy. All complete eBird checklists are required to have a measurement of event duration.

Number of observers might also influence counting accuracy, so we included the reported number of observers for each eBird checklist. The R^* and Ref2 counts were made when WDR was alone more than 99% of all dates.

Time-of-Year Characteristics

Date influences the number of species present as well as the abundances of each species. Both richness and abundance could influence counting accuracy so we included day of year in our models. Because we hypothesized that total number of all waterbirds combined may influence counting accuracy, we included R^* counts of all 20 study species and the combined daily total of all waterbirds in our model sets. In that way, we established the baseline numbers of waterbirds known to be present as a function of date. In calculating total waterbird abundance, we used data limited to the 20 study species and excluded a subset of species known to have high daily variability in counts, such as geese, which occurred primarily as fly-overs. The other species excluded from our focal group of 20 species were numerically rare. Further, to determine if percent error was influenced by the number of each particular species as opposed to overall waterbird abundance, we included R^* of each relevant species in our model sets.

We hypothesized overall waterbird species richness present at the site on a given date may influence counting accuracy. A higher number of species to identify could reduce focus for achieving accurate counts, particularly for the more regularly-occurring and common species (e.g., Mallards, Northern Shovelers). Therefore, we included in our models the total waterbird richness detected by WDR each day. Our analyses indicated that richness observed by WDR and total waterbird richness detected by all eBird contributors were highly correlated. We calculated daily *Percent Richness* based on the 35 possible waterbird species at the site and included that richness in our models (see **Supplementary Text** for a list of species). The other 15 species that formed our set of 35 waterbird species included: Snow Goose (*Anser caerulescens*), Greater White-fronted Goose (*Anser albifrons*), Cackling Goose (*Branta hutchinsii*), Canada Goose (*Branta canadensis*), Blue-winged Teal (*Spatula discors*), Eurasian Wigeon (*Mareca penelope*), Redhead (*Aythya americana*), Tufted Duck (*Aythya fuligula*), Greater Scaup (*Aythya marila*), White-winged Scoter (*Melanitta deglandi*), Black Scoter (*Melanitta americana*), Long-tailed Duck (*Clangula hyemalis*), Common Goldeneye (*Bucephala clangula*), Barrow's Goldeneye (*Bucephala islandica*), and Common Merganser (*Mergus merganser*).

Observer Experience

Observer experience at the site could also be influential, so we compared percent error in counts from the ten observers contributing the most eBird checklists at our study site with the R^* and Ref2 counts.

Data Analyses

We used the "lmer" package in R (R Core Team, 2020) to run mixed-effects models. Our overarching goal was to identify factors informative for explaining variation in *Percent Error*, our dependent variable in all models. We included observer ID and species as random effects to account for observer- and species-specific error when appropriate. We included four categorical species characteristics as fixed effects in our model sets: Dabbler or Diver; Sexually Dichromatic or not; Doppelganger or not; and Aggregated or not. Five checklist-related characteristics were included as fixed effects: start time (minutes since midnight), difference in start time between WDR's first count of a day and each eBird checklist, duration (minutes), number of observers, and day of year. Four fixed-effects related to time-of-year were also included: R^* (WDR's reference count of each species, which varied seasonally), waterbird abundance (aggregated across all species), total waterbird species richness and percent richness, our index of observer skill at species identification. We included models with the quadratic effects of species-specific abundance, waterbird abundance, waterbird richness, duration, number of observers, day of year, and percent richness to examine potential non-linear shapes of their effects.

Before running mixed effects models, we scaled and centered all numeric variables. We assessed model performance through BIC and propagated best-performing shapes for each variable to multi-variable models. We used a forward stepwise approach and added additional potentially influential variables to the best-performing model until a stable (i.e., model remained the top model after the inclusion of additional variables) top-performing BIC model was identified.

Although *count difference* was normally distributed, *percent error* was not. Non-detections of species that were detected by WDR (eBird counts of zero) equal negative 100 *percent error*. Non-detections caused a bimodal distribution of *percent error* with a second peak at negative 100 percent. We removed non-detections to create a unimodal distribution of percent error. When non-detections were removed, *percent error* was heavily right-skewed due to the high number of negative *percent errors* and the few very large positive *percent errors*. To adjust skew, we added a constant to make all values positive and log (base 10) transformed percent error. In addition to adjusting skew,

removal of non-detections improved the focus of our analyses on count errors, reducing chances that inclusion of zero counts of species might actually be species detection or identification problems instead of counting errors. Our restriction of counting error analyses to species detected in numbers of 3 or greater probably limited most effects of zero counts. In this paper we focus on analyses of data excluding non-detections but report some analyses in **Supplementary Materials** to show the effects of including non-detections (zero counts) on results. It is possible that an unknown number of zero counts were a result of reporting errors (data entry mistakes), but we assume this type of error is relatively rare.

Species-Specific Model Sets

To understand the (in)consistency of variables influencing species-specific percent error, we ran standardized linear model sets of the effects of the explanatory variables described above on *transformed percent error* for each species. As above, we included models with quadratic effects of species abundance, waterbird abundance, waterbird richness, duration, number of observers, day of year, and percent richness. As each model set was species-specific, we excluded variables of species characteristics from these model sets. We included observer ID as an explanatory variable to examine its comparative influence. In these standardized model sets, we included separate models of the main effect of each variable and propagated the best shape for each variable into more complex models. Since start time and difference in start time were highly correlated, we use the top-performing of the two in subsequent models. We used a forward step-wise approach to determine the top-performing model of checklist covariates. We then ran models with pairs of all non-checklist explanatory variables with and without the variables in the top checklist covariate model. We used BIC to compare model performance and select top models.

Non-metric Multidimensional Scaling (NMDS)

To compare the overall communities described in eBird checklists, we conducted ordination in species space with NMDS on count data. We grouped checklists by observers to simplify the analysis. To visualize differences in community characterization, we chose to contrast January and October because January represents a time of year when waterbird migration is minimal and so daily numbers are relatively stable, whereas migration is at its peak during October, so richness is high and volatility in numbers can be high. To evaluate how characterization of waterbird abundance at these times varied with respect to eBirder checklists, we first removed all checklists that included an "X" for the count of any of our 20 study species. We then calculated the mean and median values of species counts across checklists for each observer during each month. To evaluate the idea that group collective contributions of multiple eBird checklists might characterize the waterbird community more similarly to R^*, we calculated mean counts of species across observers in January and October to create combined count values, which we call the Borg number ($\overline{B}$). We similarly aggregated WDR's first-visit species counts as a Reference community. To ensure that our $\overline{B}$ NMDS positions in species space were not driven overwhelmingly by an eBirder with the largest number of checklists, we reran the NMDS without checklists from the top-contributing observer included in $\overline{B}$. We used two dimensions and a maximum of 20 iterations to run NMDS with the "vegan" package in R (version 3.6.1).

RESULTS

We compared benchmark counts of waterbirds (R^*) and at least one eBirder on 672 dates, representing a total of 1,406 comparisons (checklists). eBird checklist contributions varied seasonally with lows during winter and summer and highs during migration periods (**Supplementary Figure 1**). Our analyses included 246 different eBirders who contributed from 1 to 321 checklists.

Benchmark Count (R^*) Error

Comparisons of R^* counts with photographic evidence indicated a mean percent error across 17 species of −0.4% (SD = 2.1%; N = 222 comparisons) indicating that R^* counts were lower, on average, than numbers revealed in photos. The median differences varied from 0% for multiple species with counts below 200 to −1.2% for Northern Shoveler. We assume temporal consistency in counting errors for the duration of the study because the R^* count data were gathered by the same observer using the same methods. Another estimate of R^* count errors can be inferred from comparisons with Ref2 counts, which averaged −8%. Ref2 counts occurred throughout the 10-year duration of the study.

Percent Error

Across all twenty species, 76 percent of all counts were less than R^* (**Figure 1** and **Supplementary Figure 2**), indicating that count data in the eBird checklists regularly contained apparent counting errors. eBird checklists with species non-detections excluded (that is, no counts of zero included, even if the species was known to be present that day) had counts below R^* values by a median of 29.1% but count differences were quite variable across species (**Figure 1A**), with median absolute deviations of *percent error* averaging 44.6% (**Supplementary Table 1**). At the extremes, count differences across waterbird species ranged from negative 99% for severe under-counts to more than 3,788% too large. In real numbers, count differences ranged from being too low by 1,443 to too high by 1,048 (both for Northern Shoveler; **Figure 1B**). Median percent error was negative, indicative of undercounting, for all waterbird species except the uncommon Surf Scoter (0%; R^* was at most 11).

Percent error, when averaged across species and all observers, was fairly consistent at 30% when counts were 30 or greater. Below 30, counts were more accurate, being closest to zero error when counts were of 8–10 birds (**Figure 2A**). Percent error was related to the percent richness (our index of observer skill where higher percentages indicated an observer included more of the species known to be present that day on their checklists) in a curvilinear fashion. Checklists including the lowest richness tended to overcount (**Figure 2B**). Those including

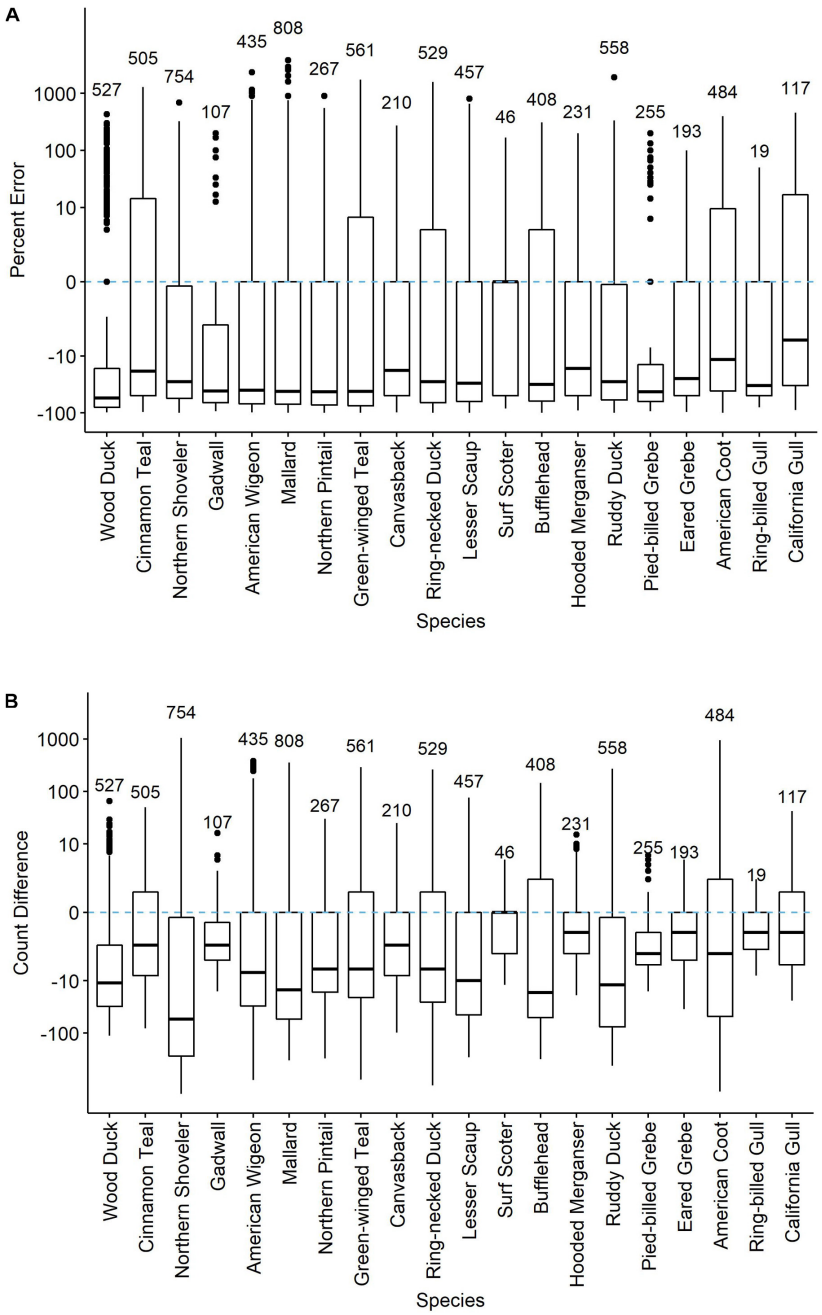

FIGURE 1 | Percent error **(A)** and count differences **(B)** in counts of 20 waterbird species reported on eBird checklists at the Philomath Ponds, Oregon USA, 2010–2019. Medians, quantile plots and outliers are indicated, as well as number of checklists reporting counts of each species. Only checklists reporting counts greater than zero were included. For checklists including counts of zero on dates when R^* counts were non-zero, see **Supplementary Figure 2**.

50% of the expected species undercounted by 50% on average, while checklists including 90% or more of the species reported on R* checklists averaged deviations of 15% or less in count.

BIC Top Models

In our multi-species mixed-effects model set, our top model garnered 70 percent of the model weight and was over four BIC from the next most competitive model (**Table 2**). Our BIC top model indicated that a quadratic effect of R^* and a linear effect of percent richness best explained variation in percent error.

Seasonality in bird numbers was also captured when the second-order R^* was included as the most informative variable predicting *percent error*. Numbers of all species varied considerably across each year (**Figure 3**). Likewise, total waterbird abundance varied several-fold from its

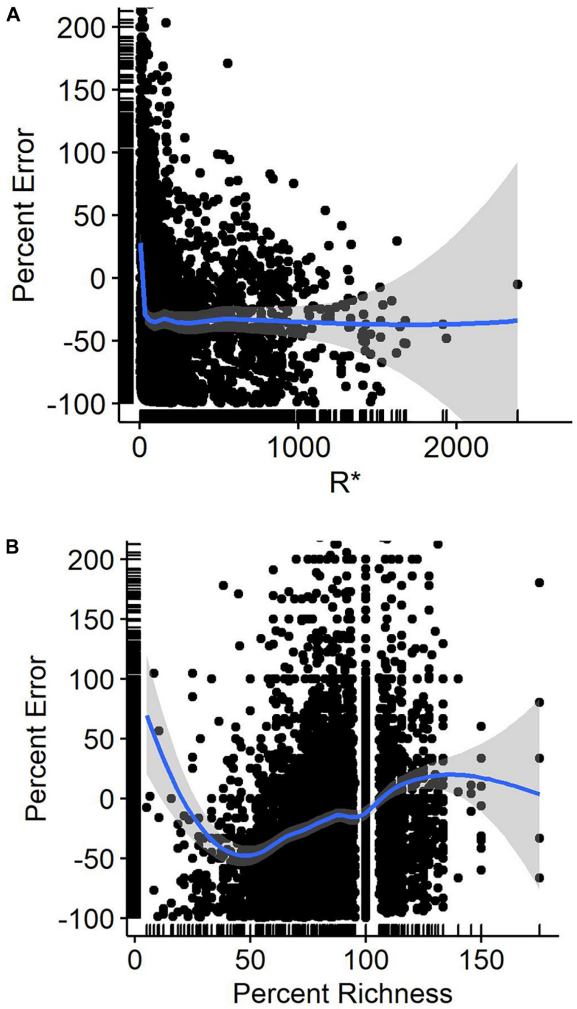

FIGURE 2 | Percent error in eBird waterbird counts as a function of **(A)** reference (benchmark) counts (*R**), and **(B)** percent richness of waterbird species detected at Philomath ponds. Lines are Loess regression lines with 95% confidence intervals.

nadir in June to a maximum in October and November (**Supplementary Figure 3**). Yet, total waterbird abundance was rarely an informative variable in our model sets. Only in counts of American Coot did it appear in the most parsimonious models (in combination with percent richness). In California Gull, waterbird abundance appeared as an informative variable but only in a weakly competitive model (19% of the model weight).

Within the species-specific model sets, the combination of *R** and percent richness carried most of the model weight (mean = 0.83, *SD* = 0.18) in 13 of our 18 non-gull species (**Supplementary Table 2**). For gulls, top models struggled to outcompete the null. Altogether, *R** and/or percent richness were in the top model sets for 17 of 18 non-gull waterbirds.

Associations With Bird Characteristics

Within our full model, bird characteristics were rarely influential on percent error (**Table 2**). Similarly, species-specific models rarely discovered bird traits to be informative variables (**Supplementary Table 2**).

Observer Effects

Our models often identified percent richness as an influential variable on percent error, so we related percent richness to percent error as means across all checklists contributed by each observer (**Figure 4A**). The two were positively related, yet only six of the 246 observers averaged *percent errors* of less than 10%. The range in percent error for observers detecting 90% or more of waterbird species was actually greater than the range for observers who detected less than 60% of species, indicating that percent error alone is an unreliable predictor of counting accuracy. The relationship was not necessarily driven by site experience because four of the six observers with the most accurate counts were contributing very few checklists (**Figure 4B**).

We then selected checklists from the ten observers who contributed the most. Those checklists also showed evidence of undercounting. In nearly all 20 species, percent error was 10–60% greater than even the Ref2 counts (**Figure 5**). Percent error was highly variable across species. In some species, such as American Coot, three of the 10 observers reported counts averaging very near the Ref2 counts, whereas in others, such as Pied-billed Grebe, all observers undercounted by at least an average of 20%. Again, percent error was highly variable in all species even when median percent error did not deviate far from zero.

TABLE 2 | Model results of variables most influential on percent error.

	df	Log likelihood	BIC	Delta	Weight
*R**2_percent richness	7	−9751.3	19565.0	0	0.696
*R**2_percent richness_duration	8	−9749.0	19569.4	4.44	0.075
*R**2_percent richness_starttime	8	−9749.2	19570.1	4.72	0.066
*R**2_percent richness_dichromatic	8	−9749.4	19570.4	5.19	0.052
*R**2_percent richness_date2	9	−9745.0	19572.7	5.41	0.047
*R**2_percent richness_prorichness	8	−9750.7	19573.0	7.79	0.014

*R*2 is the quadratic of the daily reference (benchmark) count; percent richness is the fraction of the waterbird species present each day that were included on each eBird checklist; duration was the length (minutes) of eBird checklist observation period; starttime was time of day each checklist was initiated; dichromatic was whether each waterbird species exhibited plumage dichromatism or not; date2 was the quadratic of day of year; and proRichness was the total species detected by WDR on each date. See* ***Supplementary Materials*** *for the full model results.*

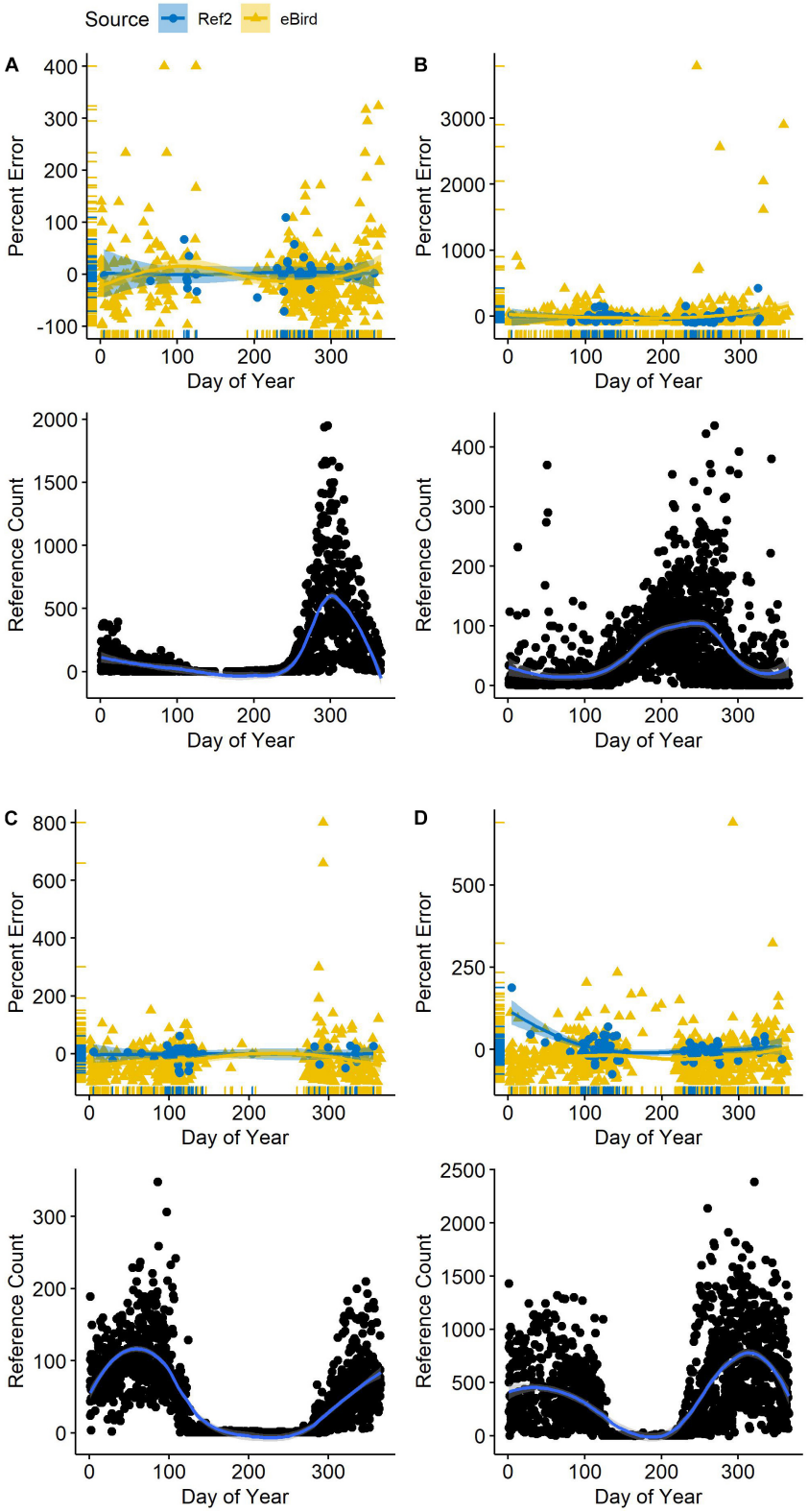

FIGURE 3 | Variation in reference (benchmark) counts (*R**) as a function of date (lower panel) and counts reported in eBird (gold triangles in upper panel) alongside second-visit counts (Ref2; blue circles) at Philomath ponds, Oregon USA, 2010–2019. Counts in the upper panels are indicated with respect to the *R** count (zero line) each day. Loess regression lines with 95% confidence intervals are included. **(A)** American Coot; **(B)** Mallard; **(C)** Lesser Scaup; **(D)** Northern Shoveler.

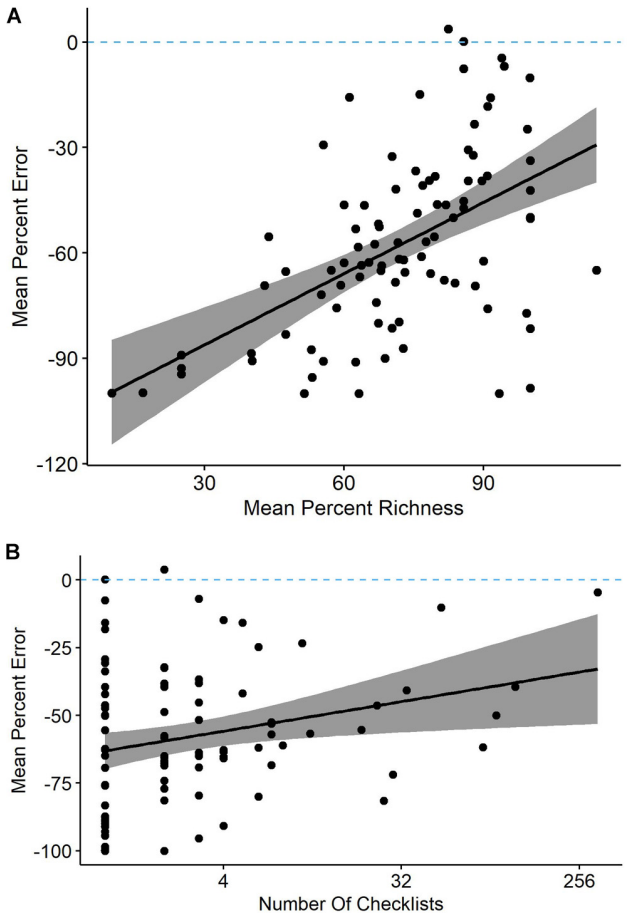

FIGURE 4 | (A) Observers reporting a greater percentage of waterbird species present at Philomath ponds, Oregon USA, tended to have lower percent counting errors in their eBird checklists (linear regression and 95% confidence intervals; y = −110 + 0.68x). **(B)** Observers submitting more total checklists tended to have lower counting errors (y = −60 + 0.17x). Note that these are means of all applicable checklists for each observer, so each point represents a unique observer.

Community Visualization

We visualized characterization of the richness and abundance of the daily waterbird community with NMDS through ordination of checklists (grouped by observer) in species space. Observers characterizing the community and its species abundance patterns similarly to R^* fell nearer to R^* whereas those positioned increasingly further from R^* described the community in increasingly dissimilar details. In both January (**Figure 6A**) and October (**Figure 6B**) high inter-observer variability in how their checklists characterized the waterbird community led to a general lack of clustering near R^*. In both months, observers reporting more species, contributing more checklists, and surveying for more time tended to group nearer R^*. The collective average, $\overline{B}$, was nearer R^* than any individual observer during January but one observer was closely positioned near $\overline{B}$ during October. Removal of checklists from the observer contributing the most data had minimal effects on results.

DISCUSSION

Benchmark data are often designed to understand temporal change in biodiversity (Curtis and Robinson, 2015; Curtis et al., 2016; Robinson and Curtis, 2020). Here, we show that they can also be used to establish standards that aid in quantification of count accuracy in citizen-science data. Through comparisons with such a standard, we discovered that bird count data contributed to eBird from our study site were consistently biased toward undercounting. Counts averaged approximately 30% too low whenever benchmark counts were of 30 or more birds. By comparison, estimates of errors in the R^* data averaged −0.4 (SD = 2.1%) based on comparisons with photos. Importantly, however, eBird count data exhibited high variability across species and observers. Because of the magnitude of count deviations and the high variability, standards like our benchmark data are needed to inform decisions regarding what subsets of abundance data should be selected to most rigorously address particular scientific questions or management decisions, analogous to how checklist calibration indices help researchers choose suitable eBird checklists based on site- and time-specific expectations of species richness (Yu et al., 2010; Kelling et al., 2015; Johnston et al., 2018). Yet, situations in which such informative standards may be developed and compared appear to be rare currently.

Our study site presented a unique opportunity to compare bird count data contributed to a citizen science database (eBird) with benchmark reference data collected by a professional observer focused on generating accurate daily counts. Characteristics of the site, where all birds were in the open and identified by sight, minimized issues of availability and therefore the need for detectability adjustments to compare counts. Data were contributed by 246 observers and included 676 dates across 10 years, providing an unusual opportunity to explore patterns and potential sources of error. Although the extent to which our results may be generalized to other sites remains unclear given the rarity of opportunities like this one, the situation probably represents a best-case scenario because birds were in the open and easy to observe. Despite the advantages, count differences in 20 species of waterbird were highly variable across the calendar year, species, and observer. Coefficients of variation were high, averaging 6.6 across the 20 species and ranging from 1 to 35.6. For comparison, in an experimental study of observer counting errors of singing birds, which should have been much harder to detect and identify but had a lower range of abundances than our waterbird community, coefficients of variation averaged 0.1 (Bart, 1985).

An assessment of count differences between benchmark data and citizen science contributions will be most robust when estimates of count variability exist for both sets of counts. Estimates of variability in counts from citizen science data are easier to generate because of the large number of visits by multiple observers. Our benchmark (R^*) data were gathered by one professional ornithologist beginning in 2006 prior to widespread eBird use by the birding community (only data since 2010 are included here). The goal was to use those waterbird count data to track population trends and to be able to detect

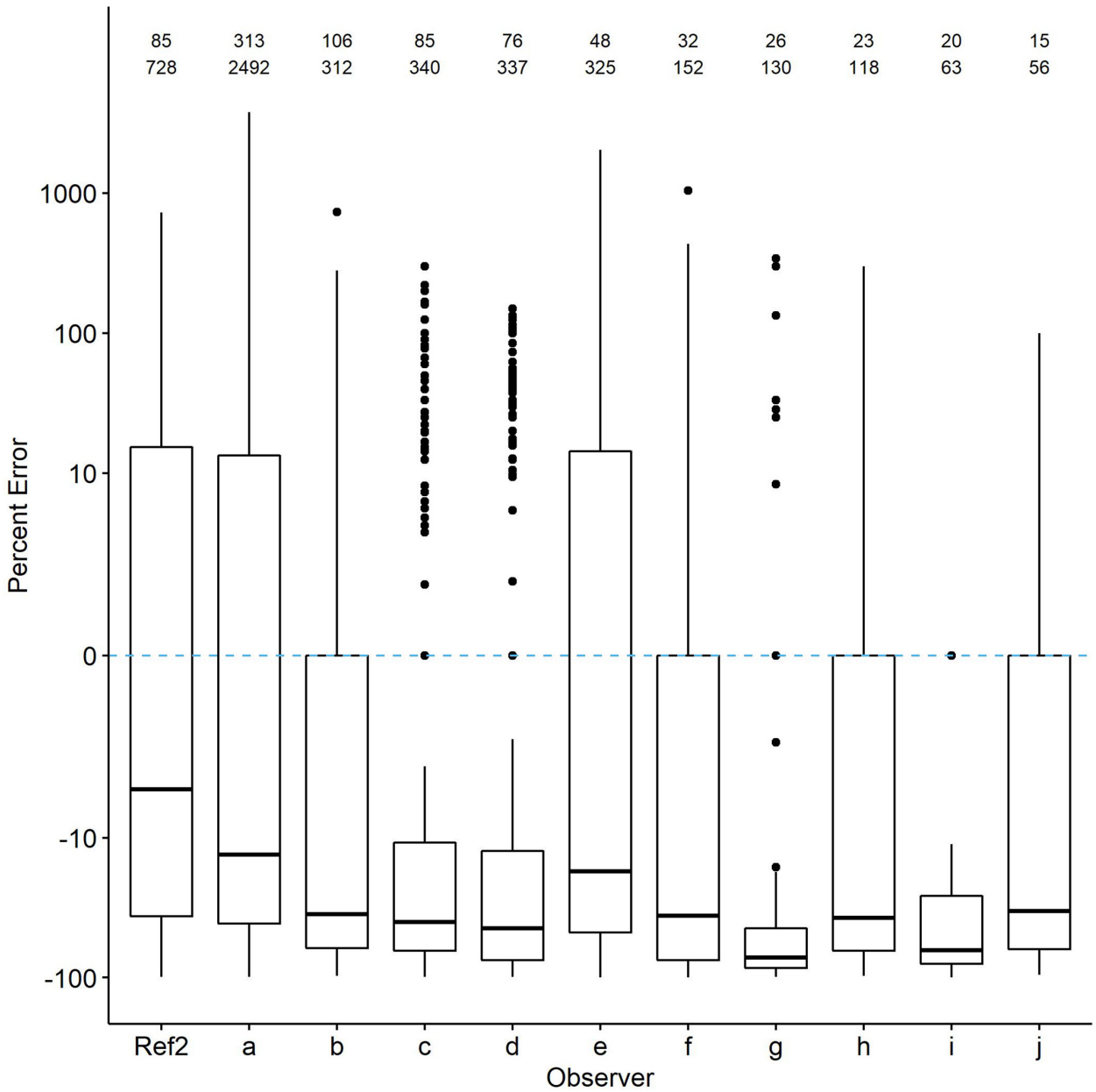

FIGURE 5 | Comparison of percent count errors in eBird checklists contributed by the 10 observers with the most checklists (top row of numbers) and waterbird observations (second row of numbers; each checklist includes multiple species). The zero line is R^*. Ref2 is the second-visit data from WDR. Quantile plots show the median, 25th percentiles as boxes and whiskers, plus outliers. Species-specific plots are available from the authors upon request.

annual changes as small as 2%, thus a high degree of count accuracy was required. No internal check of R^* counting errors was implemented consistently, in part because of the unique circumstances of the study site where all birds were in the open and easy to detect. On 90% of the days, the number of birds of 18 of our 20 species present averaged less than 15 individuals on each pond, increasing the likelihood of accurate counts. Average numbers were higher for Northern Shoveler and American Coot and during 5 weeks of peak fall migration. Our commonest measure of potential variability in the benchmark data derives from same-day counts (Ref2) by the same observer. Those counts from a later time on the same day averaged 8% lower. However, use of the same observer's second counts addresses repeatability of count data, not necessarily count accuracy. To assist in quantification of errors, our implementation of comparisons of R^* counts with photographic evidence revealed average counting errors of −0.4%. Involvement of additional experts counting independently could be an alternative solution to use of photos to effectively quantify counting errors in benchmark data, which we encourage when circumstances allow such opportunities. Overall,

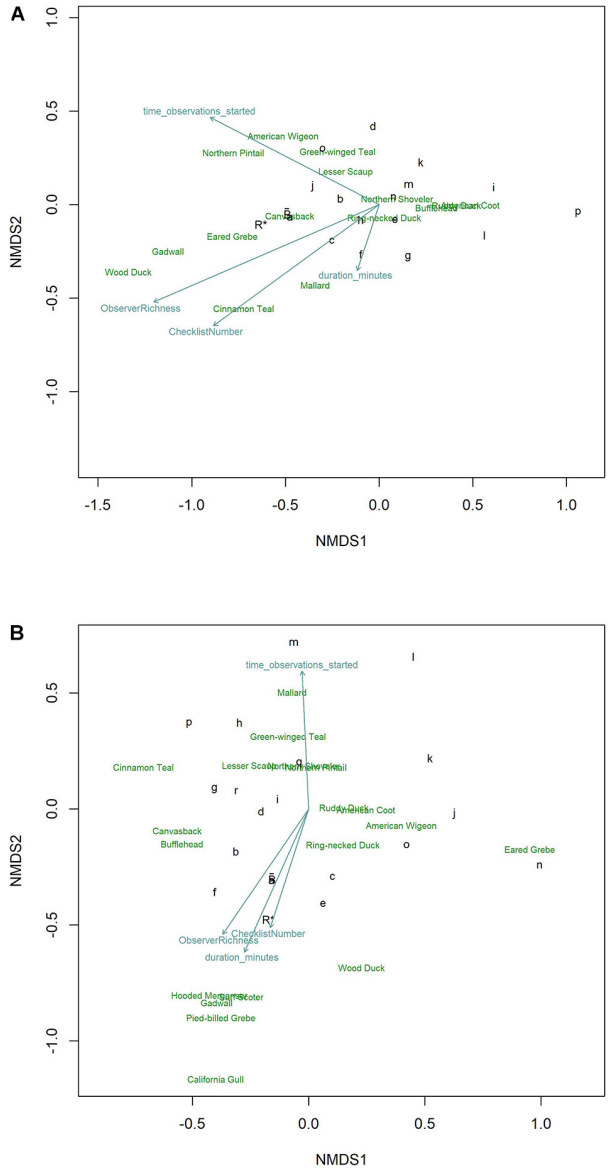

FIGURE 6 | Ordinations using NMDS of eBird checklists's characterization of the waterbird community during **(A)** January and **(B)** October at Philomath ponds, Oregon USA, 2010–2019. The most influential vectors included Observer Richness (percent of known richness reported on each checklist), Checklist Number (total number of checklists per observer), observation start time each day, and the duration of each observation period. Relative positions of species in species space are noted by species English names. Benchmark counts are noted by R^*. Individual observers are noted by lower case letters; those nearest to R^* produced characterizations of the waterbird community most like R^*. $\overline{B}$ is the collective average of eBird checklists, showing that from the perspective of generally characterizing the community, averaging across checklists contributed by many observers aligns more closely with R^* than do checklists from most individual observers, although observer *a* occupies nearly the same location in species space.

our efforts to estimate error in R^* data reveal that those counts may be 0.4–8% under-counts, on average, compared with eBird average count deviations of approximately 30% under-counts.

Our quantification of count differences in eBird data is conservative because we excluded counts of zero on eBird checklists, even for species known to be present. We did so to minimize the potential confound of misidentifications and reporting errors (failing to enter a count for a species that was actually observed) from our analysis of counting errors. Yet, it is possible that some fraction of 100% undercounts were indeed counting errors in the sense that the species was one that observers were knowledgeable enough to identify but failed to count or report. The median percent error across the 20 species was −48.6 plus or minus 50.9% (MAD) when zero counts were included vs. −29.1 plus or minus 44.6% when zero counts were excluded. Inclusion of zero counts, therefore, has a large influence on the median, but percent errors were highly variable regardless.

Our top overall mixed-effects model carried nearly 70% of the model weight and contained only two variables. The species-specific R^* count as a quadratic, which captured the seasonality in numbers present at the site, was the most informative variable when combined with a linear effect of percent richness. The inclusion of R^* indicates that eBird count data were related to the benchmark numbers but that other factors were also influential. Checklists with a more complete list of the species known to be present each day had lower counting errors. Yet, checklists including 100% of expected species still undercounted by an average of 15%. Count differences on checklists from the ten observers who most often visited the site were still exhibiting undercounts even compared to the Ref2 values, which were benchmark counts made later each day during weeks with high levels of migratory movements.

We documented strong directional bias toward undercounts and also a smaller percentage of large overcounts, leading to inconsistent patterns in count differences across species. Our comparisons revealed that undercounting was pervasive, yet very large numbers of a species being present sometimes led to severe overcounting as well. Interestingly, the influence of number of birds appeared to be species-specific. The total number of waterbirds of all species present on a given day was not an influential variable in our overall model explaining percent error, except for one species, American Coot. This pattern suggests that count differences were unlikely to have been caused by observers being overwhelmed by the total number of birds to observe, identify and count. Instead, it appears that each species presented different challenges to observers. Given that our models rarely identified species' traits as being informative, it remains unclear what species-specific factors are responsible.

The degree of variability across species in count differences should influence potential decisions regarding use of eBird count data. Our analyses clearly reveal that off-the-shelf acceptance of count data for assessments of absolute abundance should be done with great care and thoughtfulness. In addition, if researchers wish to avoid focus on absolute abundance by instead evaluating relative abundance, our results suggest further caution is warranted. We found great interspecific variability in count differences. That is, although bias was nearly uniformly directional toward undercounting, the magnitude of undercounts varied substantially across species indicating that processes generating errors are inequivalent across species. Therefore,

judging differences in one species' abundance relative to others requires careful thought. If explorations of relative abundance are focused on within-species changes across sites, care is also warranted because we found substantial differences among observers in count accuracy. If different sites have different observers, then error/bias processes will be expected to be different as well. Effective use of relative abundance data depends on assumptions of consistent errors across species and sites, which appears to be largely untrue in our data. Further exploration of techniques to determine the degree to which assumptions of similar counting errors across species might be relaxed to preserve the utility of relative abundance analyses are warranted. The use of abundance categories could be explored to maximize the information content gleaned from count data.

What role might species misidentifications have played in counting errors? Count differences were regularly so large that we conclude species misidentification was unlikely to be an important factor. Probably the most challenging identifications involved female or eclipse-plumaged ducks, which observers might ignore and exclude from checklists if identification is uncertain. We consider such omissions to be unlikely for at least three reasons. First, degree of dichromatism was uninformative in our models explaining percent error. Second, assuming that females represent approximately half of each species present during most months of a year, count differences might be expected to average 50% if males were counted accurately but females were not. Instead, percent error varied widely across species. Finally, count differences of monochromatic vs. dichromatic species were not obviously different. However, it is possible that observers were more accurate for some species than others because of paying greater attention to unusual or favorite species (Schuetz and Johnston, 2019). At our site, most charismatic species of great interest to birders are rarities and so were not included in our analyses. Counts of Surf Scoter, a species that occurs during a narrow window of time in fall, were generally accurate, but we cannot attribute the accuracy to celebrity alone given its occurrence in such small numbers.

Aside from a predominantly directional bias toward undercounts, we found few consistent species-specific patterns in percent error. Errors differed in magnitude across species, observers, and time of year. Therefore, development of some type of calibration effort, where checklist numbers are adjusted to more closely approximate species-specific abundances poses an interesting challenge. The variability in raw count data suggests that tracking trends across time without additional steps to filter data or analytically adjust for noise could be especially problematic. Depending on the particular scientific question of interest, needs for precision might decline, so other analytic approaches could be implemented. For example, if abundances can be binned into categories and approaches such as ordinal or quantile regression used (Ananth and Kleinbaum, 1997; Koenker and Hallock, 2001; Howard et al., 2014), less precisely defined trends over time might be identified. Furthermore, our observation that percent richness, which we assume to be a correlate of observer experience, was often an informative variable, suggests that additional exploration of count calibration approaches for data contributed by the most experienced observers might be informative.

If questions about patterns in abundances among species in the waterbird community are of interest, our NMDS ordination results suggest that combining checklists across multiple observers rather than selecting data from any single contributor may produce results closer to those generated by professional benchmark data. The vectors in NMDS results may also inform decisions about which criteria to use when filtering data to maximize inclusion of checklists with the greatest value for specific scientific questions. For example, the waterbird community at our site was better characterized by observers who included more species on their checklists, invested more time searching the site each time, and contributed more checklists overall. Although species-specific numbers remained inconsistently related to the R^* counts, the level of general characterization of the entire community was improved. In a detailed comparison of eBird data with structured survey results near Sydney, Australia, overall characterization of the bird communities was similar as well, but the collectively greater effort expended by eBirders resulted in discovery of a greater number of uncommon species (Callaghan et al., 2018).

Determining the extent to which results from our site and observers may be generalized more widely will require identification of other sites with benchmark data sets. We also recommend further investigation of approaches for identifying checklists with higher probability of having the most accurate count data. New approaches for categorizing checklists based on expected numbers of species have recently been developed but it remains unclear if these same criteria also apply to bird counting accuracy (Callaghan et al., 2018). Our index of checklist quality was based solely on the percent of species reported on checklists that were also detected that day by the professional observer. Percent richness was regularly in top models, so it does have explanatory influence on count differences. Yet, direct comparisons of data from those observers and the R^* and Ref2 numbers still showed substantial differences, primarily of undercounting.

If a sufficiently detailed benchmark data set is available, however, adjustments for seasonal fluctuations in numbers of each species could conceivably be implemented. Such calibrations might be conducted more effectively if individual observers exhibited consistency in counting errors, an issue we have not explored here. It is unknown if observers improve their counting skills over time in the same way that observers are expected to improve abilities to detect species or if temporal stochasticity drives counting errors. A goal could be to develop a count calibration metric for each observer so that it can be extended and applied to counts from sites lacking benchmark data if those sites are likely to have similar species composition and relative abundances. However, given the high level of variability in count data we quantified across observers, species and time, such calibration metrics may be quite challenging to develop. Complex models such as the Bayesian hierarchical models using Markov chain Monte Carlo approaches implemented with Christmas Bird Count data (Link et al., 2006), might be helpful in the absence of additional information on checklist accuracy and reliability.

Our community ordination results suggested that combining data across multiple checklists from multiple observers (the group collective effort) might more closely approximate the community characterization than most single contributors did. Further exploration of similar approaches and sensitivities to checklist characteristics could identify necessary checklist quality criteria that must be met prior to use in such analyses. In the end, use of any checklist count data will be influenced strongly by each project's specific objectives (Isaac and Pocock, 2015).

We hypothesize that the high variability in species count information on eBird checklists could be influenced by common aspects of birder behavior. Prior to the advent of eBird, most birders, in North America at least, focused their efforts on listing species and watching behavior (Eubanks et al., 2004). Intentional counting was done by a small percentage of particularly avid observers, while most others only counted during organized activities such as Christmas Bird Counts (Boxall and McFarlane, 1993). A much smaller percentage contributed count data to scientific projects with structured protocols such as the North American Breeding Bird Survey. eBird has revolutionized the degree of attention birders pay to numbers of birds around them (Wood et al., 2011). It has pushed birders to value data beyond the day's species list. The novelty of this effort to count all birds every time one goes birding, may contribute to the variability in quality of the count data. Contributors are largely untrained about best practices for counting, especially when birds are present in large numbers, flying, or inconspicuous because they are secretive or available only by sound. We encourage development of additional training opportunities for eBird contributors to improve their knowledge of the value of accurate count data as well as their counting skills. Training improves data quality even for professional observers (Kepler and Scott, 1981).

An indication on checklists in the eBird database that such training had been accomplished might facilitate selection of checklists by researchers who wish to use count data only from trained observers. Furthermore, the addition of a qualitative categorization of counting accuracy for each checklist, designated by the observer at time of checklist submission to eBird, might be useful. Currently, users may code species using presence-absence information instead of counts or select a checklist protocol (incidental) indicating that not all species detected were included in the list. A count accuracy designation could allow observers to rate their own level of confidence in the accuracy of their counts or the level of attention they paid to counting accurately, which could serve as additional criteria by which researchers might choose checklists for their particular scientific question. Given that many contributors may not focus on producing accurate counts but have a variety of other motivations (Boakes et al., 2016), allowing observers to categorize quickly and easily their personal confidence in their count data would be useful.

Finally, exploration of the sources of variation in count data needs additional attention (Dickinson et al., 2010). The potential value of the vast quantities of information from citizen science databases is great. Such data have the potential to be effective at informing conservation and management decisions (McKinley et al., 2017; Young et al., 2019), but a thorough understanding of sources of error should be a priority before their use (Lewandowski and Specht, 2015). An additional strategy that may contribute to refinement of information on count data quality in citizen science databases could be development of a network of sites with trained counters. These marquis sites could be chosen to represent major habitat types where citizen science data are often gathered or where researchers specifically need high-quality information. Creating a network of high-quality benchmark sites would have the added advantage of leaving a legacy of reliable abundance data for future generations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because purely observational.

AUTHOR CONTRIBUTIONS

WR conceived the project, gathered the data, helped analyze the data and wrote the manuscript. TH contributed to data collection and management, analyzed the data, and contributed to writing the manuscript. RH contributed to data interpretation and to writing the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

WR and TH were supported by the Bob and Phyllis Mace Professorship and the College of Agricultural Sciences. RH was supported by the NSF Grant 1910118.

ACKNOWLEDGMENTS

We thank the many birders who contributed their data to eBird and the many scientists who created, maintain, and continue to improve eBird. Dennis Lewis and Philomath Public Works permitted access to the site, not only to us but to more than 250 birders. We thank our Reconfiguration Grant Group for helpful discussions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.568278/full#supplementary-material

Supplementary Figure 1 | Number of eBird checklists contributed for the study site at Philomath Ponds, Oregon USA, 2010–2019, as a function of day of year.

Supplementary Figure 2 | Counts of waterbirds in eBird checklists included in our analyses as a function of their percent error.

Supplementary Figure 3 | Relationship between mean percent error on eBird checklists (blue line) and mean waterbird abundance (green line) as a function of day of year at Philomath ponds, Oregon USA, 2010–2019. Waterbird abundance is the mean of all the counts (R^*) of all of the possible 20 study species present each day across the 10 years.

Supplementary Table 1 | Species-specific measurements of central tendency and variation in percent counting errors. **(A)** excluding species non-detections from checklists; **(B)** including species non-detections (zero counts) in checklists.

Supplementary Table 2 | Species-specific BIC model results. Full model results are presented for each species alphabetically.

Supplementary Table 3 | Full mixed-effects model results supplementing the abbreviated results presented in **Table 2**.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 02 March 2021
doi: 10.3389/fevo.2021.579230

Climate Change and Local Host Availability Drive the Northern Range Boundary in the Rapid Expansion of a Specialist Insect Herbivore, *Papilio cresphontes*

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Species distributions, abundance, and interactions have always been influenced by human activity and are currently experiencing rapid change. Biodiversity benchmark surveys traditionally require intense human labor inputs to find, identify, and record organisms limiting the rate and impact of scientific enquiry and discovery. Recent emergence and advancement of monitoring technologies have improved biodiversity data collection to a scale and scope previously unimaginable. Community science web platforms, smartphone applications, and technology assisted identification have expedited the speed and enhanced the volume of observational data all while providing open access to these data worldwide. How to integrate and leverage the data into valuable information on how species are changing in space and time requires new best practices in computational and analytical approaches. Here we integrate data from three community science repositories to explore how a specialist herbivore distribution changes in relation to host plant distributions and other environmental factors. We generate a series of temporally explicit species distribution models to generate range predictions for a specialist insect herbivore (*Papilio cresphontes*) and three predominant host-plant species. We find that this insect species has experienced rapid northern range expansion, likely due to a combination of the range of its larval host plants and climate changes in winter. This case study shows rapid data collection through large scale community science endeavors can be leveraged through thoughtful data integration and transparent analytic pipelines to inform how environmental change impacts where species are and their interactions for a more cost effective method of biodiversity benchmarking.

Keywords: biotic interactions, benchmarking biodiversity, citizen science, species distribution models, climate change

OPEN ACCESS

Edited by:
Umesh Srinivasan, Centre for Ecological Sciences, Division of Biological Sciences, Indian Institute of Science (IISc), India

Reviewed by:
Jamie M. Kass, Okinawa Institute of Science and Technology Graduate University, Japan
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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *02 July 2020*
Accepted: *03 February 2021*
Published: *02 March 2021*

Citation:
Wilson JK, Casajus N, Hutchinson RA, McFarland KP, Kerr JT, Berteaux D, Larrivée M and Prudic KL (2021) Climate Change and Local Host Availability Drive the Northern Range Boundary in the Rapid Expansion of a Specialist Insect Herbivore, Papilio cresphontes. Front. Ecol. Evol. 9:579230. doi: 10.3389/fevo.2021.579230

INTRODUCTION

Biodiversity benchmarking is fundamental to both basic and applied ecological research offering insights into the biological processes shaping species and their interactions. Benchmarking is a labor intensive endeavor, often limited by participation and training. Recent advances in sensing technology and communication have led to a diverse and plentiful data landscape coordinating and improving biodiversity community science efforts at scale so that they can be used in meaningful ways for benchmarking efforts (e.g., Sullivan et al., 2009; Prudic et al., 2017). Observational web platforms and smartphone applications, automated camera arrays, and machine learning-assisted identifications have also changed how biodiversity data is collected, processed, and verified (e.g., Sullivan et al., 2009; Prudic et al., 2017) although challenges remain (Bonney et al., 2009). These technologies have expedited the rate of understanding and changed the research focus to exciting new areas where an informatics toolkit is now a necessity (Feng et al., 2020). One new aspect of benchmarking biodiversity is to evaluate where species are and which species they co-occur with, or species distributions and their changing interactions (e.g., Bueno de Mesquita et al., 2016; Palacio and Girini, 2018).

Species distributions are known to be greatly influenced by climate (Brown et al., 2016). Climate-related range shifts have been and are continuing to be documented globally across taxa and systems: terrestrial (Parmesan and Yohe, 2003), marine (Poloczanska et al., 2013), and aquatic (Rahel and Olden, 2008). With current changes in global climate, species range shifts (Parmesan et al., 1999) and extensions in both altitude and latitude are being observed (Roth et al., 2014; Kerr et al., 2015). While many studies have examined the ongoing changes in climate and their effects on biodiversity and species ranges, most consider only abiotic factors in their analyses, missing the potential importance of local interspecific interactions once a species moves into a novel environment beyond its previous range (Blois et al., 2013; HilleRisLambers et al., 2013; Wisz et al., 2013).

Several interspecific interactions are known to play important roles in shaping range boundaries including competition (Connell, 1961; Huey et al., 2009; Stanton-Geddes et al., 2012), mutualism (Chalcoff et al., 2012; Moeller et al., 2012), facilitation (Bader et al., 2007; Stueve et al., 2011; Ettinger and HilleRisLambers, 2017) and natural enemies (Freeman et al., 2003; Speed et al., 2010). When a species extends into a new local environment, there are a few main scenarios it can encounter (Holt, 2003; Urban et al., 2007; Sexton et al., 2009): (1) ecological conditions are similar enough to previous conditions that there is little immediate effect on fitness and population growth rate, (2) the new local environment may possess biotic or abiotic conditions that differ from the original local environment and can accelerate (e.g., competitive or predatory release; or (3) decelerate (e.g., nutrient or nesting limitation) range expansion.

For insect herbivores, climate change can influence abundance and distribution through direct mechanisms (physiological impacts on growth, development and reproduction that impact fitness) and indirect mechanisms (impacting biotic factors such as host plant quality or predator abundance) (Bale et al., 2002; Deutsch et al., 2008; Kingsolver et al., 2011; Robinson et al., 2017). How and when climate change will affect herbivorous insect dynamics has received considerable attention generating a diversity of observed responses, especially in the pest management literature (Porter et al., 1991; Cannon, 1998; Harrington et al., 2001; Altieri et al., 2015; Castex et al., 2018). Some species are expanding in ranges and abundance (Battisti et al., 2005; Robinet and Roques, 2010; Robinson et al., 2017) while others are retracting and decreasing in numbers (Robinet and Roques, 2010; Zvereva et al., 2016; Sánchez-Bayo and Wyckhuys, 2019). Host plant abundance and distribution play a key role in generating these patterns as herbivorous insects are often limited by larval food resources (Dempster and Pollard, 1981; Pearson and Knisley, 1985; Ylioja et al., 1999). Exactly how host-availability translates into patterns of distribution, abundance, and range shifts for insect herbivores is still contentious and particularly complex when combined with direct effects on physiology (Louthan et al., 2015; Lany et al., 2018). Our understanding of the determinants regulating species distributions are becoming more nuanced as we begin to incorporate information on species' dispersal capacity, population abundance trends, and climatic variables into our models (Elith and Leathwick, 2009).

In this study, we investigate the role of host availability and climatic variables on the range expansion of the specialist giant swallowtail butterfly (Papilionidae: *Papilio cresphontes*) in northeast North America over the last 60 years (1959-2018), with an emphasis on the perceived accelerated expansion of the last 18 years. We combine evidence from raw occurrence data with a series of species distribution models for *P. cresphontes* and associated host plants to evaluate the rate and direction of range changes in relation to both abiotic and biotic factors. While other studies have incorporated biotic variables as model inputs (Bueno de Mesquita et al., 2016; Palacio and Girini, 2018), our approach was to model the distribution of the insect herbivore and host plants separately and using these independent models to make *post hoc* inferences and comparisons of ranges. Because both this insect and its primary larval host plants (the common prickly ash [Rutaceae: *Zanthoxylum americanum*], southern prickly ash [Rutaceae: *Zanthoxylum clava-herculis*] and common hop tree [Rutaceae: *Ptelea trifoliata*]) are conspicuous, they are often reported in systematic biological surveys and museum collections. In this study, we bring together a combination of museum collection, survey, and citizen science data to understand how host plant availability, climate changes, and butterfly abundance are influencing the rapid expansion of an herbivorous insect as a case study. This study is one of few to demonstrate the interplay of both climate change and biotic interactions in shaping range limits while focusing on the ecologically important role of herbivores.

MATERIALS AND METHODS

Study Region and Time Interval

We focused on eastern North America (study area bounded by −94° and −65° longitude and 25° and 55° latitude) where *Papilio cresphontes* has been reported to be expanding rapidly (Finkbeiner et al., 2011; Breed et al., 2012) and data are readily available for both *P. cresphontes* and larval host plants, (*Zanthoxylum americanum, Zanthoxylum clava-herculis* and *Ptelea trifoliata*). Though records of *P. cresphontes* exist further west than −94°, we set this cutoff to minimize complications of misidentification and complex species boundaries with its congener *P. rumiko*. We categorized and compared two time periods: T1 (1959-1999) representing the period prior to the beginning of the rapid range expansion and T2 (2000-2018) as the period when the rapid range expansion to the north began. This cutoff point was determined from raw occurrence data (**Figure 1**).

Data Sources

Butterfly and Host Plant Data

Papilio cresphontes (Papilionidae) is a sub-tropical butterfly widely distributed across North America. *P. cresphontes* and host plant occurrence data were obtained from a variety of sources: iNaturalist[1], n = 3,007, Global Biodiversity Information Facility (GBIF[2]), n = 14,181, the Maine Butterfly Atlas[3], n = 11, the Maritime Canada Butterfly Atlas[4], n = 6, Massachusetts Butterfly Club, n = 512, Butterflies and Moths of North America[5], n = 1,188, and eButterfly[6], n = 3,083. Data from iNaturalist and GBIF were downloaded using the *spocc* package for R (Chamberlain et al., 2016). We filtered iNaturalist data to include only research-grade records before combining with other data sets. Combined data were filtered for time frame, duplicates, and study area extent (see below) before further analysis and model building. In total, we used 8,051 occurrence records for *P. cresphontes* and 2,697 occurrence records (combined) for all three host plant species.

Environmental Data

We used the TerraClimate data set (Abatzoglou et al., 2018), a 4 km × 4 km resolution gridded set of monthly climatological data from 1958 to 2017 (at the time of writing this) to generate environmental predictor variables for modeling. We calculated a set of yearly summaries of 19 bioclimatic variables (Fick and Hijmans, 2017), frequently used in species distribution modeling, using the *dismo* package in R (Hijmans et al., 2017) for each

[1]www.inaturalist.org

[2]www.gbif.org

[3]https://mbs.umf.maine.edu

[4]http://accdc.com/mba/index-mba.html

[5]www.butterfliesandmoths.org

[6]www.e-butterfly.org

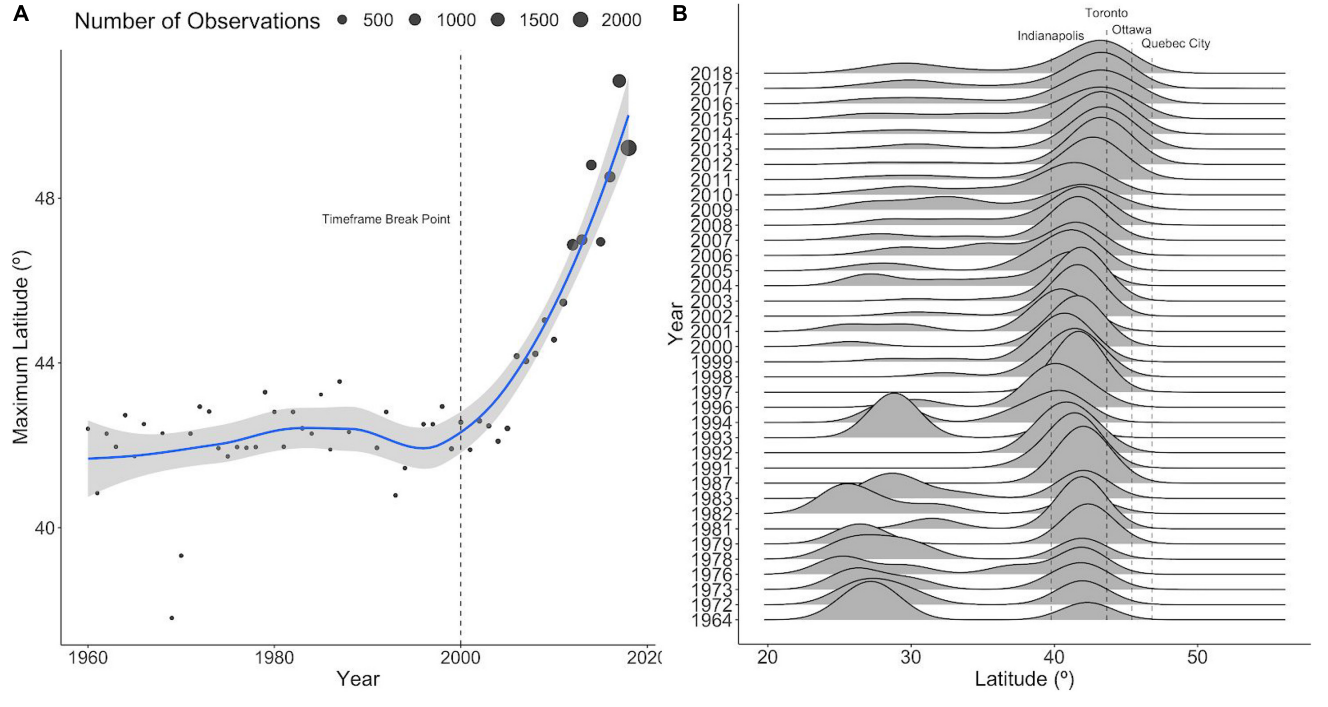

FIGURE 1 | Evidence of northward range shift of *P. cresphontes* from raw occurrence data. **(A)** The maximum latitude of a recorded occurrence of *P. cresphontes* by year. Larger circles indicate years with higher numbers of occurrence records (high numbers in more recent years are due to increased citizen-science activity). The dashed lines represent the breakpoint between T1 and T2. Three years with extremely low maximum latitudes (<35) were omitted for clarity. The blue line and gray bar represent the loess smoothing curve and 95% confidence interval. **(B)** A ridge-plot of kernel density estimates of occurrences for *P. cresphontes*. Vertical dashed lines represent latitudes of major cities within the range. Years with <5 occurrence records were removed from plotting.

year in each time period (T1 and T2) and then averaged these summaries across each time period to provide temporally appropriate climate summary for each set of models. We included all 19 bioclimatic variables as predictors for modeling.

Species Distribution Models

Distributions of *P. cresphontes* and host plants were estimated using MaxEnt 3.4.0, a machine learning algorithm based on the principle of maximum entropy (Phillips and Dudík, 2008; Elith et al., 2011; Phillips et al., 2017). MaxEnt is a presence-background method, which is considered to perform well when modeling climatic niches across a variety of sample sizes (Wisz et al., 2008). We used the *ENMeval* package for model-building, testing, and tuning (Muscarella et al., 2014), ultimately building 8 total models (*P. cresphontes* and three host species for each time period).

We used a combination of geographically structured and regular k-fold cross validation for model testing and tuning. We generated 10,000 random background points per species-time period combination (within the geographic extent outlined by the occurrences across both time periods – a rectangle defined by the minimum and maximum latitude and longitudes of occurence points) per model and used the *blockCV* package (Valavi et al., 2019) to divide our study area into 400 km × 400 km blocks. Blocks were randomly assigned to folds 1-5 over 250 iterations to determine a block design that maximized evenness of occurence and background points spread across all folds. This procedure was repeated for every model (8 times in total). Occurrence and background points from folds 1-4 were used as training data for MaxEnt cross-validation and tuning, while fold 5 was reserved as a set of out-of-sample test data for final model evaluation. Throughout the manuscript, we refer to these data as test data. We used another set of random fivefold cross validation within the training data to tune model parameters (within the *ENMeval* package). Throughout the manuscript, we refer to these data as validation data. We tested linear, quadratic and hinge features (and all combinations) as well as a set of regularization multipliers (0.5-4 in 0.5-step increments). We examined models using a range of evaluation metrics (**Supplementary Figures 1–8**), but eventually chose the model with the highest area under the receiver operating characteristic curve (AUC) on validation data. All evaluation metrics were reported for the separate set of spatially explicit test data generated by *blockCV* (**Table 1**). AUC values typically range between 0.5 and 1, and can be used for relative comparisons between models with the same data (with higher values closer to 1 indicating models with better predictive capacity (Lobo et al., 2008). Once the optimal parameters for a given species and time-frame were determined, we built full models using all available occurrence data to generate predictions for subsequent visualizations and analyses. We mapped the "cloglog" MaxEnt output, which can be interpreted as probability of occurrence under the assumption that the species presence or absence at nearby sites are independent (Phillips and Dudík, 2008; Elith et al., 2011; Phillips et al., 2017). Importance of predictors was assessed using the permutation contribution metrics generated when building full models. These metrics are built as MaxEnt steps through modifications of coefficients for single features. For each variable, values are randomly permuted on training data and a model is reevaluated on the permuted data. Then, the resulting drop in AUC scores are tracked and normalized to percentages (Phillips et al., 2006). Thresholds for binary presence-absence maps and presence distributions were generated using the maximum test specificity plus sensitivity (Liu et al., 2005). For all models, we used species-specific (but not time-specific) geographic extents during model building and tuning, as well as making predictions for graphical outputs. Kernel density plots are used to show latitudinal distributions of model predictions and northern range limits.

MaxEnt has become a popular modeling resource because of its predictive power, ease of use, and a well-detailed literature to get researchers started (Phillips and Dudík, 2008; Elith et al., 2011; Phillips et al., 2017). However, this framework has also received criticism, with researchers advocating for more explicit examinations of tuning parameters, evaluation metrics, and the incorporation of tools to deal with sampling bias (Radosavljevic and Anderson, 2014). Recent software additions have addressed some of these challenges, and opened up the "black-box" of MaxEnt (Phillips et al., 2017), though issues remain, particularly in the transparency of researchers' hyperparameter tuning and evaluation (Morales et al., 2017). To this end, we implemented recently developed tools (*ENMeval* and *blockCV* packages in R; (Muscarella et al., 2014; Valavi et al., 2019) to explicitly outline tuning (**Supplementary Figures 1–8**), and to incorporate a spatially independent evaluation design to minimize overfitting (along with the built-in regularization in MaxEnt).

TABLE 1 | Model parameter set and evaluation metrics on geographically structured test data.

Species	Timeframe	Occurrences*	Feature classes**	Regularization multiplier	AUC (test data)	Threshold	Num. non-zero coefficients
P. cresphontes	T1	219	QH	1	0.957	0.113	97
	T2	7,832	LH	1	0.892	0.212	114
Z. americanum	T1	153	LQH	0.5	0.901	0.134	84
	T2	1,170	LQH	2	0.884	0.177	109
Z. clava-herculis	T1	9	LQH	0.5	0.871	0.066	98
	T2	364	LQH	0.5	0.902	0.130	166
P. trifoliata	T1	139	LQH	0.5	0.872	0.297	182
	T2	862	H	0.5	0.893	0.149	240

**Full number of occurrences, not the number of occurrences within the test set. **Feature classes tuned in MaxEnt (L, linear; Q, quadratic; H, hinge, and combinations).*

Northern Range Limits

We calculated the distance between the northern limit modeled for *P. cresphontes* for T1 and T2 using a longitude class approach (Leroux et al., 2013). For each 4-km longitude class (i.e., each "column" of 4 km of longitude across the entire study area), we determined the latitude of the northernmost grid cell where the species was predicted to be present during T1 and T2. We selected the latitude-pairs (pairs of data for a single latitude at T1 and T2) for which we had grid cells with occurrence for *P. cresphontes* in both time periods for each longitude class and tested whether the average northern limit distribution of *P. cresphontes* differed between T1 and T2, using a paired *t*-test. We used similar methods to determine differences between northern range limits of *P. cresphontes* and *Z. americanum* for both time periods.

RESULTS

Evidence of Northward Range Shift of *P. cresphontes* From Raw Occurrence Data

Patterns of occurrence (as opposed to the predictive outputs from species distribution models) indicate a strong trend of a rapid and recent northward range expansion in *P. cresphontes* since the earliest recorded records of the species in our dataset (1959). The butterfly's highest recorded latitude in a given year has increased dramatically since 2000 (**Figure 1A**), and the predicted suitability has shifted from low to high in many cities close to the current northern edge of the range (**Figure 1B**).

Predictive Accuracy of Species Distribution Models

Maxent models with optimal complexity settings were chosen via hyperparameter tuning, and a variety of evaluation metrics were calculated (**Supplementary Figures 1–8**), but ultimately the feature classes and regularization multiplier of the model with the highest average validation AUC was used for each species-time period pair. Once the final parameter set was chosen, models were evaluated on spatially explicit out-of-sample test data created by *blockCV*. Overall, models had high predictive accuracy on test data, with AUC scores ranging from 0.871 to 0.957 (**Table 1**). Generally, models were complex and incorporated combinations of feature classes paired with regularization multipliers (**Table 1**). Final models were generated using the parameter set (feature classes and regularization multiplier) described above, but built with the full set of data (training + test) to generate predictive maps (**Figures 2**, **3**) and distributions (**Figures 4**, **5**).

Papilio cresphontes Has Expanded Northward Due to Recent Climate Warming

Predictive maps generated from MaxEnt models clearly show a change in the distribution of *P. cresphontes* between T1 and T2, with a northward expansion since 2000 (**Figures 2A,B**). Kernel density estimate plots generated from threshold occurence predictions mirror this result (**Figure 4**), and highlight that different parts of *P. cresphontes*' range match host plant use. *Z. americanum* closely matches *P. cresphontes* in the north, while the middle and southern part of the range is defined by the presence of *Z. clava-herculis* and *P. trifoliata*.

Host Plant Range Shifts

Overall, host plants (*Z. americanum, Z. clava-herculis* and *P. trifoliata*) demonstrated more complex changes in distribution between T1 and T2 compared to *P. cresphontes* (**Figures 3A,B**). Historically, the species were split latitudinally (with significant overlap) with *Z. americanum* occupying the northern part of the study area, *P. trifoliata* the middle, and *Z. clava-herculis* in the far south (**Figure 3B**). However, this pattern changes subtly in T2, with a range expansion of *Z. americanum* northward, but also westward to the boundary of our study area. Distribution changes in other host plants were more complex, with complicated range changes for *P. trifoliata* in the middle latitudes of the study area, and small range contraction of *Z. clava-herculis* to the south.

Northern Range Limits for *P. cresphontes* Have Shifted Northward and Closely Match *Z. americanum*

The northern range limit of *P. cresphontes* was significantly higher in T2 compared to T1 ($t = -38.181$, $df = 560$, $p < 0.001$; **Figure 5A**) where the median northern-most occurence for T2 (median = 46.1875 ± 0.675°) was 2.917° (∼324 km) higher in latitude than T1 (median = 43.2708 ± 1.692°). *Z. americanum* also demonstrated a significant (but small) northern range shift between T1 and T2 ($t = -6.5717$, $df = 5510$, $p < 0.001$; **Figure 5B**) where the median northern-most occurence for T2 (median = 45.5208 ± 0.914°) was 0.458° (∼51 km) higher in latitude than T1 (median = 45.0625. ± 1.667°). We also tested whether the northern range limits of *P. cresphontes* and *Z. americanum* differed from each other during each time period. In each time period, there was a significant difference between the northern range limits of *P. cresphontes* and *Z. americanum* (T1: $t = -17.485$, $df = 550$, $p < 0.001$; T2: $t = 16.771$, $df = 551$, $p < 0.001$). The difference between median butterfly and host plant northern range limits shrank from 1.75° (∼194 km) in T1 (with *Z. americanum* having a higher northern range limit) to 0.77° (∼85.47 km) in T2 (with *P. cresphontes* having a slightly higher median northern range limit; **Figures 5B,C**).

Climatic Variation in the Study Area Between T1 and T2

Overall, T2 had a higher mean annual temperature (9.45 ± 6.20° C) than T1 (8.67 ± 6.27°C) ($t = -45.274$, $df = 534850$, $p < 0.001$). Bioclim variables 10 and 11 [mean temperature of warmest quarter (breeding season) and mean temperature of the coldest quarter (pupal overwintering season)] had the biggest impacts on predicting *P. cresphontes* distribution, while variables 9 (mean temperature of driest quarter), 10 (mean temperature of warmest quarter) and 3 (isothermality) had the biggest impacts across both time periods for *Z. americanum*.

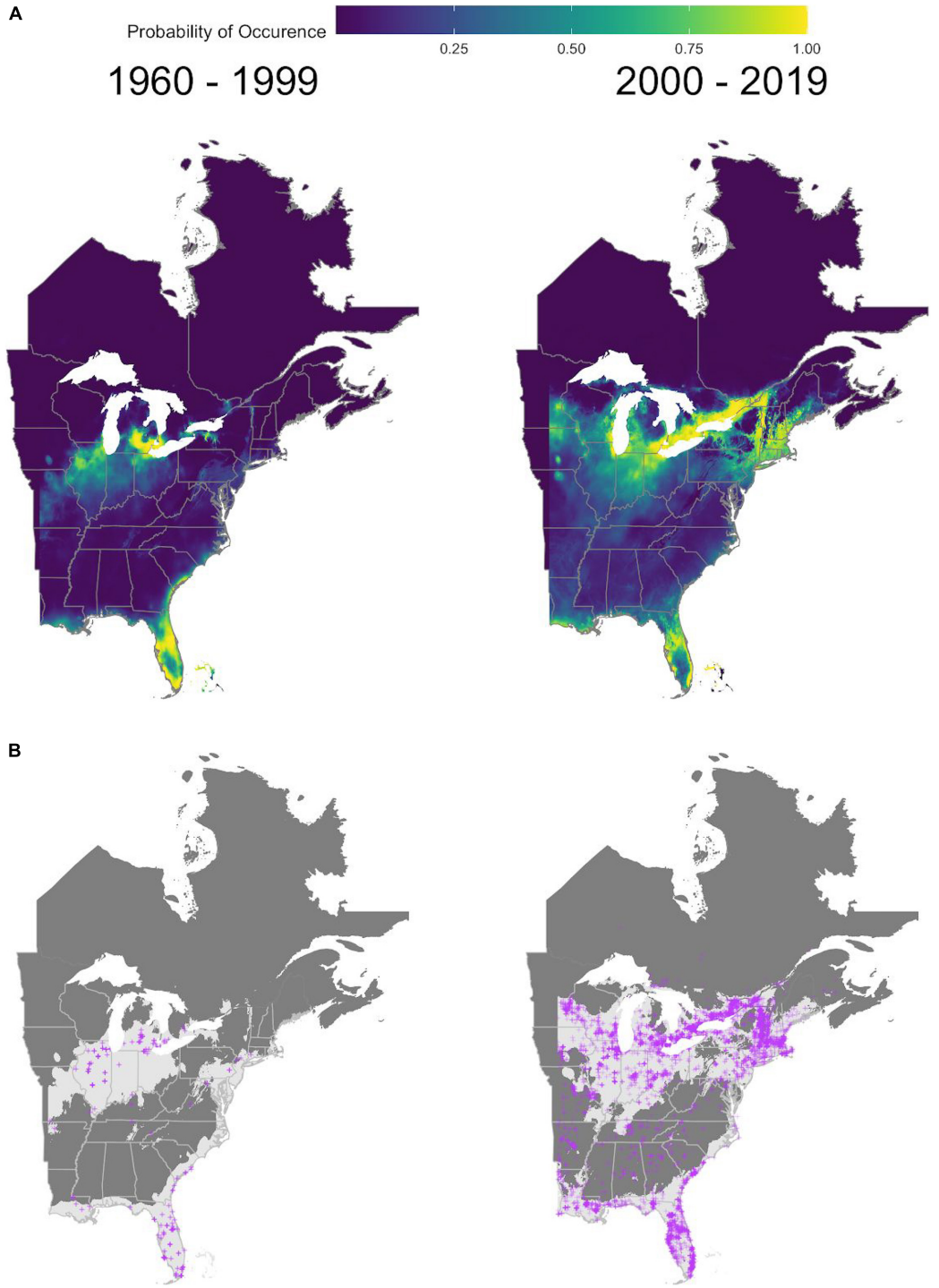

FIGURE 2 | MaxEnt model predictions for the giant swallowtail butterfly, *P. cresphontes* for t_1 (1959-1999) and t_2 (2000-2018). **(A)** cloglog transformed output from full MaxEnt models for two time periods. Lighter yellow areas denote higher probabilities of occurrence. **(B)** Threshold maps of presence absence for the two time periods. Light areas represent predicted occurrence and dark gray represents predicted absence. Purple crosses represent actual occurrence data for each time period.

Other host plants had multiple bioclim variables across time periods that impact distribution models (**Figure 6**). Variables that commonly had high permutation importance scores showed significant differences between T1 and T2 on average across our study area, with an overall trend of warmer patterns from 2000 to 2015 (T2) compared to 1959-1999 (T1) (**Table 2**).

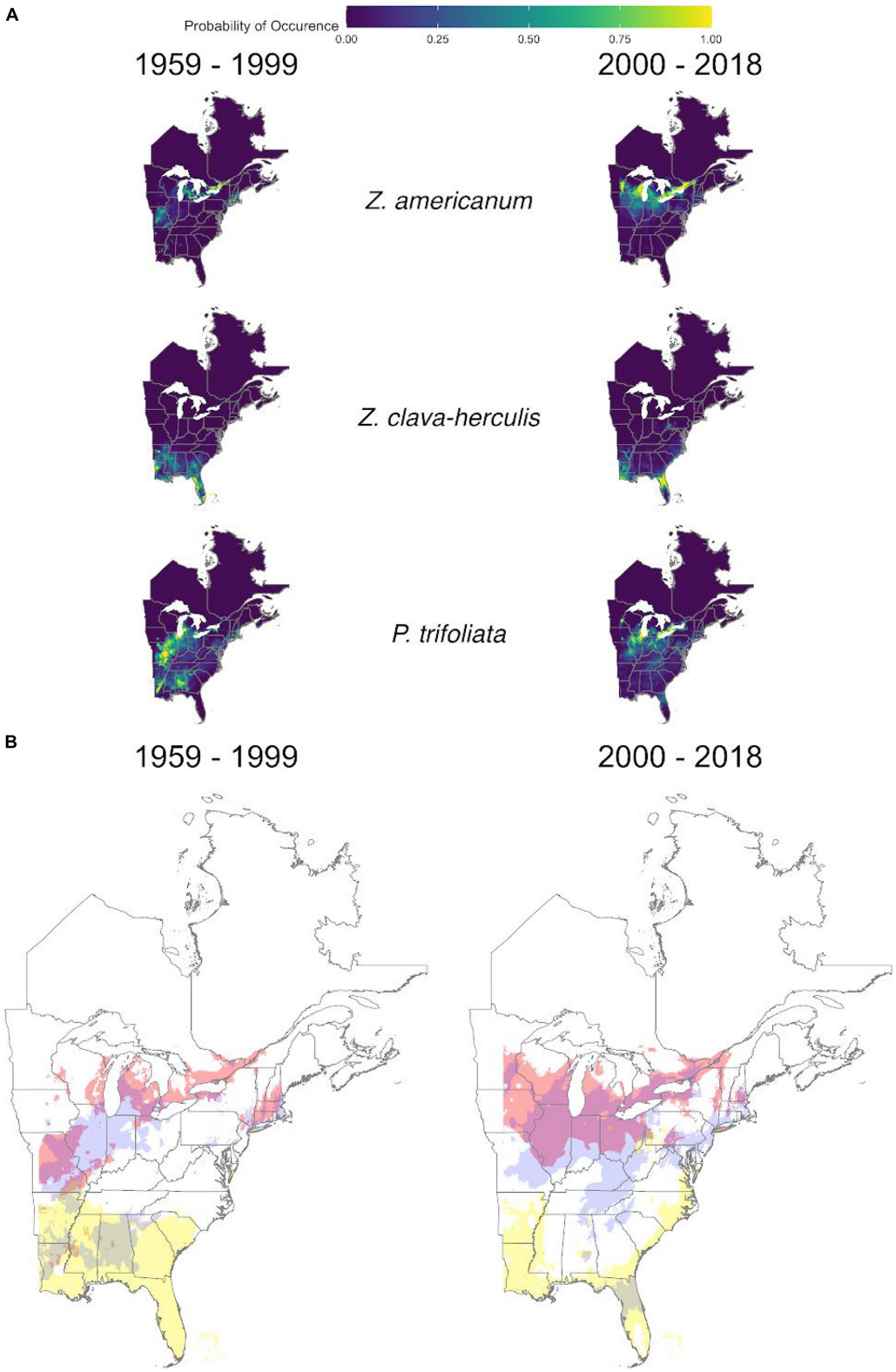

FIGURE 3 | MaxEnt model predictions for predominant giant swallowtail butterfly host plants (*Z. americanum, Z. clava-herculis* and *P. trifoliata*) for T1 (1959-1999) and T2 (2000-2018). **(A)** cloglog transformed output from full MaxEnt models for each host plant across two time periods. Lighter yellow areas denote higher probabilities of occurrence. **(B)** Threshold maps of presence absence for the two time periods. Different colors (red, blue, and yellow) represent areas of predicted occurrence for each host plant and white represents predicted absence. Mixed colors indicate areas of overlap.

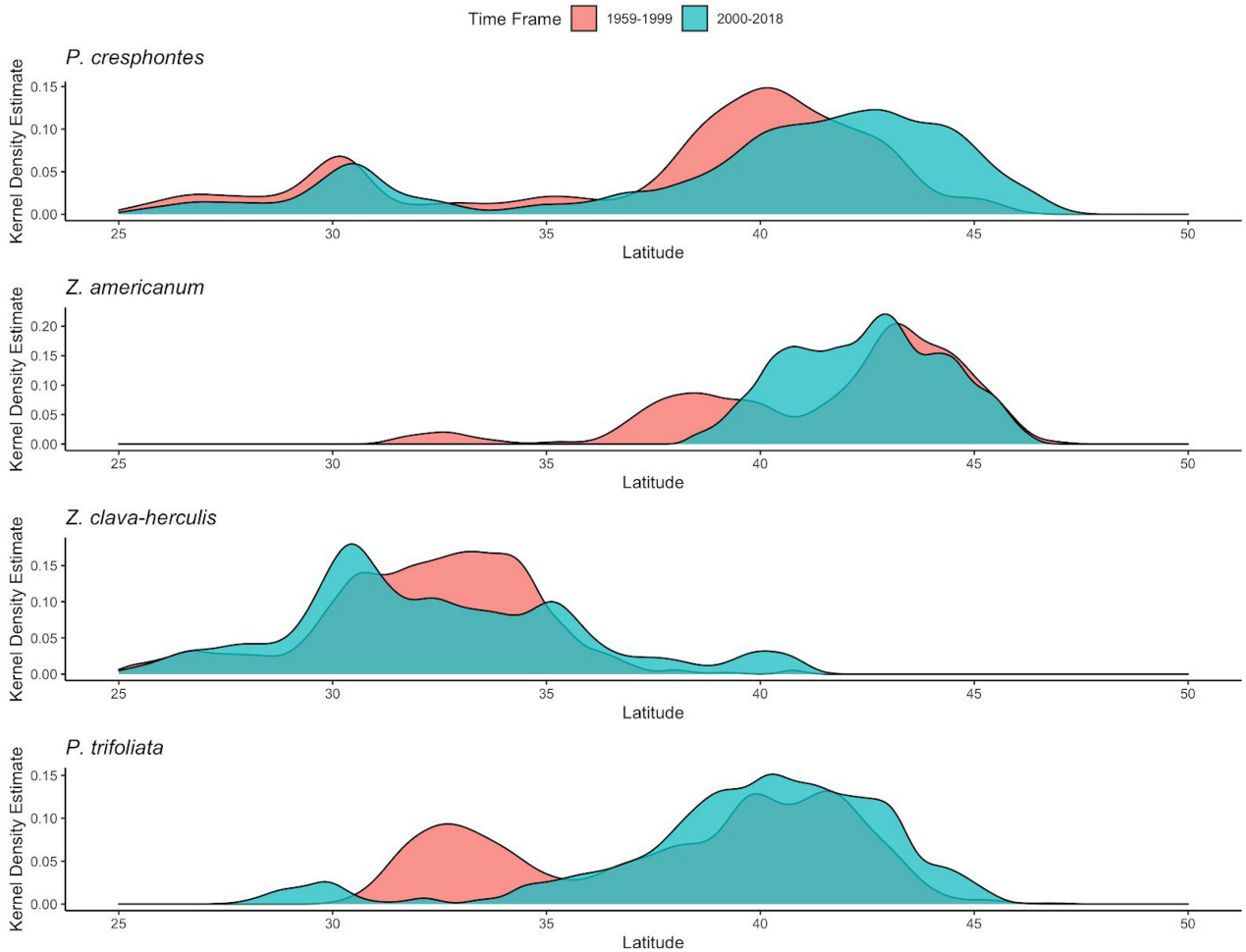

FIGURE 4 | Kernel density estimates for modeled predicted presence of giant swallowtail butterfly *P. cresphontes* and host plants (*Z. americanum, Z. clava-herculis* and *P. trifoliata*) for T1 (1959-1999) and T2 (2000-2018). Red plots are from T1 (1959-1999) and blue plots are from T2 (2000-2018).

DISCUSSION

The determinants of species distributions have long been debated not just because they are essential in ecology and evolutionary biology, but also because where organisms are and where they will be is central to successful conservation and restoration practices in light of rapid climate change (Buckley et al., 2013; Gallagher et al., 2013; Robillard et al., 2015). Our study details a recent and rapid northward range expansion by *P. cresphontes* between 2000 and 2018 (**Figure 1**). We also model the distributions of the butterfly's naturally occurring larval host plants, which, when combined with analysis of *P. cresphontes* range, result in different conclusions for the future distribution of this butterfly than if we had relied on abiotic variables alone (**Figures 2**, **3**). Recent climatic shifts, particularly warmer, wetter temperatures during breeding season and warmer temperatures during pupal overwintering season, have allowed *P. cresphontes* to rapidly expand northward to now match or even surpass the slower moving northward range expansion of the northernmost host plant, *Z. americanum*, with further northward expansion of *P. cresphontes* now limited by host plant range, not climate (**Figure 4**). Our results highlight the importance of including biotic interactions (and interactions between herbivorous insects and host plants in particular) in examinations of range shifts and their speed, an idea often highlighted, (Urban et al., 2016) but infrequently implemented (Lemoine, 2015; Dilts et al., 2019; Svancara et al., 2019).

Poleward range shifts in herbivorous insects, particularly butterflies, have been documented for a number of species (Parmesan et al., 1999; Warren et al., 2001; Pöyry et al., 2009; Breed et al., 2012). Additionally, northward expansions of other butterfly species have been shown to have dramatic impacts on community composition through linked biotic interactions (Audusseau et al., 2017), which could be happening in this system as well, but would require further examination to determine. While studies demonstrating range shifts in multiple taxa provide valuable insights into the magnitude and direction of shifts for different taxa, gaps in knowledge remain (Pöyry et al., 2009). Namely, (1) how has warming acceleration affected recent range shifts during the last 10-15 years in poleward latitudes, and (2)

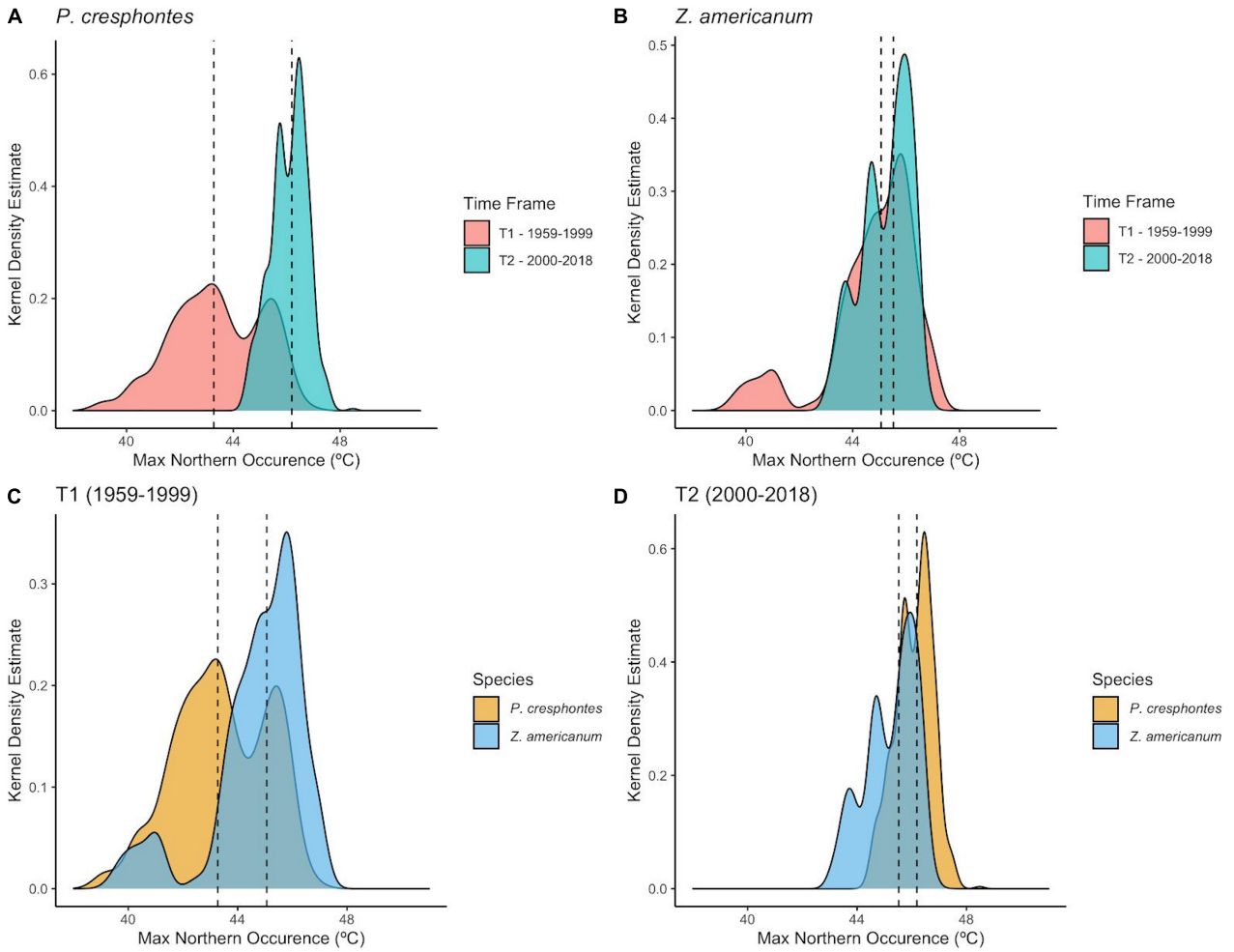

FIGURE 5 | Predicted maximum northern occurence for *P. cresphontes* and host plants for T1 (1959-1999) and T2 (2000-2018). Dashed vertical lines represent the median value for each group. **(A)** *P. cresphontes* northern range limit between the two time periods. **(B)** *Z. americanum* northern range limit between the two time periods. **(C)** Northern range limit comparison for T1 (1959-1999) for *P. cresphontes* and *Z. americanum*. **(D)** Northern range limit comparison for T2 (2000-2018) for *P. cresphontes* and *Z. americanum*.

how do abiotic and biotic factors interact to shape range shifts? Our study addresses both of these questions and provides a scalable, data acquisition and analytic pipeline by focusing on a single herbivore and multiple host plant species. We show an usually rapid northward range shift in this insect herbivore, *P. cresphontes,* over the last 18 years (predicted most northward occurrences differ by 2.917° of latitude (∼ 324 km) between T1 and T2, or a northward expansion of 180 km/decade) that is more than 27 times faster than the average of northward movement of global meta-analyses for plants, lichens, birds, mammals, insects, reptiles and amphibians, fish and marine organisms (Parmesan and Yohe, 2003) and over nine times faster than all butterfly species in Britain (Hickling et al., 2006). These observations are associated with warmer, wetter climate conditions during active flight times and overwintering. Our findings largely follow (Pöyry et al., 2009), who postulate that mobile species utilizing woody host plants like *P. crespontes* should exhibit large and fast range shifts northward, and that habitat availability and dispersal capacity largely determine success. We have laid the groundwork for one way to gather large amounts of data and analyze it as scale for future work across all butterfly and host plant species.

Interestingly, the northward incursion of *P. cresphontes* in northeastern North America is not a new phenomenon. Accounts detail movement into the region 145 years ago that lasted several decades (Scudder, 1889). In 1875, *P. cresphontes* were found in southern New England and by 1882 there are documented records just south of Montreal, Quebec. By the 1930s, the species had apparently retracted southward and were considered "extremely rare" in Massachusetts (Farquhar, 1934) and did not push northward into the region again until the last 8 years. Multiple long-term climate reconstructions (paired with historic instrument data) for the 145-year incursion period indicate a strong warming trend compared to the previous century (Marlon et al., 2016). However, this warming trend continues through the 1930s, so it is unclear which factors may have resulted in a retraction, though hydroclimatic reconstructions indicate

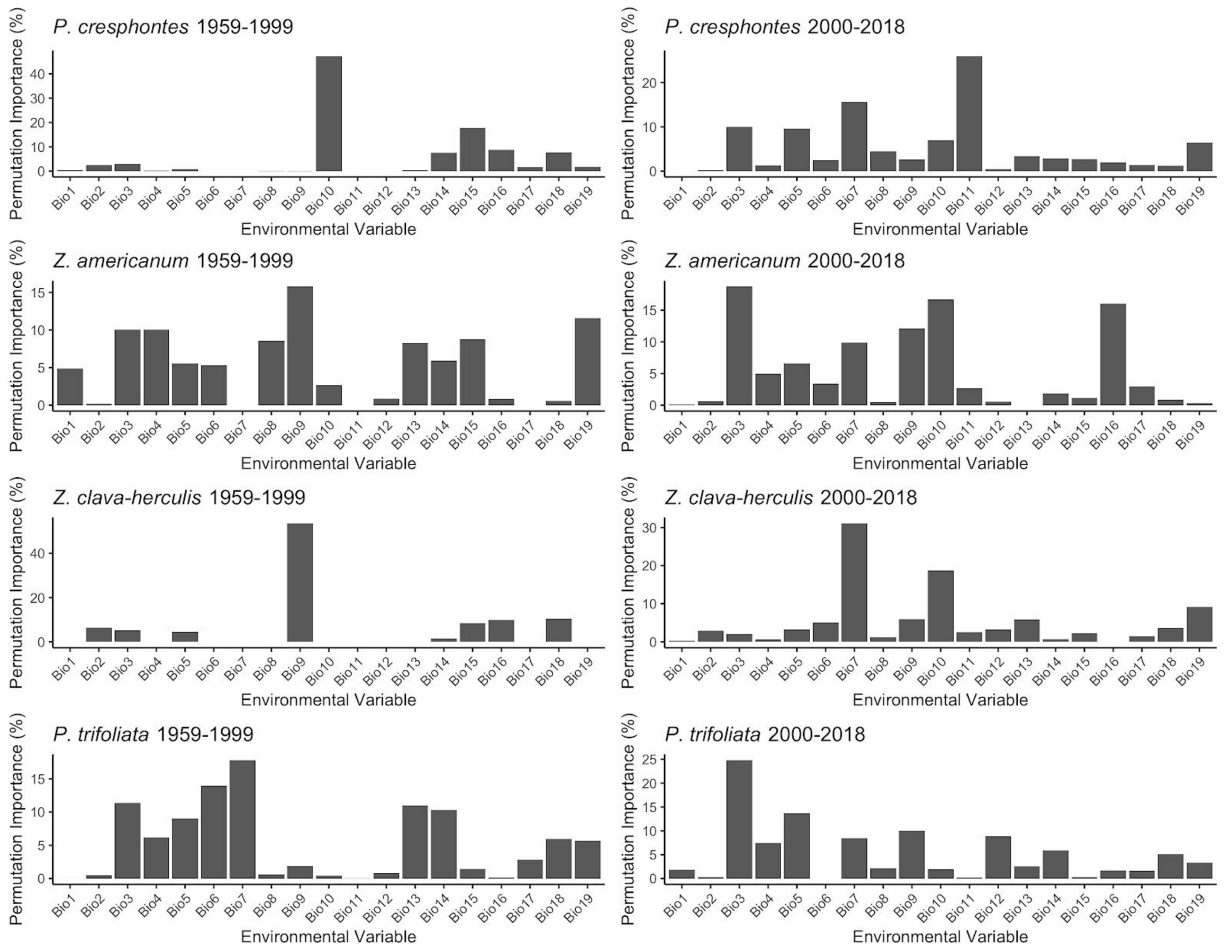

FIGURE 6 | Percent contribution of each of the 19 bioclim variables to final models for each species and time period. Percentages are computed from MaxEnt model training – as predictive gains increase, environmental factors contributing to feature generation are calculated and summarized in the final model. Common major contributors across many models include BIO9 (mean temperature of the driest quarter), BIO10 (mean temperature of the warmest quarter) and BIO11 (mean temperature of the coldest quarter).

TABLE 2 | Bioclimatic shifts in Bioclim variables between T1 (1959-1999) and T2 (2000-2015) that impact butterfly and host plant distributions.

Bioclim variable	T1 Median	T2 Median	*t*	df	*p*
1 (Mean annual temperature)	6.18 ± 7.19°C	7.000 ± 7.06°C	–2734.1	329112	<0.001
9 (Mean temperature driest quarter)	−2.04 ± 11.61°C	−1.307 ± 11.81°C	–229.86	329112	<0.001
10 (Mean temperature warmest quarter)	18.28 ± 4.75°C	18.873 ± 4.67°C	–1805.0	329112	<0.001
11 (Mean temperature coldest quarter)	−7.08 ± 10.21°C	−6.03 ± 10.07°C	–2732.7	329112	<0.001

an increase in drought in the northeastern United States over this time period, which likely had strong impacts on host-plant/nectar-plant distributions and quality through the range of *P. cresphontes* (Marlon et al., 2016), not to mention direct impacts on insect survival.

Our work also highlights the importance of including biotic interactions when predicting and projecting range shifts. *Papillio cresphontes*' current northern range now closely matches the northernmost host plant (*Z. americanum*) (**Figures 3**, **5D**) and this butterfly species is now limited by the ability of *Z. americanum* to expand its range northward. Because of the differences in life-history strategies, dispersal capabilities, reproductive outputs and environmental tolerances between insect and host plant, the northern expansion of *P. cresphontes* appears to now be largely curbed as the host plant is much more sessile and has much longer generation times. Though sightings of the winged adult stage of *P. cresphontes* will likely continue to be seen further north than the naturally occurring host plant range (**Figure 5D**), without a suitable host plant, further northward expansion seems unlikely but may be facilitated by recently documented *P. cresphontes* occurrences in horticultural settings. *Papilio cresphontes* lay eggs and larvae feed successfully

on two non-native garden plants, garden rue (*Ruta graveolens*) and gas plant (*Dictamnus albus).* Common hoptree (*P. trifoliata*), is increasingly planted as an ornamental in the Northeast yet is a native species from central and southeastern North America. Although these exotics are not distributed uniformly across the region, dispersing *P. cresphontes* have an uncanny ability to find host plants in complex environments, perhaps further enabling them to expand their range in urban and suburban areas as abiotic conditions allow.

Data from community science sources continue to grow as platforms become more popular, and can provide tremendous boons to researchers across disciplines (Bonney et al., 2009, 2014; Dickinson et al., 2010), including those interested in creating species distribution models (Kéry et al., 2010; Yu et al., 2010). There has been debate about the quality and veracity of community science data, but recent work has demonstrated that citizen science initiatives can reliably produce research quality data though it often has similar biases to professionally-gathered data (Kosmala et al., 2016). Here, we use community science data sources supplemented by data from museum collections to generate species distribution models using the well-established MaxEnt modeling framework (Phillips and Dudík, 2008; Elith et al., 2011; Phillips et al., 2017), and advocate for continued development and use of community science data and its pairing with museum collection data in developing species distribution models in ecology and conservation.

Though we focused mostly on the distributional changes of *P. cresphontes*, there were also surprisingly large range shifts in host plant species (**Figure 3**). In contrast to the straightforward northward expansion of *P. cresphontes*, the distributional changes in host plants were more complex and nuanced. *Z. americanum* and *P. trifoliata* have both shifted northward between the two time periods in slightly different patterns (**Figures 3**, **4**). While *P. trifoliata* appears to have shifted mostly northward (primarily gone from a large southern zone in T1), *Z. americanum* has undergone a northward and westward shift, and occupies areas that overlap with the range of *P. trifoliata* (**Figure 3**). The potential effects of this overlap on *P. cresphontes* (i.e., population dynamics, apparent competition, selection for oviposition behavior) are to our knowledge currently unknown, and warrants further examination in light of *P. cresphontes* westward expansion and previous work demonstrating significant within-population variation in oviposition behavior in *Papilio* (Thompson, 1988). Interestingly, mean temperature and annual temperature range (Bioclim variables 1 and 7) had the strongest impact in predicting the distribution of *Z. americanum* in T2, highlighting the impact that temperature may have in shaping and limiting current distribution. In contrast, the range of *Z. clava-herculis* appears to have contracted slightly in the southern United States. Compared to pre-2,000 distributions, available host plants to *P. cresphontes* are more widely distributed with greater overlap, but with notable gaps throughout portions of the southern United States. These complex distributional changes are likely driving part of the overall range shift northward for *P. cresphontes* (**Figure 1B**) and could also be potential drivers of speciation, and the evolution of specialization or host plant switching now and in the future (Descombes et al., 2016).

CONCLUSION

Multiple biotic interactions have evolved between insects and other species to create a wide variety of ecosystem services including herbivory and pollination (Losey and Vaughan, 2006). Anthropogenic climate change and habitat loss are creating a growing urgency for quantifying range size, understanding range boundaries, and assessing range shifts across insect species in order to preserve the integrity of future ecosystem function. Our work outlines the power of using increasingly abundant citizen science data, as well as the importance of including biotic interactions alongside environmental factors when developing analytical pipelines for biodiversity benchmarking studies. Future work should also incorporate climate change estimates into modeling efforts to project future distributions for both herbivores and host plants across many more butterfly and plant species. Incorporating both abiotic and biotic interactions in biodiversity benchmarking will provide a deeper, more nuanced understanding of temporal and spatial overlap among species, guiding conservation and management practices in a rapidly changing climate.

STATEMENT OF DATA ARCHIVING

Data and R scripts for all analyses are archived on Zenodo (https://doi.org/10.5281/zenodo.4476735).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Zenodo DOI: 10.5281/zenodo.4476735 (also in mansucript under Statement of Data Archiving).

AUTHOR CONTRIBUTIONS

ML, KM, KP, DB, and JK conducted the project conception. JW carried out analyses (data acquisition, model building, statistical analyses, and visualization) with initial explorations of data and analyses from NC and support from RH. JW led manuscript preparation, with initial pieces in place from NC and DB. All authors supported in editing, commenting and adding material to the manuscript.

FUNDING

RH was supported in part by the National Science Foundation under grant no. 1910118.

ACKNOWLEDGMENTS

We would like to thank our reviewers and editors and Jeff Oliver for helpful comments and suggestions on the

manuscript and analyses. Specifically, we would like to thank the Global Biodiversity Information Facility, the Maine Butterfly Atlas, the Maritime Canada Butterfly Atlas, the Massachusetts Butterfly Club, Butterflies and Moths of North America and eButterfly for the use of their data. We would also like to thank Scott Laurie and Ken-ichi Ueda at iNaturalist for their web platform, and all those relentless volunteers in various citizen science programs, past and present, who contributed these data for the greater good. This manuscript has been released as a pre-print at *bioRxiv*, (Wilson et al., 2019).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.579230/full#supplementary-material

Supplementary Figure 1 | Hyperparameter tuning and model evaluation of *P. cresphontes* distribution during the T1 period.

Supplementary Figure 2 | Hyperparameter tuning and model evaluation of *P. cresphontes* distribution during the T2 period.

Supplementary Figure 3 | Hyperparameter tuning and model evaluation of *Z. americanum* distribution during the T1 period.

Supplementary Figure 4 | Hyperparameter tuning and model evaluation of *Z. americanum* distribution during the T2 period.

Supplementary Figure 5 | Hyperparameter tuning and model evaluation of *Z. clava-herculis* distribution during the T1 period.

Supplementary Figure 6 | Hyperparameter tuning and model evaluation of *Z. clava-herculis* distribution during the T2 period.

Supplementary Figure 7 | Hyperparameter tuning and model evaluation of *P. trifoliata* distribution during the T1 period.

Supplementary Figure 8 | Hyperparameter tuning and model evaluation of *P. trifoliata* distribution during the T2 period.

Supplementary Table 1 | Bioclim variable definitions.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 08 April 2021
doi: 10.3389/fevo.2021.622603

Current and Forthcoming Approaches for Benchmarking Genetic and Genomic Diversity

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The current attrition of biodiversity extends beyond loss of species and unique populations to steady loss of a vast genomic diversity that remains largely undescribed. Yet the accelerating development of new techniques allows us to survey entire genomes ever faster and cheaper, to obtain robust samples from a diversity of sources including degraded DNA and residual DNA in the environment, and to address conservation efforts in new and innovative ways. Here we review recent studies that highlight the importance of carefully considering where to prioritize collection of genetic samples (e.g., organisms in rapidly changing landscapes or along edges of geographic ranges) and what samples to collect and archive (e.g., from individuals of little-known subspecies or populations, even of species not currently considered endangered). Those decisions will provide the sample infrastructure to detect the disappearance of certain genotypes or gene complexes, increases in inbreeding levels, and loss of genomic diversity as environmental conditions change. Obtaining samples from currently endangered, protected, and rare species can be particularly difficult, thus we also focus on studies that use new, non-invasive ways of obtaining genomic samples and analyzing them in these cases where other sampling options are highly constrained. Finally, biological collections archiving such samples face an inherent contradiction: their main goal is to preserve biological material in good shape so it can be used for scientific research for centuries to come, yet the technologies that can make use of such materials are advancing faster than collections can change their standardized practices. Thus, we also discuss current and potential new practices in biological collections that might bolster their usefulness for future biodiversity conservation research.

Keywords: benchmarking biodiversity, biological collections, genetic benchmarks, genomic diversity, geographic sampling, long-term change

OPEN ACCESS

Edited by:
David Jack Coates, Department of Biodiversity, Conservation and Attractions (DBCA), Australia

Reviewed by:
Alexandra Pavlova, Monash University, Australia
Mario Barbato, Catholic University of the Sacred Heart, Piacenza, Italy

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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *28 October 2020*
Accepted: *01 March 2021*
Published: *08 April 2021*

Citation:
García NC and Robinson WD (2021) Current and Forthcoming Approaches for Benchmarking Genetic and Genomic Diversity. Front. Ecol. Evol. 9:622603. doi: 10.3389/fevo.2021.622603

INTRODUCTION

Almost every form of human activity is directly or indirectly connected to the alteration or loss of natural habitats, leading experts to define this current era as the "Anthropocene" (Lewis and Maslin, 2015; Waters et al., 2016). Paleontological records show that we are currently undergoing a higher rate of species extinction than in any previous transition between geological eras

(Waters et al., 2016). During this time of rapid biological change, museum specimens collected decades or even centuries ago can be used as baselines to document more recent, human-related changes in species numbers and their distributions, in phenotypes and in genetic variability (Billerman and Walsh, 2019). The utility of the specimens for these purposes is often dictated by the type of specimens that were collected, particularly before molecular techniques were developed or when those techniques were still under development. We now have the ability to study genetic changes at vast scales, as we can produce enormous amounts of data across the entire genome for large numbers of individuals and species.

"Biodiversity" is a blanket concept relevant across different levels of biological organization. The genetic/genomic level is increasingly relevant in this time of planetary change, as a healthy pool of genetic diversity helps populations evolve and adapt (Templeton, 1994). When current technologies are combined with comprehensive genetic sampling it is possible not only to survey genetic information in populations at the entire genome scale, but also to explore the genetic basis of different adaptive or non-adaptive phenotypes. Understanding phenotype-genotype relationships is one of the longest-standing questions in biology in general (Orgogozo et al., 2015), and is also highly relevant for conservation efforts (Allendorf, 2017). Our rapidly improving abilities to search for candidate genes with adaptive value will be advantageous for the conservation and management of species in changing environments.

This is therefore a critical time to focus efforts and resources into creating and maintaining collections that allow us to benchmark current genetic and genomic diversity using current and future techniques, i.e., to establish genomic diversity "baselines." This will allow us to better understand what could be lost, and to predict what may be lost if we do not take conservation actions. Given that sample collection and preservation are costly, it is prudent to prioritize the collection and curation of certain samples over others. Here, we offer ideas that can guide sample archiving for genetic benchmarking of vertebrates. Any sample collection should, of course, be well-designed and follow statistical sampling protocols when relevant (Hayek and Buzas, 2010). Our list is not exhaustive, because questions of interest evolve over time, just as techniques do. The development of new techniques opens new frontiers of interesting questions, which may in turn reveal additional opportunities to leverage the benefits of genetic benchmarking (Lawson Handley, 2015; Allendorf, 2017).

Ideally, sample collection can serve dual purposes of answering questions of current interest while preserving samples as genetic benchmarks for future research. This duality of immediate and legacy benefits helps justify the substantial effort required to collect and curate samples. But many questions of implementation remain. Where should samples be collected? What populations or species should be targeted? We address these core issues in this review, and we suggest that effective genetic benchmarking could fall into at least eight broad topics of investigation: rare species; undescribed and/or cryptic species hotspots; naturally fragmented populations and isolated populations due to changing landscapes; species with continuous geographic ranges; habitat specialists vs. generalists, and range-restricted vs. widespread species; hybrid zones; newly colonizing and reintroduced populations; and changing landscapes. The ideal sources of genetic material are samples associated with vouchered individuals (Rocha et al., 2014). However, lethal collection can be impractical in certain situations, as is the case when working with endangered species, or when the research question requires dense sampling of different individuals from the same population. Therefore, we also review recent research using alternative means of obtaining genetic material, such as historical museum specimens. But since these older specimens were typically not collected for the purpose of obtaining genetic material, extracting it in sufficient quantities and qualities can be challenging (McCormack et al., 2017). We contemplate the analogous possibility that the samples we are acquiring today may be suboptimal for technologies that are developed in the future, such as those focusing on analysis of proteomic data. We conclude by analyzing steps to maximize the use of samples collected today by anticipating new techniques that will likely be broadly deployed in the near future. Given our personal backgrounds and expertise, we focus on samples from birds and other vertebrates, yet many of the topics and ideas we discuss are relevant to other kind of organisms.

UNDERSTANDING THE EXTENT OF GENETIC AND GENOMIC DIVERSITY LOSS IN A RAPIDLY CHANGING WORLD

Here we discuss a series of categories and situations where sample collection should be prioritized, with the intention of providing a basic overview of possible justifications for purposeful collection of genetic benchmark samples. These categories are, of course, incomplete. The details of sampling designs will ultimately depend on the specific research questions being addressed, the types of organisms, and the complex considerations of logistics, permissions, and time and expense tradeoffs that pertain to any genomic benchmarking situation.

Rare and Declining Species

A fundamental need for effective conservation and management of rare and declining species is accurate estimates of both census and effective population sizes, past and present (Frankham et al., 2014; Waples, 2016). The effective population size has been defined as the size that an *idealized* population (i.e., one in which random mating, equal sex ratio, discrete and non-overlapping generations, and random variation of reproductive success all occur) should have to be experiencing the same rate of genetic change as the natural population of interest (Caballero, 1994). In contrast, census population size is commonly noted to be the complete count of individuals in a population. The relationship between census and effective population sizes can be informative of demographic processes within the population (Pierson et al., 2018). They can both be genetically estimated (Luikart et al., 2010), though some caution should be taken, considering several factors and conditions that may influence these estimates (see Box 2 in Hoban et al., 2020). Genetically

derived estimates of census population sizes are increasing in number, as they are often cheaper and perhaps easier than traditional field-derived estimates, such as mark-recapture studies (Sabino-Marques et al., 2018; Bourgeois et al., 2019). Additionally, they could be perceived as being more ethical if they do not require direct interaction with individuals of endangered animals (Solberg et al., 2006; Arandjelovic and Vigilant, 2018). Estimates derived from hair or fecal samples, for example, may require 2–3 times more samples than the expected number of animals in the population to arrive at acceptably precise estimates; however, the most recent technological developments may make it possible to even obtain whole-genome level coverage from these "poor-quality" samples (Taylor et al., 2020). In addition, the same fecal samples may be analyzed with metabarcoding methods to discover information about diet and roles of animals in ecological networks (Barba et al., 2014; Barnes and Turner, 2016).

Genomic methods can inform us of recent changes in effective population size (Luikart et al., 2020) and also of historic changes, offering the potential to provide long-term perspectives on the effects of anthropogenic change on genomic diversity (Gattepaille et al., 2016; Oldeschulte et al., 2017; Bi et al., 2019). No vertebrate long-term monitoring programs date back more than a century, and most are only a few decades old. Therefore, these studies may be missing long-term cycles, as the declines of some species they detect may be occurring after abundances responded positively to widespread habitat alteration prior to the advent of such surveys (Hallman et al., 2020). The apparent declines, therefore, may not directly relate to immediate conservation problems, but fit within a longer-term pattern of abundance fluctuations. Therefore, there has been an increase in studies incorporating the perspective on longer-term changes in population sizes applied to conservation and management decision-making (Ardren and Kapuscinski, 2003; Brüniche-Olsen et al., 2018; Sato et al., 2020). Genetic techniques provide opportunities to understand historic context of temporal changes at much longer time scales. Past population bottlenecks can be detected as well as precipitous declines hundreds and thousands of generations ago (Ramakrishan et al., 2005; Oldeschulte et al., 2017), and currently, assesments of single nucleotide polymorphisms (SNPs) across the entire genome allow exploration of these questions even when very few samples are available (Brüniche-Olsen et al., 2018; Sato et al., 2020). The gray whale (*Eschrichtius robustus*), for example, already lost its Northern Atlantic populations (probably due to environmental change and/or by commercial whaling), and it is now only found in the Northern Pacific Ocean (Alter et al., 2015). The western gray whale population (near the coast of Asia) is estimated to be less than 200 individuals (Cooke, 2018). Brüniche-Olsen et al. (2018) used samples from two western and one eastern gray whales, to obtain whole-genome sequences at very deep coverages (between 27X and 30X) and were able to infer that these species show lower autosomal nucleotide diversity than most other marine mammals, but the decline of effective population size and the extent of inbreeding, is greater in the Western Pacific than in the Eastern Pacific populations. Interestingly, according to niche modeling, the authors also found future climate change could open new migratory routes that could allow gene flow and subsequent genetic recovery in the western population (Brüniche-Olsen et al., 2018).

Preservation of genetic samples for benchmarking purposes could allow retrospective analyses as techniques improve and allow more precise estimates of population size and the temporal scale over which such changes occurred (Bi et al., 2019). In addition, with rapidly improving techniques and ideas in the realm of de-extinction options, cryopreservation of gametes and other reproductive tissues, even for extant yet rare populations, adds potential insurance against complete population extinction (Saragusty et al., 2016; Corlett, 2017). Cryopreserved somatic tissues could serve this purpose as well: the San Diego Zoo recently announced (September 2020) the birth of a Przewalski's horse cloned from the tissue of a male preserved 40 years ago[1].

Dry storage may offer an interesting alternative, considering some of the disadvantages of cryopreservation, such as complex and expensive logistics, and the need of constant supply of energy and maintenance by trained personnel (Saragusty and Loi, 2019). Both gametes and somatic cells can also be preserved through different drying techniques, and while they may not remain viable after rehydration, DNA is preserved almost intact (Saragusty and Loi, 2019). Collection and preservation of genetic samples from rare species of conservation concern should be a priority.

Hotspots of Undescribed or Cryptic Species

One of the basic criteria for defining priority geographic areas for protection is the number of species an area harbors, in particular the number of endemic species, as these cannot be found elsewhere if such areas are damaged or lost (Giam et al., 2012; Ennen et al., 2020). An increasing number of studies are also starting to move the focus from species richness to phylogenetic diversity, a proxy that may represent aspects of biodiversity beyond that captured by species richness (Gumbs et al., 2020). In either case, the operational units used in these studies are usually already described species and do not consider estimates of undiscovered and undescribed species (Vieites et al., 2009). One of the biggest challenges in this respect is the fact that there is a large proportion of unknown biodiversity that will undergo extinction before being scientifically described ("crypto-extinctions," Giam et al., 2012). Undescribed species usually have very restricted ranges and are therefore particularly susceptible to extinction (Vieites et al., 2009).

Quantitative estimates of undescribed biodiversity are heterogeneous across taxa and geographic areas. In general, vertebrate taxonomy is much better known than that of any invertebrate taxa (Stork, 1993), and within vertebrates the estimated proportion of undescribed species is significantly higher for amphibians than for mammals (Giam et al., 2012). Proper species delimitation requires integrated assessment of genetics, phenotypic and behavioral data. However, such assessments at large scales to define priority areas can be impractical and time-intensive. Both promoting more geographically comprehensive sampling and the implementation

[1]https://reviverestore.org/projects/przewalskis-horse/

of genetic tools to analyze such samples become critical for estimating the amount of undescribed biodiversity.

The greatest numbers of undescribed species are probably found in tropical forests of the Neotropical, Afrotropical and Indomalayan regions (Giam et al., 2012). The Amazonia is the largest lowland rainforest in the world, probably harboring vast numbers of undescribed anurans (Fouquet et al., 2007; Funk et al., 2012; Ferrão et al., 2016). Vacher et al. (2020) used a platform for high-throughput sequencing for small DNA fragments (Illumina MiSeq, Quail et al., 2012) to assemble a database of short mitochondrial sequences from approximately 4,500 samples of amphibians. They combined these newly generated data with approximately 6600 accessions from the NCBI online repository and showed that the number of species could be almost twice the currently recognized for the area (876 species vs. 440 listed by the IUCN Red List). While the selection of a species concept could impact these estimates, a strength of this study is that authors started working with OTUs (Operational Taxonomic Units, solely based on genetic clustering), and then proceeded to contrast their results with recently described, valid species finding high levels of coincidence, and supporting the idea that their number of estimated new species was accurate.

This study focused on the Eastern Guiana Shield of Amazonia, where the authors recovered three bioregions altogether and determined that up to 82% of the OTUs found in this area are endemic. Interestingly, the Eastern Guiana Shield has been considered as a unique bioregion based mostly on avian species (Naka, 2011). This highlights that, while birds are among the best-known vertebrate groups in terms of taxonomy and distribution, they may not be a good proxy for other terrestrial groups given their much higher mobility.

Knowing the number of species and their abundances is an essential step in benchmarking our planet's biodiversity, but we lack this basic information for many of the most biodiverse areas of our planet. The study by Vacher et al. (2020) is just one example of how recently developed genetic and genomic techniques can help us tackle these problems, by detecting genetic variation across large spatial scales to reveal cryptic biodiversity.

Fragmented Populations Due to Natural and Anthropogenic Causes

While the description of new species is key for conservation efforts, there is general consensus that protecting the genetic diversity contained within species, in recognized subspecies or isolated populations, should also be a priority, even in widespread species still not considered as vulnerable or endangered (Thakur et al., 2018). New high-throughput sequencing techniques not only allow production of massive amounts of short DNA sequences from thousands of individuals, but they also make it possible to scan entire genomes to study more subtle patterns of genetic variation, such as those found in some fragmented populations.

The emperor penguin (*Aptenodytes forsteri*), for example, is considered "Near Threatened" by the IUCN because "is projected to undergo a moderately rapid population decrease as Antarctic sea ice begins to disappear within the next few decades owing to the effects of climate change." (BirdLife International, 2020). These birds form breeding colonies on sea ice at the majority of their known colony locations (Fretwell et al., 2012) and previous studies found conflicting results in terms of the population structure between the colonies, ranging from complete demographic isolation of breeding colonies (Barbraud and Weimerskirch, 2001) to species-wide panmixia (Cristofari et al., 2016). A better understanding of the current connectivity between these colonies will inform risk assessments and management plans, since these colonies are sensitive to fluctuations in the extent and duration of the sea ice (Trathan et al., 2011; Fretwell et al., 2014). A more recent study sampled eight colonies around Antarctica and used Restriction Site Associated (RAD) sequencing to obtain almost 4,600 genome-wide SNPs from 110 individuals (Younger et al., 2017). The colonies sampled were divided into at least four metapopulations, with the colonies in the Ross Sea being one of them. The world's largest breeding colonies of both emperor (Fretwell et al., 2012) and Adèlie (Lynch and LaRue, 2014) penguins are located in the Ross Sea, which is also the only region with a predicted stable or increasing population of emperor penguins (Jenouvrier et al., 2014). Genetic tools revealed that the assumption of all colonies being demographically connected was incorrect (Younger et al., 2017). Thus, an extensive sampling across fragmented populations combined with genome-wide sequencing techniques can also provide a benchmark for the degree to which apparently connected populations may be demographically isolated, influencing long-term population resilience.

Genetic change may occur especially quickly in landscapes where composition and configuration are being altered by humans (Athrey et al., 2012; Aleixo-Pais et al., 2019; Pelletier et al., 2019), leading to genetic structuration and loss of genetic diversity across populations (Amos et al., 2014; Schlaepfer et al., 2018). Therefore, sampling across landscapes that are changing due to anthropogenic causes should also receive particular attention in genetic benchmarking efforts. Effects of habitat isolation vary strongly among species, often most strongly affecting levels of connectivity and gene flow among spatially disjunct populations (Allendorf, 2017). The transformation of large portions of territories into agricultural, urban and industrial lands (and the development of traffic infrastructure to connect them), is one of the main causes of habitat loss, fragmentation and pollution (Gill and Williams, 1996; Rouget et al., 2003; Gallant et al., 2007; Rompré et al., 2008). Therefore, it is urgent to understand how they affect the genetic diversity of both endangered, declining and not yet endangered species (Bani et al., 2015; Lenhardt et al., 2017).

The pace at which genetic responses to recent anthropogenic isolation appear has been difficult to measure in the past. The availability of more sensitive assays being developed by advancing technology and the possibility of sequencing entire genomes may improve our abilities to detect small changes, including evidence of inbreeding, other small-population effects, and restricted dispersal across different forms of habitat barriers (Corlett, 2017; Kozakiewicz et al., 2019; Maigret et al., 2020). For example, based on a dataset of approximately 2000 SNPs

for the copperhead snake (*Agkistrodon contortrix*), Maigret et al. (2020) were able to detect evidence for subtle genetic structuring closely following the path of a highway that experienced high traffic volumes between 1920 to 1970 in eastern Kentucky, United States, but has now lost most traffic to a newly constructed alternative route. Their results add evidence revealing subtle impacts of anthropogenic fragmentation of landscapes, but also highlight the importance of temporal factors in landscape genetics, showing that temporal lags may impact our ability to detect the detrimental effects of land use change. The ability to detect subtle genetic structure across populations can help implement conservation management plans earlier, and therefore improve chances of successful protection of genetic diversity (Ralls et al., 2018).

Our ability to detect effects of land use change on population connectivity also depends on spatial scale of analysis. Kozakiewicz et al. (2019) sampled 271 bobcats (*Lynx rufus*) obtained from five populations in southern California, between Los Angeles and San Diego. Based on more than 13,000 SNP loci, landscape genomic effects were most frequently detected at the study–wide spatial scale, as predicted. However, negative effects of urban land cover on connectivity were also revealed when analyzing each population separately, with these negative effects being particularly strong in one population where stream habitat had been lost (Kozakiewicz et al., 2019). This is particularly interesting because knowing which landscape features can mitigate reduced connectivity in urban areas, such as riparian corridors in this case, can make the case for better conservation planning when continued urbanization is unavoidable.

Transecting Geographic Ranges

An unresolved question regards the patterns of genetic diversity across species' geographical ranges, even when distribution is or seems continuous. The long-standing but still controversial central-marginal hypothesis (CMH) suggests that genetic diversity should decline as one moves from the middle of the range, where species tend to be most common, to along the periphery, where the species' distribution becomes more fragmented, presumably because habitat conditions become less suitable and population sizes decline (Eckert et al., 2008; Pironon et al., 2017). Evidence for the hypothesis has been mixed (Sinai et al., 2019; Ntuli et al., 2020). The definition of "marginal," whether it be geographical, ecological or genetic, influences evaluations (Eckert et al., 2008; Pironon et al., 2017).

From the genetic perspective, the amount of data used may affect inference about diversity patterns across geographical space. In a metanalysis of almost 250 studies published between 1968 and 2014, the probability of detecting a center–marginal pattern was not related to the genetic methods used by the studies considered (Pironon et al., 2017). However, our abilities to produce genetic data have increased dramatically since 2014. The studies discussed in the previous section are only two of many examples of how larger datasets, both in terms of sampled individuals and SNPs scanned, can detect previously shallow but significant genetic differentiation, undetected with previously available methods (Chattopadhyay et al., 2016; Aguillon et al., 2018; Clucas et al., 2019; Nascimento et al., 2019). We anticipate even greater sensitivity to small but important genetic differences as technology improves, which will certainly be helpful for understanding the bigger and more challenging question of which processes led to these observed patterns.

A recent study used approximately 30,000 SNPs to test the main predictions of the CMH in the ongoing invasion of the cane toad (*Rhinella marina*) in Australia (Trumbo et al., 2016). The authors defined populations in the northern and eastern Australian coasts as the "core" populations and then collected samples along 6 continuous transects into interior Australia, where arid habitats and cold temperatures currently limit their distributions. Their results were mixed, with only some transects revealing what was predicted by the CMH, and highlighted the importance of environmental and climatic factors on shaping the patterns of variation in genomic diversity within continuous population ranges (Trumbo et al., 2016). Lower genetic diversity in edge populations could be one of the reasons such populations cannot evolve traits that would allow them to expand their ranges.

Most studies have assumed greater population sizes near range centers and not explicitly linked genetic data with population size estimates. Indeed, genetic diversity could simply be greater where abundance is greater (Hague and Routman, 2016; Allendorf, 2017), but alternative hypotheses suggest peripheral populations, if they are spatially distinct from central populations and experience limited gene flow, may be more genetically distinct because selective pressures in marginal environmental conditions are intense and differ from pressures in the center of the range. Peripheral populations may possess abilities to respond to changes and therefore may be key to a species' abilities to respond to climate change and other stressors (Lavergne et al., 2010). An interesting case is that of the redbelly yellowtail fusilier (*Caesio cuning*), an Indo-Pacific reef fish with a bipartite life history, first as pelagic larvae and later settling on coral reefs as juveniles. Adults depend on reef structure for protection at night, and do not migrate. Altogether, this suggests that long distance dispersal in this species requires a strong oceanographic conduit. Using approximately 2,500 SNPs generated from RAD sequencing, Ackiss et al. (2018) found evidence of reduced genetic diversity in the peripheral populations of this species in relation to the Kuroshio Current, a powerful western-boundary current in the Pacific Ocean. The authors found that sites closest to the periphery exhibited increased within-population relatedness and decreased effective population size, and potential for local adaptation. Further studies analyzing both genetic variability and population effective sizes could help us better understand differences in the genomes of central and peripheral populations. Therefore, thoughtful selection of species to sample along transects from the center of current ranges to margins, could help future scientists to understand what aspects of genomes have changed through time and to identify which locus or loci may have been under the strongest selection and favored success or failure to adapt and persist (Macdonald et al., 2017). In addition, more complete sampling across carefully chosen suites of species could better inform current basic questions about patterns of genetic diversity, such as the central-marginal hypothesis. We already have extensive evidence of shifts in geographic ranges associated with climate change for many

species (Shoo et al., 2006; Chen et al., 2011; Pecl et al., 2017) and also forecasting models that have generated predictions of how geographic ranges are expected to change (Lawler et al., 2009; Guisan et al., 2013). Such models could form the basis for selection of taxa for further genetic study, which in turn can better inform future models, as some inconsistencies arise between predictions and observations (Walsh E.S. et al., 2019). One possible cause of such inconsistencies is that traditional modeling does not account for the ability of some species to adapt to change, which recent models are trying to incorporate and improve predictive accuracy (Nadeau and Urban, 2019; Peterson et al., 2019).

Habitat Specialists Versus Generalists

Comparatively little is known about relationships between genetic diversity, niche breadth and adaptability of vertebrate populations to environmental change. Most studies to date have focused on plants (Sexton et al., 2017), though some studies in animals show similar trends: specialist species tend to have deeper and finer-scale phylogeographic structure and stronger demographic fluctuations when compared to closely related generalist species (Silva et al., 2017; Engelbrecht et al., 2019).

Extreme specialists offer interesting models to study the genetic basis of certain phenotypes, and to better understand how changing conditions can affect different species and their interactions. The saltmarsh (*Ammospiza caudacutus*) and Nelson's (*A. nelsoni*) sparrows are two recently diverged species (∼600,000 years; Rising and Avise, 1993) commonly considered marsh endemics. However, the saltmarsh sparrow is a narrow niche specialist, while the Nelson's sparrow can be found in a broader range of habitats (see Walsh J. et al., 2019 and references therein). Lower nesting success in tidal marshes have been reported for the Nelson's sparrow, suggesting adaptive differences between these species (Maxwell, 2018). Walsh J. et al. (2019) analyzed genome-wide divergence between these species and found several candidate genes to be linked to adaptation to tidal marsh environments, including genes linked to osmotic regulation, circadian rhythm, and plumage melanism.

We generally assume that habitat generalists should have advantages in dynamic environments, but what is the underlying genetic basis for niche breadth variation and ability to adapt to changing conditions? Genetic benchmarks establishing current levels of diversity, along with measurements of niche breadth generated from field observations and habitat analysis, would improve our understanding of the temporal plasticity in niche characteristics and how that plasticity associates with dynamics of the genome.

Range-Restricted Versus Widespread Species

The relationship between extent of geographic range and niche breadth is generally positive (Slatyer et al., 2013), resulting in some species having expansive geographic ranges, whereas others are restricted to small areas of geographical space. Given this relationship, one might predict greater resilience to environmental change in widespread species and higher levels of genetic diversity; while those restricted to disappearing habitats and already in low abundance require immediate attention.

Identification and analysis of relevant functional loci and how those vary across time and space could facilitate accurate assignment of populations to conservation-relevant risk categories. The willow flycatcher (*Empidonax trailli*) is an interesting case, because the entire species is widespread across North America, but one of its four subspecies, the southwestern willow flycatcher (*E. t. extimus*), is native to the Desert Southwest of the United States, and restricted to riparian woodlands along waterways (Sedgwick, 2020). These habitats probably provide a refuge against the extreme temperatures of these region (Chen et al., 1999); and with the loss of these habitats this subspecies has been undergoing a steady decline, with an estimate of no more than 500 breeding pairs in an assessment from 20 years ago (Sogge et al., 1997). Temperature increases due to climate change is expected to worsen the situation, which motivated Ruegg et al. (2018) to use genomic techniques to study local adaptation in the southwestern willow flycatcher to extreme temperatures and asses its vulnerability to future climate change. The authors assembled a reference genome for the species and then analyzed more than 100,000 SNPs from more than 150 individuals across 22 localities (Ruegg et al., 2018). By incorporating a series of climate variables to their analyses, they were able to identify a set of genes of potential importance for thermal regulation, and to assess the "genomic vulnerability" to predicted climate change of the different lineages within the willow flycatcher. As expected, the already endangered southwestern willow flycatcher will be the most vulnerable lineage to the anticipated increases in heat waves (Ruegg et al., 2018).

How dynamic are these relationships across time and across spatially variable environmental conditions? Are there underlying genomic differences across lineages that might reveal mechanisms allowing greater tolerance to environmental variability? Again, comprehensive sampling may be required to answer these questions, keeping in mind that current representation of organisms in museum and biological collections may be biased toward species with broader distributional ranges (Boakes et al., 2010; Vale and Jenkins, 2012).

Hybrid Zones

Hybrid zones, where the ranges of two lineages exchanging genes meet, inform us of the pace, pattern and process of speciation (Hewitt, 2001; Gompert et al., 2017). They may be relatively stable in location or displace (Buggs, 2007). The genomic dynamics of hybrid zones vary across lineages and certainly through time and space. Monitoring these movements generally requires genetic data, as phenotypes will rarely reflect many of the genomic dynamics very readily. What is more, certain areas of the genomes can be more resistant to gene flow than others (Wolf and Ellegren, 2017). Although current locations of many vertebrate hybrid zones are well-known, many are sparsely sampled, particularly where phenotypic signals are cryptic among poorly known taxa (Allendorf et al., 2001). Geographically structured samples collected to provide benchmark genetic data

can help us quantify the temporal and spatial patterns of gene flow, introgression, and inference as to the ancestral origins of genotypes by establishing additional historical bases for comparisons (Carling et al., 2011). Methods for investigating hybrid zones and current research directions have been recently summarized (Gompert et al., 2017). In addition, as landscape characteristics change along hybrid zones, patterns of gene exchange may also change.

Temperature shifts, for example, can have significant effects on species distributions and the dynamics of hybrid zones (Taylor et al., 2014; Ryan et al., 2018). Particularly susceptible to temperature changes are ectotherms, such as North American box turtles (*Terrapene* sp.). Martin et al. (2020) assembled a dataset of samples from more than 350 individuals across two well-studied zones of hybridization within this genus: one in South Eastern United States, between the woodland (*T. carolina carolina*), Gulf coast (*T. c. major*), three-toed (*T. carolina triunguis*) and Florida (*T. bauri*) box turtles, and the other one in Midwestern United States, between one subspecies of the ornate box turtle, *T. ornata ornata*, and *T. c. carolina* (see Martin et al., 2020 and references therein). Based on these replicated instances of contact at the intra and interspecific levels, the authors were also able to study the contrasting effects of selection and migration on hybridization. Analyzing more than 10,000 unlinked reference-mapped loci, they found that while in the midwestern contact area hybrids are present in low numbers and restricted to F1 generations only, the southeastern contact area included many backcrosses and F2 individuals, providing evidence of higher levels of introgression between the taxa. Interestingly, they found a set of specific loci with steep genomic clines between taxa, strongly correlated with temperature variables, but not with any precipitation or wind-related variables (Martin et al., 2020). The authors interpreted this as evidence of thermal gradients having a strong effect on introgression patterns and predicted that future changes in temperature could significantly affect the integrity of species boundaries within this genus of turtles (Martin et al., 2020).

A modern offshoot of natural hybrid zones involves the potential intermixing of genes from wild versus captively raised and released animals (Kitada, 2018). This is particularly true for economically important fish species such as salmon (Einum and Fleming, 1997; Clifford et al., 1998; Glover et al., 2017). Genetic benchmark samples of less economically important populations may provide similar opportunities to understand potential introgression and gene flow between native and released populations, especially given the extensive movement of organisms out of their native range by humans (Vitousek et al., 1997; Costello and Solow, 2003).

Newly Colonizing Populations and Reintroductions

Changes in allelic diversity that allow some populations of vertebrates to survive and thrive in new environments can be explored if samples are collected relatively soon after colonization is detected. Most colonizations and reintroductions fail, whether they are natural or anthropogenic in origin (Blackburn and Duncan, 2001). Reasons are many, but data on the specific roles that functional locus or loci may play in enhancing probability of success are sparse. Genetic benchmarks of newly arriving populations may reveal drivers of success or failure, and help identify situations where recolonization of eradicated invasive species is less likely (Purcell and Stockwell, 2015).

Describing the genetic characteristics of organisms utilized in translocation or reintroduction programs, and then resampling the population several generations later could help identify important information about who established successfully and who failed. Such information could improve efficiency in choice of individuals for future conservation translocation projects (Barba et al., 2010). The alpine ibex (*Capra ibex*) is a species of European wild goat that recovered from less than 100 individuals to approximately 50,000 in a century (Grossen et al., 2018). After genotyping more than 100,000 SNPs from 170 individuals, Grossen et al. (2018) could detect the footprints of their reintroduction strategy. Despite this encouraging recovery in numbers of individuals, the authors found that all reintroduced populations had lower levels of genetic diversity than the source population, both individually and combined. This could be related to the reintroduction plan used with this species, which consisted of initial reintroductions from captive breeding followed by secondary reintroductions from established populations. This is a nice example of how genetic benchmark samples can serve an immediate purpose of ensuring a sufficiently diverse sample of individuals is being introduced, perhaps reducing chances of inbreeding issues developing, and can also inform us of patterns of success when comparing the initial benchmark samples with future samples.

Genetic assessment of individuals prior to their use in reintroduction programs is also necessary to avoid including those that show signs of hybridization with other species. A particular problem arises when domesticated species are not reproductively isolated from their wild relatives, as is the case of several ungulate species in Europe (Iacolina et al., 2019). Genetic benchmarks could help avoid introgression of artificially selected variants into wild populations. The European mouflon (*Ovis aries musimon*), the wild relative of the domestic sheep, became extinct from mainland Europe by the Neolithic, but remnants from the first wave of sheep domestication that brought them to the Mediterranean isles of Corsica and Sardinia established feral populations (Chessa et al., 2009). Now considered "historically autochthonous," the species is protected by regional laws after almost becoming extinct due to intense hunting and erosion of its habitat (Somenzi et al., 2020). There has also been evidence of extensive hybridization with domestic sheep since Roman times, with confirmed adaptive introgression of loci related to immunity mechanisms from mouflon to sheep, but not the other way round (Barbato et al., 2017). Yet, as individuals are being relocated within the islands and to mainland Europe, it would be important for future management to know the ancestry of individuals. Somenzi et al. (2020) used a machine learning procedure to screen more than 50,000 SNPs from

non-admixed mouflons and sheep form Sardinia, and from confirmed admixed individuals, generating panels of reduced numbers of SNPs which could be used as Ancestry Informative Markers (AIMs). These AIMs represented fast, low-cost tools to identify the ancestry of a given individual, therefore the study provided both a tool to contribute to the conservation of this species, and also a new methodology that can be applied to the conservation of other wildlife in risk of hybridization with domestic species.

Species Benefiting From Anthropogenic Novelty

All habitats created by humans are novel on evolutionary time scales. Our agricultural habitats may mimic some natural habitats in structure, but plant species composition is shifted dramatically. This undoubtedly changes food resource availability as well as distribution and abundance of reproductive niches. Furthermore, novel chemicals are encountered as they are applied to control pests. Likewise, urban and suburban habitats in the modern era are home to sets of species that probably rarely co-existed in the past, including pathogens that may challenge immune function in novel ways.

While many organisms experience population fragmentation and loss of genetic diversity due to urbanization (see before), others actually may benefit from "urban facilitation" depending on their life history strategy. Many invasive species become dependent on resources provided by humans and therefore thrive in cities (Hulme-Beaman et al., 2016; Johnson and Munshi-South, 2017). Urbanization thus may facilitate dispersion and expansion of invasive species, which in turn may aggravate the threats against native biodiversity. Such is the case of feral pigeons (*Columba livia*) in eastern United States, which showed higher-than-expected gene flow under an isolation by distance model within large cities (Boston, Providence, New York City, Philadelphia, Baltimore, and Washington, DC; Carlen and Munshi-South, 2021). This means that the development of large human settlements and their increasing connectivity are facilitating the expansion of an invasive species, and the same is probably true for many other "human commensals" (Johnson and Munshi-South, 2017).

Samples collected to establish genetic benchmarks in time provide opportunities to understand the evolution of plasticity in response to human modification of habitats. What genetic mechanisms allow some species to be "winners," adjusting to and even thriving in human-altered landscapes, while other species decline and disappear?

We have proposed several broad subjects of study to be considered as priorities for future collection of genetic benchmark samples. We also recognize the importance and encourage the publication of Data Papers with appropriate and extensive metadata to alert future researchers to the existence of vertebrate genetic samples and facilitate their appropriate future use (Deck et al., 2017). Such tools and papers will be helpful for development of formal prioritization and assessment processes, similar to efforts to identify collection priorities aimed at preserving wild crop plant genetic diversity (Castañeda-Álvarez et al., 2016).

SURVEYING PAST AND CURRENT GENOMIC DIVERSITY FROM NON-INVASIVE AND HISTORICAL SAMPLES

Collection of samples with an associated voucher is scientifically the best the option by far because it maximizes the potential information obtainable from each specimen (Rocha et al., 2014; Webster, 2018). However, some species in urgent need of genetic analyses are already endangered and the only available sources of genetic material are non-invasive samples. The possibility to transition from "conservation genetics" to "conservation genomics" raised a potential issue, as some of the technologies collectively referred to as "next-generation sequencing" techniques required higher concentrations of DNA than are usually possible to obtain from non-invasive samples (Allendorf et al., 2010). But as technologies progressed and costs decreased, attempts to reduce sample size requirements have improved. For example, Russello et al. (2015) used non-invasive snares to collect hair samples from American pika *Ochotona princeps*; after extracting DNA they followed a nextRAD genotyping-by-sequencing method that allowed them to identify and genotype 3,803 high-confidence SNPs from 67 out of the 96 hair samples. The American pika is a small lagomorph with a discontinuous distribution along mountainous areas throughout western North America. Still considered of "least concern" by the IUCN Red List (Smith and Beever, 2016), it has become a focal species for studies of population dynamics and extinction risk due to climate change (Peacock and Smith, 1997; Stewart et al., 2015). Contrary to previous results across elevationally distributed sites in British Columbia, Canada (Henry et al., 2012), Russello et al. (2015) found that sites at the lower fringe of American pika distribution in North Cascades National Park exhibited significantly lower levels of gene diversity and heterozygote deficit likely due to inbreeding.

In many other cases, minimally invasive but non-lethal sampling is a possibility. As indicated earlier, collection of samples with an associated voucher is widely considered to be best practice, but we emphasize that not being able to associate a voucher with a genetic sample should not necessarily discourage collection of material for DNA extraction. Blood extraction from birds, for example, followed by release of the individual is a good option when it is impractical to euthanize individuals (**Figures 1A,B**). This is particularly the case for vertebrates other than mammals, whose red blood cells do possess a nucleus and are therefore a good DNA source. In such cases lacking traditional voucher specimens, the production of some type of "e-voucher" (i.e., electronical voucher, such as a photograph, Astrin et al., 2013) becomes a priority. Electronic vouchers such as photographs are often obtained in non-controlled environments where it may not be possible to follow the steps of high-quality protocols, such as using

FIGURE 1 | Collection of samples ranges from harvesting eDNA freely available in the environment to euthanizing animals. Here we show scientists obtaining some of the different types of samples from birds that can be used for genetic benchmarking. **(A)** Left, Pablo Lavinia taking a blood sample from a Hudson's black-tyrant (*Knipolegus hudsonii*, full body shot of the bird to the right). **(B)** Gustavo S. Cabanne banding a straight-billed reedhaunter (*Limnoctites rectirostris*) after taking a blood sample. **(C)** Gemma Clucas taking a tissue sample from a chinstrap penguin (*Pygoscelis antarcticus*) found dead on the South Sandwich Islands. **(D)** Dario Lijtmaer taking a toe-pad sample from a specimen of glaucous macaw (*Anodorhynchus glaucus),* a probably extinct species from South America, at the Museo Argentino de Ciencias Naturales. Photos by Ana Barreira [**(A)**, left], Pablo Lavinia [**(A)**, righ], Jazmín Safarin **(B)**, Jim Wilson **(C)** and Pablo Tubaro **(D)**.

standard lighting. However, including low-cost size and color references is a simple way of increasing the scientific value of the e-voucher. Also, depending on the taxa, more than one photograph may be required, providing details of different parts of the body containing diagnostic characters. Therefore, members of collecting expeditions should familiarize themselves with such requirements to produce proper e-vouchers, following published protocols or designing and archiving their own.

Biological collections often welcome salvaged specimens (i.e., those found dead, **Figure 1C**) as they can produce viable samples for DNA extraction, and often some type of voucher can be associated to them. Salvaged specimens can be found by scientists during collection expeditions, but many are found by citizens or recovered by authorities from illegal hunting, pet trade, etc. Barone et al. (2020) assessed the relevance of avian tissues obtained from donated and confiscated materials for the National Ultrafrozen Tissue Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia." They found that, out of a total of almost 10,300 avian tissues deposited at the collection, over one third come from donations and confiscated specimens, i.e., specimens found and donated by citizens or other institutions or confiscated by authorities from illegal trade. Interestingly, 18% of the species in the tissue collection are represented only by samples that come from donations and confiscated material (Barone et al., 2020).

Another alternative for assessment of genetic benchmarks when collection of new samples is limited are existing specimens in biological collections (**Figure 1D**). Museums have been accumulating biological collections for over two centuries, but for the largest proportion of that time there were no means and no intention to preserve tissue for genetic analysis (as the majority of the specimens in the world's biological collections were obtained before the discovery of the role of DNA). Yet these collections represent the only resources to study the genomic diversity of extinct species, or of species that can no longer be collected for other reasons. Methods to extract DNA from museum specimens have been under development for decades, with the challenge that historical DNA is degraded by fragmentation. The previously available techniques were designed to target specific regions of the genome to accurately copy long (typically 300–1,500 bp) stretches of DNA and require steps of DNA amplification which are very susceptible to contamination (Hykin et al., 2015; McCormack et al., 2017). The development of high-throughput sequencing brought new hope, as these typically produce sequences of as few as 50–150 nucleotides per read, making it easier to recover genetic data from old specimens, especially those preserved as dry preparations (Yeates et al., 2016; McCormack et al., 2017; Ruane and Austin, 2017; Pierson et al., 2020).

Historical specimens can complement fresh tissues to assemble geographically comprehensive datasets, which are critical to detect genetic structure within a clade and inform conservation plans. The red-tailed black-cockatoo *Calyptorhynchus banksii*, is an Australian species with five currently recognized subspecies based on body and bill size and

plumage color patterning (Ford, 1980). Despite being common in many locations, the rarity of some of its populations and subspecies combined with its wide geographic range makes the assemblage of a species-wide set of samples challenging. Ewart et al. (2019) used a restriction site-associated DNA marker approach (DArTseq^{TM}, Diversity Arrays Technology, Australia) to obtain thousands of SNPs from 113 fresh tissue samples and 29 (out of 47 included) toepads, with a mean age of 44 years, ranging from 5 to 123 years. Using two different pipelines to process and filter the data, the proprietary DArTsoft14 and STACKS (Catchen et al., 2011), the authors obtained 6,389 SNPs (with 4.19% missing data and 2,745 SNPs with 11.6% missing data), respectively. Interestingly, the authors also combined fresh and historical samples in different datasets to evaluate how the inclusion or not of the old samples affected their results. They found that, while most data sets showed the same patterns of differentiation among the five populations based on Fst values, both the bioinformatic pipeline and the samples included in SNP calling had a large effect on Fst values obtained, which lead to considerable variation in estimates of the scale of population differentiation (see Table 3 and Supplementary Tables 3, 4 in Ewart et al., 2019).

Historical DNA can also be extremely useful to evaluate changes in genetic diversity over time in highly endangered taxa. van der Valk et al. (2019) were able to infer genomic changes in the last century in the two subspecies of eastern gorillas, Grauer's (*Gorilla beringei graueri)* and mountain gorillas (*G. b. beringei)*. The authors first performed a low-depth sequencing with historical DNA extracted from teeth and dried soft-tissue samples of 59 gorilla specimens and followed a series of steps that ended in seven Grauer's and four mountain gorilla samples collected between 1910 and 1962 with adequate coverage (3.1–10.8 X) to assess genomic changes. The Grauer's gorilla has a historically higher genetic diversity than that of the mountain gorillas, which the authors attribute to a period of population growth and expansion between 5000 and 10000 years ago. However, in the short time period spanned by this study (about 100 years, corresponding to 4–5 gorilla generations), the Grauer's gorillas showed evidence of a significant reduction of genetic diversity as well as an increase in inbreeding and genetic load (van der Valk et al., 2019). Those results may be related to reduction of 80% of its population size down to less than 4,000 individuals in the last 20 years. The much smaller population of mountain gorillas, in contrast, has experienced little genomic change in the studied period. While they have also experienced demographic changes, their population size has remained small and more stable, decreasing from less than 1,000 individuals to approximately 250 between the 1950s and the 1980s, and then recovering to ∼450 in 2013. On the one hand, this study demonstrates the negative genomic consequences that severe population declines during the last century can have, even in a species with long generation times. On the other, it suggests that conservation efforts unable to prevent population declines but slow them instead can still be useful to alleviate the negative genomic impacts of population declines.

Recovering genetic data from old specimens preserved as dry preparations has become routine (Payne and Sorenson, 2010; Lim and Braun, 2016; McCormack et al., 2016; Tsai et al., 2020). But many extinct, endangered and secretive amphibians and non-avian reptiles have been preserved mostly as formalin-fixed and fluid-stored specimens, from which obtaining genetic data remains challenging or impossible (Simmons, 2014; Hykin et al., 2015; Pierson et al., 2020). The DNA contained in formalin-fixed specimens is highly degraded by fragmentation, base modification and cross-linkage within the DNA or between DNA and proteins. High-throughput sequencing technologies that required short DNA fragments, combined with bioinformatic tools that allow detection and filtering of low-quality sequences, may provide the opportunity to obtain genomic information from fluid-preserved specimens. Snakes are among the poorest studied clades within vertebrates for reasons inherent to their biology (their habits make them difficult to find and collect) and also related to their threatened status that limits collecting opportunities (Ruane and Austin, 2017). For many species the only potential source of genetic material are old specimens that were formalin fixed immediately after collection. Recently, Ruane and Austin (2017) presented a modified DNA extraction protocol which, combined with high-throughput sequencing, allowed them to recover DNA from 10 formalin-fixed and fluid preserved snakes for which there are little or no modern genetic materials available in public collections. Including specimens collected more than 100 years ago, the authors were able to sequence ultraconserved elements (2318 loci), which they combined with data from modern samples to build a phylogeny that included some enigmatic and poorly known species for the first time, such as the Günther's Mountain snake *Xylophis stenorhynchus* (endemic to the Western Ghats, India) and the Bougainville coral snake *Parapistocalamus hedigeri* (restricted to Bougainville Island in the North Solomon Islands group, Papua New Guinea). Both species have very restricted ranges and are categorized as "Deficient Data" by the IUCN Red List (Hamilton et al., 2013; Srinivasulu et al., 2013).

MOVING FORWARD: COLLECTING AND PRESERVING SAMPLES TODAY PLANNING FOR THE FUTURE

Most protocols for collecting and preserving samples are developed and adapted according to the needs of the technologies available at the moment of collection, yet their objective is to make the material useful to the generations to come. As we see scientists working hard to develop new tools and protocols to obtain DNA from material that was collected even before the DNA molecule was described, an important question arises: is there a way to reverse the story and collect and preserve biological material in a way that anticipates the technologies or applications of the future?

Documenting genetic diversity does not stop at finding variant sites in the genome, as that is only one of the dimensions of variation at the molecular level. In an interesting essay about the role of museums in the Anthropocene, Campagna and Campagna (2012) reflect that museums can only preserve "anatomical" structures and not "functions": the plant can be

preserved, but not its photosynthetic process. While strictly this is still true, we are already preserving the RNA (transcriptome) and proteins (proteome) which are results of the expression of the coding parts of the genome. We can get closer to preserving biological functions by establishing and preserving cell cultures (Wong et al., 2012; Yohe et al., 2019). Yet tissue collection nowadays is not routinely done in a way compatible with RNA and protein sequencing and analysis, mostly due to methodological difficulties and increased costs of preserving tissues in such manner (**Supplementary Material**). Corlett (2017) provides a comprehensive list of conservation problems with current or potential genomic solutions, and some of the most interesting ones include sequencing RNA to make better informed decisions when selecting populations for reintroduction, optimizing *ex situ* conservation efforts and assessing acclimation potential (see Table 1 in Corlett, 2017).

The Bat1K consortium is a pioneer in this respect, with the recent publication of a detailed methodological paper describing recommended best practices to collect tissues in manners compatible with all three "-omics" analyses (genomics, transcriptomic and proteomic) and even cell culture (Yohe et al., 2019). As the authors stated, the main motivation to develop this detailed protocol was *"to maximize the amount of potential molecular and morphological data for each bat and suggest* ***optimal ways to preserve tissues so they retain their value as new methods develop in the future.****"* Many bat species are endangered, and bats live longer and produce offspring at much lower rates than other similar-sized mammals such as rodents or shrews. This makes bat populations slow to recover and particularly susceptible to specimen collection. The specimens and tissues that can be obtained nowadays are limited and for some species may be last to ever be collected. Ensuring we maximize information from each specimen should be a priority.

CONCLUSION/FINAL REMARKS

Genetic or genomic samples can be used to establish benchmarks in time of organismal, evolutionary, and even population processes that may augment and surpass the value of "traditional" museum specimens. While biological collections have been sampling tissues for genetic analyses for decades, we consider it a priority that future collecting expeditions incorporate as one important objective the acquisition of samples that contribute to create these "baselines" of genomic diversity. Though not exhaustive, the series of criteria that we proposed here can help defining sampling priorities from now onward. Thoughtful collection of samples with respect to collection locations and populations of particular biological interest will not only serve this purpose but will certainly enhance the value of these samples over time.

We recommend future collecting efforts consider not only the criteria we discussed here in relation to *what* and *where* to collect but also *how*. Careful planning of which tissues will be extracted and how they will be preserved (immediately and long term) can help anticipating the inevitable improvement in biotechnology and analytical techniques and minimize the types of analyses for which samples will become "obsolete." In addition to the technical aspects of collecting and preserving a sample, its value is strongly attached to the information surrounding its acquisition. Therefore, collection and curation should adhere to best practices for linking samples with detailed metadata (Eymann et al., 2016). Lastly, while the original samples are irreplaceable and therefore worth effort and resources to be properly preserved, we also consider of great importance the long-term preservation and sharing of the knowledge derived from such samples (for example, by depositing sequences obtained in repositories such as GenBank) to develop complete and comprehensive benchmarks of the world's genetic and genomic diversity.

ETHICS STATEMENT

Written informed consent was obtained from the individuals appearing in **Figure 1** for the publication of any potentially identifiable images included in this article.

AUTHOR CONTRIBUTIONS

NG and WR conceived the idea, wrote the manuscript, and approved it for publication. Both authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We would like to thank Irby Lovette and the Lovette Lab members for their useful comments and suggestions during the preparation of this manuscript. We would also like to thank two reviewers for their valuable comments and suggestions that significantly improved this manuscript. NG was supported by the Imogene Postdoctoral Teaching Fellowship, Cornell Lab of Ornithology. WR was supported by the College of Agricultural Sciences and the Bob and Phyllis Mace Professorship.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.622603/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

PERSPECTIVE
published: 21 May 2021
doi: 10.3389/fevo.2021.645820

Benchmarking Taxonomic and Genetic Diversity After the Fact: Lessons Learned From the Catastrophic 2019–2020 Australian Bushfires

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OPEN ACCESS

Edited by:
W. Douglas Robinson, Oregon State University, United States

Reviewed by:
Natalia Cristina Garcia, Cornell Lab of Ornithology, United States
Tyler Andrew Hallman, Oregon State University, United States

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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *24 December 2020*
Accepted: *21 April 2021*
Published: *21 May 2021*

Citation:
Catullo RA, Schembri R, Tedeschi LG, Eldridge MDB, Joseph L and Moritz CC (2021) Benchmarking Taxonomic and Genetic Diversity After the Fact: Lessons Learned From the Catastrophic 2019–2020 Australian Bushfires. Front. Ecol. Evol. 9:645820. doi: 10.3389/fevo.2021.645820

Environmental catastrophes are increasing in frequency and severity under climate change, and they substantially impact biodiversity. Recovery actions after catastrophes depend on prior benchmarking of biodiversity and that in turn minimally requires critical assessment of taxonomy and species-level diversity. Long-term recovery of species also requires an understanding of within-species diversity. Australia's 2019–2020 bushfires were unprecedented in their extent and severity and impacted large portions of habitats that are not adapted to fire. Assessments of the fires' impacts on vertebrates identified 114 species that were a high priority for management. In response, we compiled explicit information on taxonomic diversity and genetic diversity within fire-impacted vertebrates to provide to government agencies undertaking rapid conservation assessments. Here we discuss what we learned from our effort to benchmark pre-fire taxonomic and genetic diversity after the event. We identified a significant number of candidate species (genetic units that may be undescribed species), particularly in frogs and mammals. Reptiles and mammals also had high levels of intraspecific genetic structure relevant to conservation management. The first challenge was making published genetic data fit for purpose because original publications often focussed on a different question and did not provide raw sequence read data. Gaining access to analytical files and compiling appropriate individual metadata was also time-consuming. For many species, significant unpublished data was held by researchers. Identifying which data existed was challenging. For both published and unpublished data, substantial sampling gaps prevented areas of a species' distribution being assigned to a conservation unit. Summarising sampling gaps across species revealed that many areas were poorly sampled across taxonomic groups. To resolve these issues and prepare responses to future catastrophes, we recommend that researchers embrace open data principles including providing detailed metadata. Governments need to invest in a skilled

taxonomic workforce to document and describe biodiversity before an event and to assess its impacts afterward. Natural history collections should also target increasing their DNA collections based on sampling gaps and revise their collection strategies to increasingly take population-scale DNA samples in order to document within-species genetic diversity.

Keywords: conservation unit, cryptic diversity, undescribed species, genetic composition, taxonomic impedement

INTRODUCTION

Environmental catastrophes are becoming more common and intense due to climatic changes, such as increases in the number of days of extreme fire weather and increases in intense rainfall events. They will magnify impacts on species already subject to other threatening processes such as habitat fragmentation and invasive species (Coumouand Rahmstorf, 2012; Harris et al., 2018). Catastrophic events often affect huge areas, and in some cases, almost all of a particular ecosystem or species' distribution (Lande, 1993). Actions to promote recovery from large-scale events require two particular forms of biodiversity information: what was impacted, and how well it can rebound. While the spatial scale of impact can often be estimated from distribution and trait data (Legge et al., 2020; Ward et al., 2020), recovery is more complex to forecast. Long-term recovery needs accurate taxonomic information, and should incorporate information on genetic diversity in order to ensure the long-term persistence of recovered species (reviewed in Pierson et al., 2016).

Identifying what was impacted can be challenging when the description of biodiversity is incomplete. The presence of taxonomically unrecognised species-level diversity when coupled with loss of geographic populations (e.g., Ceballos et al., 2020) can lead to cryptic extinction (Boessenkool et al., 2009; Travouillon et al., 2019; White et al., 2019). Unrecognised species diversity is more likely to occur in low vagility organisms distributed across topographically complex biomes that have undergone regular habitat expansion and contraction over glacial cycles, which enables allopatric speciation (e.g., Hewitt, 2000). In these circumstances, species might not differ morphologically (Singhal et al., 2018), especially if mate choice is based on non-morphological traits such as mating calls or pheromones. Where an event encompasses a region and a set of taxa for which these criteria apply, careful consideration of whether taxonomic recognition of species is complete and robust is needed for impact assessments and to prevent cryptic extinction.

Similarly, within-species diversity is important in assessing impacts and recovery from large-scale events. Genetic composition is considered an essential biodiversity variable (EBV)[1] for the management of biodiversity, and the maintenance and enhancement of genetic diversity is a key goal in the maintenance of global biodiversity (Convention on Biological Diversity, 2020). In particular, genetic EBVs focus on the maintenance of genetic variation within species and between populations, and the reduction of inbreeding to protect the long-term genetic health of biodiversity. A key genetic indicator suggested for inclusion as an EBV is the number of evolutionarily viable populations, i.e., with an effective population size (N_e) above 500 (Hoban et al., 2020).

Assessing how genetic diversity across a species' range has been impacted is more complex than species-level spatial analyses distribution data alone can describe (Hanson et al., 2020). Species often comprise discrete, definable genetic units having direct relevance to conservation management (Coates et al., 2018). These units range from populations within a meta-population, where each population is considered a Management Unit, to Evolutionarily Significant Units, which represent sets of distinct meta-populations that rarely admix with others (Moritz, 1994). These genetic units are also distinct in characteristics important to long term persistence, including their genetic diversity (e.g., heterozygosity, allelic richness) and meta-population connectivity. Long term recovery of species needs to prioritise the preservation of distinct conservation units while ensuring the genetic health of each independent unit.

Ideally, comprehensive information on the population genetic structure of species prior to a catastrophic event would enable assessments of immediate impact. They would also be a benchmark for comparisons after the event. These data would then enable genetically guided restoration and translocation. However, these data do not exist for the vast majority of species on earth. Where these data do exist, they may not be publicly available, or publicly databased sequences may be poorly georeferenced (Pope et al., 2015; Miraldo et al., 2016). Here we discuss our attempt to develop genetic benchmarking following the large-scale 2019–2020 bushfires in order to aid the recovery of vertebrate species in Australia.

THE AUSTRALIAN 2019–2020 BUSHFIRES

The Australian continent is often simplistically considered a bushfire-prone landscape in which the fauna and flora are well adapted to periodic, patchy fires. However, the bushfires of 2019–2020 were unprecedented in their extent (**Figure 1a**; Boer et al., 2020; Filkov et al., 2020) and severity, and burned some areas where fire is not part of ecosystem renewal, including rainforests (Ward et al., 2020; Godfree et al., 2021). Some of these wet forests burned for the first time in recorded history. The most fire-affected state, New South Wales, reported that more than 5.4 million hectares (∼14 M acres) burned, including

[1] https://geobon.org/ebvs/what-are-ebvs/

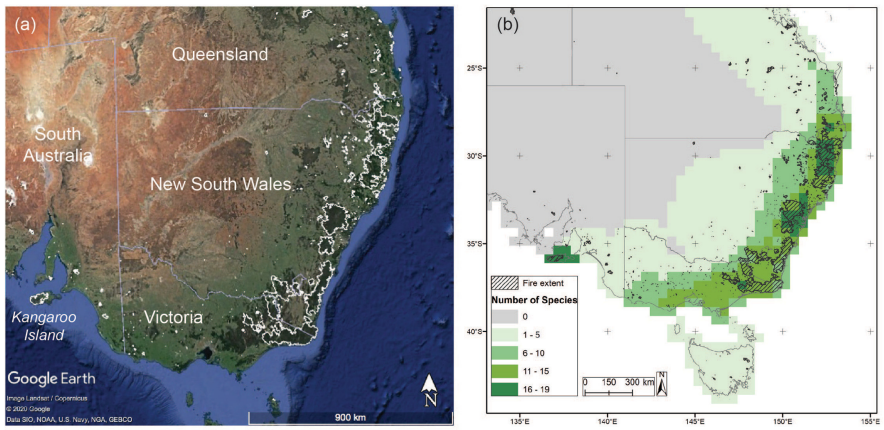

FIGURE 1 | The extent of the fires **(a)** is shown over a Google Earth satellite image. The fire extent (from the Commonwealth National Indicative Aggregated Fire Extent Dataset) is outlined in white, and dark green regions on the image represent pre-fire closed forests. Areas where conservation units could not be assigned across the 59 species assessed, due to a lack of genetic samples, are shown in panel **(b)** (from Catullo and Moritz, 2020). Colour indicates the number of species in a grid cell for which populations from that area could not be assigned to a conservation unit, with the fire extent shown in the polygons.

37% of the national park estate (State of NSW and Department of Planning, Industry and Environment, 2020). These fires significantly affected particular habitats, including more than 81% of the World Heritage listed Greater Blue Mountains, and 54% of the World Heritage listed Gondwana Rainforests. Burned regions include extensive forests along the Great Dividing Range of eastern Australia, which are highly differentiated from surrounding less mesic ecosystems (Byrne et al., 2011). Many of these wet forests were in decline prior to the fires due to a long history of habitat fragmentation and extensive drought (Lindenmayer et al., 2000; Bradshaw, 2012). As such they are home to many endemic and declining species. Conservative estimates suggest that over 1 billion mammals, birds, and reptiles were killed directly in the fires or in their aftermath, and that over 3 billion were impacted (van Eeden et al., 2020).

The forest habitats of the Great Dividing Range, and the associated coastal platform, form a series of highly structured biogeographical regions. Significant expansion and contraction of the forest habitat has been associated with Pleistocene glacial cycles (Byrne et al., 2011). A substantial number of studies identify high levels of inter- and intra-specific turnover at key biogeographic barriers along the range (reviewed in Chapple et al., 2011; Bryant and Krosch, 2016) and rainforest taxa show especially high local endemism (Rosauer et al., 2015). However, for many of the more latitudinally widespread species that are likely to be fire-impacted, spatial genetic studies have not been undertaken or have not yet been published. Therefore, it is difficult to accurately estimate the overall impact on species-level diversity, and genetic diversity within species.

In response to what has been widely considered a conservation emergency, the Commonwealth Department of Water and the Environment developed a draft framework to prioritise emergency action for all vertebrate species whose distributions were substantially bushfire-affected (Legge et al., 2020; Ward et al., 2020). This framework ranks species for conservation action based on the overlap of the species with fire, threat status prior to the fire, traits that influence during- and post-fire mortality, and the likelihood of species recovery. For example, mountain stream endemic frogs from the genus *Philoria* were ranked as a high priority due to a likely high fire impact (pre-fire conservation status of endangered, high level of fire overlap with the species' range, and potentially high mortality during and after fires) and low rate of recovery (long life spans, and low number of eggs per clutch). From this exercise, 114 species of vertebrate were rated as a high priority for urgent management intervention (Legge et al., 2020).

A key opportunity to advise on the recovery of bushfire-affected vertebrates arose as scientists within Australia were aware of taxonomic issues relevant to such species. These issues included "known unknowns" – taxonomic species known by experts to be composite in some way, either comprising multiple candidate species (i.e., one or more potential undescribed species within a currently described species) or major genetic subdivisions such as Management Units. Also, potentially over-split species or subspecies were accorded inappropriate attention. In addition, given the scale of the conservation effort being planned across the range of the fires, there is significant value in genetic health of species being incorporated in recovery plans, and in clearly defining conservation units for management and recovery teams. To this end, we organised experts across Australia to provide information from published and unpublished information to government agencies regarding:

- Taxonomic uncertainty, such as scientific support for subspecies,
- Undescribed species which needed inclusion in the formal assessment process,
- Conservation units within species where sufficient genetic data exists for this purpose, and
- Priority areas for further sample collection by species and region to better enable researchers to quantify the distribution of conservation units and species.

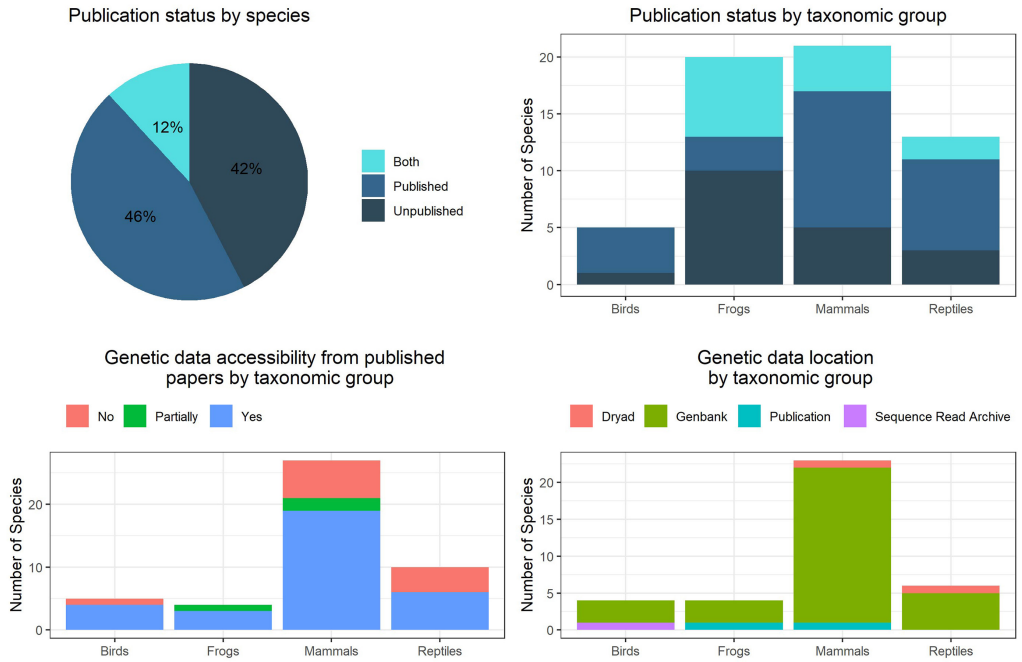

FIGURE 2 | Proportion of individual species assessments relying on published or unpublished genetic data, or a combination of both **(top left)**. Publication status of studies by taxonomic group **(top right)**. For published studies, a summary of whether the genetic data was publicly available **(bottom left)**, and if so, from where the data was available **(bottom right)**.

PRE-FIRE GENETIC BIODIVERSITY BENCHMARKING

Our primary goal was to provide individual assessments of the pre-fire taxonomy and spatial genetic diversity for each priority species, where genetic data exists. These assessments summarised the taxonomic status of species and subspecies, defined conservation units within each species, and reviewed available knowledge about genetic diversity within each conservation unit (Catullo and Moritz, 2020; now available at https://www.nespthreatenedspecies.edu.au/projects/genetic-assessment-of-priority-taxa-and-management-priorities & https://www.nespthreatenedspecies.edu.au/publications-and-tools/genetic-assessment-of-bushfire-impacted-vertebrate-species).

In the first step we worked with known taxon experts (see Acknowledgments section) to identify existing publications and unpublished data, and to identify additional researchers who may have relevant unpublished genetic data. Of the genetic data included in our assessments (**Figure 2**), 42% of species relied entirely on unpublished data held by participating researchers, and another 12% of species assessments relied on a combination of published and unpublished data. For all data sources, the evidence for multiple taxa (candidate species or ESUs) within described species was peer-reviewed at an expert workshop in April 2020 (Catullo and Moritz, 2020). Species were categorised as having sufficient data for initial assessment (N = 59), potential for multiple taxa but insufficient geographic sampling (N = 40), having no indication of strong spatial structure (N = 37), short-range endemics (N = 37), or insufficient data to form an opinion (N = 36). The relative proportion of unpublished data was highest for frogs and lowest for birds. Most of the datasets comprised mtDNA sequencing only (28%) or combined mtDNA and nuclear DNA markers (24%). High resolution nuclear DNA SNP screens were included in 28% of datasets, mostly frogs. Much of these data are included in ongoing assessments of taxonomic boundaries in morphologically cryptic species complexes; it can take many years to generate the necessary spatial sampling and complementary genetic and phenotypic data.

Published data when available often did not address questions specific to this project, i.e., they did not define conservation units and assess levels of genetic diversity. Accordingly, the benchmarking effort for this project required reinterpretation of existing data, and substantial one-on-one engagement with taxon experts. Researchers were unanimously willing to provide their unpublished data and be identified as experts in the individual assessments. Researchers acting as experts were also asked to identify the correct conservation units across each species based on a set of standardised definitions (see Catullo and Moritz, 2020), and to review and approve final individual species assessments.

This assessment process resulted in the delineation of a substantial number of conservation units, ranging from undescribed species though to Management Units (**Table 1**). Within our initial assessment of 59 taxonomic species the expert group identified 29 undescribed or candidate species among the fire-affected mammals, reptiles, and frogs. These assessments identified, proportionally, the highest number of

TABLE 1 | Change in number of conservation units identified by experts in the 59 species assessed, by taxonomic group (number of species assessed under current taxonomy).

	Undescribed species	Candidate species	Subspecies	Evolutionarily significant unit	Management unit
Frogs (18)	8	10	−2	7	>10
Reptiles (14)	1	3	−2	19	>18
Mammals (22)	2	5	1	40	>22
Birds (5)	0	0	−7	0	6

Values in each column identify the change in the number of each type of conservation unit from the number of species assessed. Negative values identify where previously described taxonomic units were not supported by genetic data. Conservation units are defined as known but undescribed species, clades that may represent undescribed species, subspecies, evolutionarily significant units, and management units. From Catullo and Moritz (2020).

undescribed species in frogs, followed by mammals, then reptiles. Evolutionarily significant genetic structure below the species level (i.e., confirmed or candidate ESUs) was identified in a substantial proportion of mammals and reptiles, with lesser values for amphibians. Overall, more birds were identified as being over-described at subspecies level, the genetic differentiation of many bird subspecies being comparable to the genetic differentiation between management units in other taxonomic groups.

There were significant biases between taxonomic groups, however. One bias was the number of species for which adequate spatial genetic data, published or unpublished, were available. The most genetic datasets were available for terrestrial mammals (N = 22) and the fewest for birds (N = 5) for which there are fewer tissue samples available (but often many skins suitable for DNA analysis).

Of the published studies used in our assessments all but a few had genetic data available online (**Figure 2**). The greatest proportion of non-downloadable data was seen in mammals and reptiles. These data were mostly available on Genbank[2], and newer studies had utilised genomics publishing data on Dryad[3] or the Sequence Read Archive[4]. Where data were published, there were still substantial challenges in accessing the required genetic data. Ideally, this would include georeferenced individuals and manipulatable results files such as phylogenetic trees. In most cases, georeferenced data on individuals is available, but often in a form that requires manual extraction from publications, and analytical outputs such as phylogenetic tree files are not available online.

Another significant challenge to this genetic benchmarking exercise was the high proportion of unpublished data that informs assessments of both taxonomic and intraspecific genetic diversity (**Figure 2**). While taxonomists have been very willing to provide unpublished data for the assessment of conservation units in target taxa, the primary challenge has been discovering whether unpublished genetic data already exists for a priority species, and which researcher has it. A necessary consequence of including unpublished data is that conservation assessments for such species were published in confidential appendices only available to agencies directly involved in the conservation effort, not to the general public as would be preferred.

During the process of spatially defining conservation units, there were significant areas where boundaries of conservation units relative to fire-impacted areas could not be defined due to geographic gaps in sampling. These areas of uncertainty are particularly important to our understanding of the confidence we can have in the conservation value of a geographic region. Therefore, we defined geographical areas of uncertainty for each species. Areas with a substantial number of undefined conservation units should be a priority for future field collection. To enable these collections, we highlighted areas without DNA samples by spatially summarising the number of species with uncertain conservation unit assignment in each grid square (**Figure 1b**). Secondly, we also provided lists of species that need collecting (i.e., were uncertain in their conservation unit designation) by protected area[5]. Through this approach we are able to both identify priority areas for future collections, and also identify the priority species for collection in each area.

DISCUSSION

The exercise of attempting to benchmark taxonomic and genetic diversity highlighted a number of important challenges to the effective and robust use of genetic diversity indicators (Hoban et al., 2020). However, the scale of previously unrecognised diversity we identified across the target species (**Table 1**) demonstrates the need for benchmarking genetic diversity for conservation and threatened species management. Identifying existing but unpublished datasets that were vital to describing diversity within many species was a significant challenge. Repurposing existing genetic datasets is not always straightforward, due to a combination of heterogenous data types, variable completeness of spatial sampling, and incomplete access to the necessary data such as georeferenced locations (Pope et al., 2015; Miraldo et al., 2016). We also learned that the benchmarking exercise is worthwhile: despite the challenges associated with identifying and summarising the data, Commonwealth and state governments are now actively incorporating this genetic information into their ongoing conservation assessments. However, in order to accomplish this effort in a time-frame useful to the conservation efforts, our effort required multiple staff working virtually full time for almost

[2]https://www.ncbi.nlm.nih.gov/genbank/

[3]https://datadryad.org/stash

[4]https://www.ncbi.nlm.nih.gov/sra

[5]https://www.nespthreatenedspecies.edu.au/publications-and-tools/genetic-assessment-of-bushfire-impacted-vertebrate-species-appendix

six months. We believe our work, and improving processes around data availability and conservation assessment, will assist in conservation funds being targeted toward the most at risk species, regardless of their current taxonomic status.

Unpublished and Missing Data

Despite Australia's rank as the world's fifth most megadiverse country (for comparison, the United States is the 16th; OECD, 2019), the taxonomic workforce has been in decline. This decline is explicitly linked to the prevalence of unpublished data and undescribed species, even in a well-populated region and in the well-studied vertebrates as considered here. Within Australia, the taxonomic workforce declined by 10 percent over the 25 years leading to 2017, during which time the Australian population increased by 40 percent (Taxonomy Decadal Plan Working Group, 2018). This lack of investment in a skilled workforce of sufficient size, relative to the scale of biodiversity, presents a significant roadblock to benchmarking biodiversity prior to and following a catastrophe. The level of undocumented biodiversity is likely significantly higher in groups such as invertebrates, plants, and fungi, all of which face potential cryptic extinction during a large-scale event. Investment from both state and Commonwealth governments in expanding and supporting a permanent taxonomic workforce would improve the ability to benchmark existing biodiversity, publish existing data, and to assess impacts following catastrophic events.

Australian natural history collections have been fundamental to any benchmarking of the genetic diversity of fire-impacted vertebrates. However, significant sampling gaps and low numbers of samples impeded genetic benchmarking for many species. While genotyping from vouchered specimens is becoming increasingly possible (Paplinska et al., 2011), the additional technical challenges mean these data need to be available at the time they are required. Museum collections can improve the ability to benchmark genetic diversity especially in rarer species through different but nonetheless complimentary strategies of voucher acquisition and acquisition of samples for DNA collection. Ideally, museums primarily collect DNA samples from vouchered specimens. While this is clearly best practice for vouchering, there is significant benefit to benchmarking genetic diversity through the collection of non-lethal replicates in populations (see García and Robinson, 2021). This is particularly true for threatened species for which extensive vouchering is not advisable (and for which genetic data may be most useful). We suggest collections aim to sample at least 10 spatially spread sites from each conservation unit within a species, ideally with 10 or more non-related samples per site to allow for estimates of within population diversity. Targeted sampling at areas where poor sampling exists across many species (**Figure 1b**) can make the collection exercise more cost effective. The effort to document the genetic diversity within species would also be supported by researchers providing subsamples of tissues to museums as standard practice.

Data Reusability

Key to enabling future biodiversity benchmarking is the availability genetic data under FAIR principles (findability, accessibility, interoperability, and reuse; Wilkinson et al., 2016), with appropriate and searchable metadata. Incomplete metadata in particular consistently frustrate efforts to quickly and bioinformatically assess diversity across geographic scales (Pope et al., 2015; Miraldo et al., 2016). Projects such as the Genomic Observatories Metadatabase (GEOME; Riginos et al., 2020) provide tools to improve uploading of effective sample metadata into DNA sequence repositories and we encourage their use. Useful analytical outputs such as phylogenetic tree files were generally not available, but should be provided through open data providers such as Dryad or TreeBase (Boettiger and Lang, 2012).

Published or unpublished, a significant issue for recent research utilizing single nucleotide polymorphisms (SNPs), is the accessibility and reusability of data sets. As a work around for the public dissemination of data, SNP data sets are often provided through supplementary materials or other file hosting sites. Where this is the case, it is often the final set of SNPs that are provided, not access to the raw sequence read data that would enable its repurposing for conservation questions. An additional issue with providing just SNPs is that different calling/filtering parameters generate inconsistent estimates of genetic diversity parameters (Wright et al., 2019), so limiting reusability. In our case, existing datasets were often designed to test for admixture between two candidate species. If these data were to be used to assess genetic diversity within each species, each species would be inferred to have a marked deficiency of heterozygotes (i.e., Wahlund effect; De Meeûs, 2018), leading to downstream issues when estimating diversity parameters. For these data to be reusable, the ability to recall SNPs data from more homogeneous sets of individuals is required.

Improving Assessments of Listing Status

Most jurisdictions assess the conservation status of species against the IUCN Red List criteria (IUCN, 2020). This coarse approach risks cryptic extinction of major components of genetic diversity and evolutionary heritage within species. In Australia, the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* is able to recognise "important populations." These are populations that are necessary for long-term survival and recovery of a species, and the designation is applied for reasons such as protecting key source populations, protecting populations that are necessary for maintaining genetic diversity, and protecting populations near the limit of the species' range that may contain unique adaptive diversity. Approaching assessments of conservation status using both the IUCN Red List criteria as well as under any regionally specific legislation can provide significant additional conservation benefits. In our initial assessment we assessed "important population" status for all ESUs or candidate species. For example, we recommended this designation for the source population of the endangered Broad-headed Snake (*Hoplocephalus bungaroides*) in heavily burnt Morton National Park, and to each ESU within the Platypus (*Ornithorhynchus anatinus*).

In summary, our recommendations to improve the ability of governments to create genetic benchmarking datasets that enable the recovery of species are:

1. Research scientists should embrace FAIR data principles (Wilkinson et al., 2016). In particular, this should include ensuring raw sequence data are available online in such a manner to enable their repurposing. These data should have accessible and integrated sample metadata including highly accurate georeferenced locality data. Publication of research should include providing analytical outputs such as phylogenetic tree files.
2. Analysis of conservation status should include assessments under the specific nation-based legislation that applies at and below the species level, in addition to species-level IUCN Red List assessments.
3. Governments should invest in a highly-skilled taxonomic workforce with the capability to describe biodiversity prior to the catastrophe, and to assist in monitoring and recovery following the event.
4. Museums and herbariums should work with ethics and scientific permitting agencies to revise collection missions to increase population-level DNA sampling as a key priority outcome, in order to document the genetic diversity of species through time.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

RC, RS, and LT compiled the data. RC and CM wrote the manuscript with editing by all other authors. All authors contributed to the article and approved the submitted version.

FUNDING

Funding for this project was provided by the National Environmental Science Programme Threatened Species Recovery Hub, Theme 8: Post bushfire recovery support, and the Centre for Biodiversity Analysis (https://biology.anu.edu.au/research/centres-units/centre-biodiversity-analysis).

ACKNOWLEDGMENTS

We thank all the contributing experts; without their time and data, our efforts would not have been possible. In no particular order: Andrew Baker (Queensland University of Technology), Anna MacDonald (Australian National University/BioPlatforms Australia), Sarah Legge (Australian National University/NESP), Emily Roycroft, Linda Neaves, Mitzy Pepper, Scott Keogh (Australian National University), Bernd Gruber, Peter Unmack (University of Canberra), Brian Arbogast (University of North Carolina Wilmington), Conrad Hoskin (James Cook University), Greta Frankham, Jodi Rowley, Matthew Lott (Australian Museum), Harry Hines (Queensland Parks and Wildlife Service), Jane Melville, Joanna Sumner, Kevin Rowe, Maggie Haines (Museum Victoria), Jason Bragg (Royal Botanic Gardens), Jennifer Pierson (ACT Parks & Wildlife), Jessica Worthington-Wilmer, Patrick Couper, Paul Oliver (Queensland Museum), Katherine Farquharson (University of Sydney), Kyle Armstrong, Steven Cooper (University of Adelaide), Michael Mahony (University of Newcastle), Sam Banks (Charles Darwin University), Simon Ferrier (CSIRO), Sophie Mazard (Bioplatforms Australia), and Steve Donnellan (South Australian Museum).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 05 October 2021
doi: 10.3389/fevo.2021.697511

Big Bird Plots: Benchmarking Neotropical Bird Communities to Address Questions in Ecology and Conservation in an Era of Rapid Change

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OPEN ACCESS

Edited by:
Umesh Srinivasan, Indian Institute of Science (IISc), India

Reviewed by:
Andres Garcia, National Autonomous University of Mexico, Mexico
William Ernest Magnusson, National Institute of Amazonian Research (INPA), Brazil

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Specialty section:
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *19 April 2021*
Accepted: *10 September 2021*
Published: *05 October 2021*

Citation:
Robinson WD, Errichetti D, Pollock HS, Martinez A, Stouffer PC, Shen F-Y and Blake JG (2021) Big Bird Plots: Benchmarking Neotropical Bird Communities to Address Questions in Ecology and Conservation in an Era of Rapid Change. Front. Ecol. Evol. 9:697511. doi: 10.3389/fevo.2021.697511

Extensive networks of large plots have the potential to transform knowledge of avian community dynamics through time and across geographical space. In the Neotropics, the global hotspot of avian diversity, only six 100-ha plots, all located in lowland forests of Amazonia, the Guianan shield and Panama, have been inventoried sufficiently. We review the most important lessons learned about Neotropical forest bird communities from those big bird plots and explore opportunities for creating a more extensive network of additional plots to address questions in ecology and conservation, following the model of the existing ForestGEO network of tree plots. Scholarly impact of the big bird plot papers has been extensive, with the papers accumulating nearly 1,500 citations, particularly on topics of tropical ecology, avian conservation, and community organization. Comparisons of results from the plot surveys show no single methodological scheme works effectively for surveying abundances of all bird species at all sites; multiple approaches have been utilized and must be employed in the future. On the existing plots, abundance patterns varied substantially between the South American plots and the Central American one, suggesting different community structuring mechanisms are at work and that additional sampling across geographic space is needed. Total bird abundance in Panama, dominated by small insectivores, was double that of Amazonia and the Guianan plateau, which were dominated by large granivores and frugivores. The most common species in Panama were three times more abundant than those in Amazonia, whereas overall richness was 1.5 times greater in Amazonia. Despite these differences in community structure, other basic information, including uncertainty in population density estimates, has yet to be quantified. Results from existing plots may inform drivers of differences in community structure and create baselines for detection of long-term regional changes in bird abundances, but supplementation of the small number of plots is needed to increase generalizability

of results and reveal the texture of geographic variation. We propose fruitful avenues of future research based on our current synthesis of the big bird plots. Collaborating with the large network of ForestGEO tree plots could be one approach to improve understanding of linkages between plant and bird diversity. Careful quantification of bird survey effort, recording of exact locations of survey routes or stations, and archiving detailed metadata will greatly enhance the value of benchmark data for future repeat surveys of the existing plots and initial surveys of newly established plots.

Keywords: Neotropical forests, bird community structure, biogeography, foraging guild, species richness, bird survey methods

INTRODUCTION

The Neotropics is the global hotspot of avian diversity (Harvey et al., 2020), but its bird communities generally lack sufficient benchmark measurements of bird species composition and abundance. Establishment of such baselines provides the historical context required for effective evaluation of change through time and is becoming increasingly important in a rapidly changing world (Magurran et al., 2010; McNellie et al., 2020). Patterns of abundance across species are a fundamental characteristic of any community, and the absence of such data presents a formidable impediment to advancement of ecological knowledge. The combination of abundance data and species traits also permits analysis of functional diversity and evaluation of ecosystem services (Şekercioğlu, 2012). The need for foundational data from the world's richest biomes can be remedied with solutions for the methodological and logistical challenges associated with thorough characterization of its rich and diverse communities.

Methodological challenges have included basic aspects of species identification in diverse and poorly studied communities, a lack of standardized counting protocols, and issues accounting for interspecific variation in detectability, which can impede accurate estimation of species' abundances (Banks-Leite et al., 2014). Reasonably complete community inventories require reliable information on taxonomy, which has greatly improved in the last few decades despite a growing appreciation that many cryptic species continue to lack formal recognition (Bickford et al., 2007). Current information, however, is certainly sufficient to produce reliable identifications for most species. Our abilities to identify birds by their vocalizations have greatly improved recently with the proliferation of freely available online sound recording archives (https://www.xeno-canto.org/ and eBird.org). Many Neotropical bird species inhabit structurally complex habitats such as forests, and are more often heard than seen, so detection from vocal cues is critical during community surveys (Celis-Murillo et al., 2012). Furthermore, even when vocalizations are learned exhaustively, low vocalization rates and population densities may hamper detectability of certain species and impede accurate abundance estimation (Anderson et al., 2015). As new techniques for handling sampling difficulties continue to be developed, we anticipate wider application of modern approaches to estimate detectability and generate reliable estimates of population density (e.g., Gómez et al., 2018).

Logistical issues have also hindered the establishment of benchmark tropical bird surveys. Recognition that most species, at least in tropical forests, occur at very low abundances, established a logistical hurdle because population densities of most species could only reasonably be estimated in large (suggested to be at least 100-ha) plots (Terborgh et al., 1990). Surveys of large plots require substantial and consistent sampling effort that may not be feasible in tropical countries where obtaining funding for long-term research has been challenging (Barlow et al., 2018). In addition, most sites selected for such large plots have been placed in relatively low-elevation and accessible terrain to facilitate plot access. No large plots yet exist in mountainous terrain, which may bias perspectives and limit generalizations about the structure of Neotropical bird communities.

The current focus by tropical ornithologists on conservation of at-risk landscapes has probably also contributed to the lack of benchmark surveys in undisturbed forest (Robinson et al., 2004). Neotropical forests, for example, have been experiencing some of the fastest rates of deforestation and habitat conversion worldwide (Kim et al., 2015; Giam, 2017). Thus, most previous research has focused on conservation-relevant topics such as the impacts of fragmentation and habitat loss on Neotropical forest bird communities (Boyle and Sigel, 2015; Stouffer, 2020). Yet, the establishment of reliable biodiversity benchmarks from intact forests has provided the opportunity to quantify long-term, gradual changes in bird communities from relatively undisturbed areas. For example, even in remote Tiputini, Ecuador, and Brazilian Amazonia, evidence for subtle changes in the avifauna over the last two decades, perhaps driven by climate change, has been found (Blake and Loiselle, 2015; Stouffer et al., 2021).

Here, we review the history of the six big bird plots surveyed in the Neotropics (**Table 1**). We summarize the motivations for establishment of these plots, the primary methodological approaches used to inventory Neotropical forest bird communities, as well as the key ecological questions addressed from the resultant datasets. After comparing the major findings from the plots, we briefly evaluate and interpret differences in community structure and organization. Because scope of inference has been limited by the small number of big plots in exclusively lowland forests, we suggest that a more extensive network of plots is both needed and feasible. As a guide to the potential of creating a larger network of bird plots, we look to the extensive conceptual advances created through the global

TABLE 1 | Summary of the ∼100-ha plots used to characterize Neotropical bird communities, including their locations, basic environmental characteristics, geographic coordinates, range of years sampled, and species richness.

Source	Plot	Location	Coordinates	Elevation (m)	Annual rainfall (mm)	Years sampled	Species richness
Terborgh et al., 1990	Cocha Cashu	Manú National Park, Peru	11° 54′ S, 71° 18′ W	400	2,000	1982, 2018	319
Robinson et al., 2000b	Limbo	Soberanía National Park, Panama	9° 9′ N, 79° 44′ W	35–80	2,600	1994–1996	252
Thiollay, 1994	Nouragues	French Guiana	4° 5′ N, 52° 41′ W	40–400	3,500	1990–1992	248
Blake and Loiselle, 2015, 2016	Tiputini (2 plots)	Yasuní Biosphere Reserve, Ecuador	0° 37′ S, 76° 10′ W	190–270	3,100	2001–2020	318, 320 (349 total)
Johnson et al., 2011	Manaus	Brazil	2° 30′ S, 60° 0′W	110–150	2,714	2006–2008	228

network of tree plots established by the ForestGEO group (Davies et al., 2021). We then connect the potential gain in knowledge from expanding the network of big bird plots with the challenges of establishing such a network. Finally, we identify and address challenges that need to be addressed if we are to reap potential benefits of an expanded network of tropical bird plots.

HISTORY OF NEOTROPICAL BIRD PLOTS

The history of large plots used to study Neotropical bird communities is paradoxically both long and brief, being initiated in the 1980s but including only a few big plots surveyed, mostly, for short, discrete time periods (**Table 1**). Perhaps inspired by the North American network of plots surveyed by volunteers (Johnston, 1990), mostly via spot-mapping, Karr recognized the absence of similar data from the Neotropics (Karr, 1971). He established a 2-ha plot in Soberanía National Park, Panama, in the late 1960s, apparently creating the first effort to go beyond simple listing of species occurrences to estimation of abundances. Observing that, at least in Amazonia, most bird species were rare and had very large home-ranges, Terborgh et al. (1990) concluded that any plot-based effort aimed at estimating population densities of most tropical forest bird species would have to be much larger. Consequently, they established the 97-ha Cocha Cashu plot near the Manú River in southeastern Peru. Completing surveys in the late 1980s, they discovered that, indeed, most species occurred at densities of less than 2.5 pairs/100 ha and had home ranges many times larger than the 2-ha Soberanía plot, validating the need for large plots. Even from surveys of nearly 100 ha, the Cocha Cashu plot was still too small to allow reliable estimates for a quarter of the species detected (Terborgh et al., 1990), but nearly all of those were assumed to be quite rare or transients. Furthermore, they realized that multiple methods were needed to survey Neotropical birds given their tremendous ecological and life-history diversity, often low rates and amplitudes of vocalizations, and variation in detectability (Robinson et al., 2000b, 2018).

The successful accomplishment of a nearly complete inventory of species alongside population density estimates allowed assessment of guild structure and biomass distribution. Linking the basic measurements of richness and abundance with species traits opened the door to test for differences in functional diversity and community structure between tropical and temperate bird communities. The idea that 100 ha is a useful plot size in which to study tropical forest birds gained traction and led to the eventual establishment of analogous plots in French Guiana (1986), Panama (1994), Ecuador (2001), and Brazil (2008) to improve and evaluate the generalizability of the Cocha Cashu results, as well as address other particular questions of interest detailed in the individual plot histories below.

Cocha Cashu, Peru —

Situated alongside an oxbow lake for which it is named, Cocha Cashu Biological Station was established in 1968 within the meander belt of the Manú River in southeastern Peru. Consequently, the vegetation throughout the meander belt represents a mix of successional stands reflecting the periodic flooding dynamics of the river. The 97-ha plot itself is in a mature floodplain forest that remains above the normal annual flooding level of the river. The plot is surrounded by the oxbow lake, fig swamps, and is contiguous to a larger tract of mature floodplain forest. An extensive trail system became the basis for a study grid system which encompasses the original 97-ha plot and has expanded to approximately 10 km^2. The trail system was converted into a grid by mapping trail markers every 25 m along all trails.

At the time of the establishment of the plot, ornithological knowledge was on the cusp of allowing reliable identification of most Neotropical bird species by sight and sound. The development of these identification criteria, which had long been a cornerstone of studies in temperate systems, allowed for the incorporation of survey techniques such as spot-mapping to evaluate the organization and structure of rich Neotropical bird communities. Multiple techniques were employed to overcome the challenges of counting the variety of species with diverse life histories and behaviors, which included traditional methods such as spot-mapping, and also employed mist-netting and visual counts to estimate the density of flocking and colonial species. Even some radio-tracking was implemented to map territories of woodcreepers. Based upon survey work of forest surrounding the Cocha Cashu plot in which just three additional species were detected, Terborgh et al. (1990) estimated that the plot

encompassed 99% of the bird community. It should be noted, however, that although the plot was estimated to be large enough to detect 99% of the bird community, this was still not adequate for estimating population density for a quarter of the species.

In sum, the original survey included >15,000 spot-map registrations which yielded an average of ∼15 detections per territory. The use of mist-nets on the plot was employed on 6 separate mist net lines and captured ∼755 different birds representing ∼80 species. This was supplemented by focal studies on Yellow-rumped Caciques (*Cacicus cela*) which yielded greater than 1,000 color-banded individuals (Robinson, 1986), and 1,173 individual group counts of monospecific flocks of parrots. Combining all these different survey efforts yielded 1,920 birds/100 ha. In total, 319 species were detected and density estimates were derived for 245 species that held territories on the plot. Most species tended to be somewhat rare, with a median of 2.5 pairs/100 ha. Among foraging guilds, insectivores had the highest species richness but accounted for the smallest fraction of overall biomass (18%), whereas granivores comprised the largest portion of biomass (43%) of any given foraging guild. Thus, as the first exhaustive survey of a Neotropical bird community at a relatively large spatial scale, the results showed the exceptional richness, domination of the community rank-abundance curve by a long tail of rare species, concentration of most avian biomass among granivores and frugivores, and a large diversity of lifestyles requiring implementation of multiple survey techniques.

Nouragues, French Guiana —

The Nouragues field station, established in 1986, was located in continuous primary lowland rainforest of the Eastern Amazonian interior of French Guiana. The Nouragues bird plot was located deep in forest interior to facilitate bird population and behavioral studies, as well as to provide a comparison of community structure with the Cocha Cashu plot. Standardized surveying commenced at the 100-ha plot in 1990 (Thiollay, 1994). The plot's location was designed to avoid habitat-edge effects, but an effort to describe internal spatial heterogeneity created by treefall gaps was included. On a central, 24-ha subplot, 78 treefall gaps that accounted for 3.7% of the area were mapped in 1991, one of the primary years of the bird counting work. Spot-mapping and mist-netting were the primary approaches used to generate population density estimates during 8 months of surveys between February 1990 and November 1992. The plot was systematically searched from dawn to dusk to map residents using a grid of 1-ha quadrats. A subset of 1-ha quadrats were scrutinized in nine 33 m × 33 m sub-squares to facilitate territory mapping and estimation of typical territory sizes. Mist-netting took place within a core 24-ha quadrat in September 1991 and March and October 1992. Twenty 12-m mist nets were placed along seven 400-m parallel trails and operated for 5880 net-hours resulting in 694 marked individuals. Data were supplemented by an earlier study (5 years of effort) where 1,353 mist-netted birds of 99 species were followed and spot-mapped. Supplementary surveys such as the use of acoustic playback, nest locations, nocturnal surveys, radio-telemetry, color-band resighting and canopy observations were conducted to quantify community composition and home-range sizes.

Collectively, J. L. Dujardin and M. Jullien mapped 6,658 individuals comprised of 248 resident species on the 100-ha plot (Thiollay, 1994). 220 species had a density of ≥0.50 pair/100 ha and 157 species had a density of ≥1 pair/100-ha. The estimated density on the entire plot was 829 pairs/100 ha (about 1,658 individuals), quite similar to the 1,910 individuals/100 ha at Cocha Cashu (Terborgh et al., 1990). According to Thiollay (1994), 441 resident species occurred in the 80,000 km^2 of the interior primary forest of French Guiana. Of these, more than half (58%) had an average density under one pair/100 ha. The species found at Nouragues included 77% (234/305) of the most forest-restricted species of the region. The community was dominated by two species that had 28 and 38 pairs on the plot and ten "subdominant" species with 14–18 pairs in the 100 ha. Those dozen species made up 31% of the estimated bird density on the plot. Defining rare species as those with <2 pairs/100 ha, Thiollay (1994) suggested the Nouragues plot had 137 rare species representing every guild, family, and ecological niche of the region; 64 were species considered to occur at densities <1 pair/100 ha. By definitions used in Terborgh et al. (1990), 37% of the 248 species were rare (≤1 pair/100 ha), including species of large body mass with large home ranges as well as patchily distributed species. Distributions on the Nouragues plot were often patchy, also a characteristic of birds in Cocha Cashu, Peru (Terborgh et al., 1990). Thiollay (1994) hypothesized that the local absences from the Nouragues plot of some common French Guianan species could be attributed simply to patchiness of distributions. Overall, the community structure was remarkably similar to that at Cocha Cashu.

Limbo, Panama —

The Limbo plot (104 ha), located in Soberanía National Park on the isthmus of central Panama, was established in 1994 following scouting for site placement in 1993 (Robinson et al., 2000b). The site had a prior history of mist-netting studies extending back to the 1960s. Those efforts were centered at the Limbo Hunt Club, a former camp site and small cabin used by military personnel during hunting trips along the Pipeline Road (Karr, 1971). A 2-ha plot was established at the site in 1968 by Karr (1971), following the success of similarly sized plots in measuring bird community structure across North America (e.g., Short, 1979). The community was studied via mist-netting and mapping observations of color-marked birds to estimate territory sizes and densities. Richness on the original 2-ha Limbo plot was 140 resident species (Karr, 1971). Extrapolation of abundances to 100-ha suggested a combined density of 1,800 pairs. The richness and density values per 100 ha were eventually questioned when the Cocha Cashu plot, nearly 50 times larger (97 ha), discovered much different community structure, with many more rare species and lower maximum abundances. Importantly, territory sizes of most Amazonian species averaged 4.5 ha, more than twice the size of the original Limbo plot (Terborgh et al., 1990). The Cocha Cashu results, therefore, suggested the Limbo 2-ha plot may have been too small to adequately characterize the community if species were too wide-ranging or if their distributions were too patchy, as reported from French Guiana (Thiollay, 1994), to be sampled by a small plot. Because of

these concerns and how they might influence interpretation of geographical differences in community structure, a larger plot was established at Limbo (104 ha) to facilitate fairer comparisons with South American plots. In addition to increasing plot size, a wider variety of survey methods, focused on extensive spot-mapping of territories, was also used. Thus, the primary questions posed by Robinson et al. (2000b) were: (1) Are results from the original 2-ha Limbo plot influenced by the small spatial scale of the initial study? (2) Is organization of the Panama bird community fundamentally different from communities in Amazonia and, if so, why?

The 104-ha plot was positioned to overlap the original 2-ha plot. It encompassed tropical moist forest ranging in age from 250 or more years old (largely on and near the original plot) to less than 15 years old along the margins of Pipeline Road and at large treefall gaps created by windstorms. Aside from the one-lane unimproved road passing through it, the plot was more than 3.5 km from edges at the eastern park boundary and Gatun Lake. Its permanently flowing Rio Limbo, a small creek arcing through the northern and eastern portions of the plot, attracted some riparian species. Otherwise, most of plot was *terra firme* forest in a relatively flat basin.

The Limbo plot was constructed of north-south transects spaced at 100-m intervals and three east-west transects, one each at the northern and southern plot boundaries and one through the plot center. Surveys were conducted largely by one observer (WDR) who walked each transect, stopping every 100 m to conduct 8-min point counts where the direction and distance of each bird was noted. The points were surveyed 8 times each in 1994–1996 to facilitate density measurements and also to establish an easily repeatable survey method to allow future re-surveys. Extensive mist-netting to color-mark birds was conducted largely by two observers at 8 different routes across the plot. Point count data and mapping of color-marked bird observations were used to spot-map, along with discoveries of nests for a subset of species (Robinson et al., 2000a,c), then clusters were identified to enumerate density. For wide-ranging and patchily distributed species, transects and encounter rates were used to estimate densities.

Altogether, more than 30,000 bird observations were mapped on the 104-ha Limbo plot, representing 252 species. Of those, 152 resident species were present in densities of at least 0.5 pairs/100 ha. The original 2-ha plot results suggested that the Panama and Amazonian bird communities were structured quite differently, a conclusion confirmed by the 104-ha plot results. The Limbo community was dominated by eight very common species whose abundances were several times greater than the most common species at the Cocha Cashu and Nouragues plots.

Tiputini, Ecuador —

Tiputini Biological Station (TBS) is located adjacent to Yasuní National Park and within Yasuní Biosphere Reserve, one of the most diverse regions of the world (Bass et al., 2010). Although TBS itself is only ∼700 ha, it is surrounded by extensive areas of intact forest. An initial site visit was made to Tiputini Biodiversity Station (TBS) in 2000 to determine the feasibility of establishing long-term study plots. The goal was to find a site that was diverse, surrounded by large expanses of forest, and reasonably accessible, where we could establish two replicate 100-ha plots, something that had not been done at other sites. Two plots provide the opportunity to compare community composition at a relatively small spatial scale. The station and nearby areas are dominated by *terra firme* forest and also include *várzea* forest, palm swamps, and various successional habitats.

Two 100-ha plots (*ca* 1 km × 1 km each) were established in *terra firme* forest during 2001. Plots are approximately 1.5 km apart at the closest point. Both plots are gridded (100 × 200-m grid lines) and marked with 1.5-m PVC tubes. The Harpia plot is characterized by more dissected upland forest while the Puma plot is flatter overall. Both areas experience partial inundation when small streams back up as the Tiputini River rises; Puma has more areas that fill with persistent standing water during the rainy season. Dominant vegetation on both plots is tall, evergreen forest.

We had several different objectives for long-term studies. At the most basic level, we wanted to investigate spatial patterns of species distribution at within-and-between-plot scales and how those patterns might change over time. By employing capture-mark-recapture analyses we wanted to be able to estimate survival rates for a diverse set of species (Blake and Loiselle, 2008, 2013). A second major focus was on behavior, spatial distribution, genetic relatedness, and seed dispersal by manakins (family Pipridae; e.g., Loiselle et al., 2007; Blendinger et al., 2008, 2011; Ryder et al., 2009), with the majority of the studies based on the two plots.

We took two approaches to sample the birds: mist nets and visual observations. Mist nets (12 × 2.6 m, 36-mm mesh) set at ground level were arranged in a series of eight sets of 12 nets on each plot (96 sites per plot). Each set of 12 nets formed a rectangle (100 × 200 m) with nets set ∼50 m apart; maximum distance between nets on a plot was approximately 920 m. Each set of nets was run for one day (∼0600–1200 h) in January (peak of breeding for many species) and one day in March (late breeding season for many species), starting in March 2001. March samples have largely been discontinued during the last few years, primarily because heavy rains precluded netting. Captured birds were identified and most were banded with aluminum leg bands. Most manakins were also marked with color bands. Blood samples were collected from many species during the first years of the project and were used to investigate occurrence of blood parasites (e.g., Svensson-Coelho et al., 2014).

To obtain a more complete picture of the community, JGB has conducted transect observations that started in 2005. Locations of all birds seen or heard were noted on scale maps of each plot while walking along transects; unknown songs were recorded for later identification. Approximately 1–1.4 km of transects were covered during a morning with starting positions distributed throughout the plots. Each plot took ∼12–13 days to cover. Transects covered the entire plot but were not repeated during a given sample, precluding the more traditional spot-map analyses. From 2013 to 2017, passive acoustic monitors were deployed on both plots to evaluate their effectiveness as a sampling tool (Blake, 2021). Results from the recordings were compared to transect counts conducted during the same periods. Finally, a long-term

camera trapping project has provided additional information on some of the larger, terrestrial species, such as tinamous (family Tinamidae) and trumpeters (family Psophidae; Blake et al., 2017).

To date (2001–2020), 180 species have been captured in mist nets (16,883 captures) on the two plots combined, including 160 on Harpia (8307 captures) and 155 on Puma (8576 captures). A total of 336 species have been detected during transect counts, with 302 on Harpia (34,249 records) and 304 on Puma (29,719 records). With both captures and observations combined, 320 species have been recorded on Harpia, 318 on Puma, and 349 with both plots combined. Patterns of species accumulation, capture rates, and observation rates are generally similar on both plots. Family and overall species composition also are similar on the two plots; the same species are the most dominant on both plots (Blake, 2007; Blake and Loiselle, 2009). Despite the overall similarities, many species showed differences in abundance (captures or observations) across the plots with differences often related to small-scale variation in topography and habitat between the plots. For example, Screaming Piha (*Lipaugus vociferans*) has a large lek on the Harpia plot but is absent from Puma. In contrast, Wire-tailed Manakin (*Pipra filicauda*) is common on Puma but has no leks on Harpia. Comparisons with other sites (e.g., Cocha Cashu, French Guiana, Panama) showed stronger similarities between Cocha Cashu than with other sites, particularly Panama (Blake and Loiselle, 2009).

Plots at Tiputini have been surveyed annually since 2001, something that has not been done at the other big plots. As such, we have a more detailed picture of temporal fluctuations in bird numbers than at the other plots. Capture rates and observations fluctuated over the first years – 2001–2009 – but showed no consistent pattern of change. Since 2009, however, captures and observations have declined by approximately 50% on both plots, in the absence of any change in local anthropogenic influences (e.g., logging, hunting) (Blake and Loiselle, 2015, 2016). Declines have occurred across many guilds and species, with insectivores particularly hard-hit. Some terrestrial insectivores (e.g., *Formicarius* antthrushes, *Sclerurus* leaftossers) largely disappeared for some years, although numbers have increased slightly in the last few years. Initial declines coincided with some strong La Niña events, which bring periods of heavy rains. Despite a lack of such strong events in the last few years, numbers of captures and observations have remained low.

Manaus, Brazil—

Research with birds has been an integral part of the Biological Dynamics of Forest Fragments Project (BDFFP) since the project began in 1979 (Stouffer, 2020). The original intent of the BDFFP was to follow biological processes, including bird communities, in fragments of rainforest that would be isolated by agricultural development in an area of undisturbed rainforest about 80 km north of Manaus, Amazonas, Brazil (Bierregaard et al., 2001). During the 1980s, research fragments were isolated, and the BDFFP established a control site of continuous forest at KM 41 of the ZF3 road, at the far eastern end of the BDFFP, connected to nearly unbroken forest to the north and east. With the gradual construction of what became a permanent camp and some 500 ha of continuous forest gridded with 100 m × 100 m trails, KM41 provided a research base for projects in unfragmented forest. At a larger spatial scale, deforestation mostly ceased by the late 1980s, with the overall BDFFP area remaining ∼90% forested to the present, maintaining unbroken connection to vast rainforest with minimal disturbance (Rutt et al., 2019).

Several projects at KM41 set the stage for the big-plot survey. Standardized mist-netting, beginning in the late 1980s, contributed to the BDFFP bird capture database. We described mixed-species flock structure and space use (Develey and Stouffer, 2001). We spot-mapped and radio-tagged terrestrial insectivores over 10 years (Stouffer, 2007). In the days before GPS could provide accurate locational information under the rainforest canopy, the accuracy of spatial data for these studies hinged on the 100 m × 100 m trail grid. During work at KM41 and elsewhere at the BDFFP we identified criteria for aging birds and assembled audio recordings of almost all bird species, eventually producing two important resources for the big plot survey (Naka et al., 2008; Johnson and Wolfe, 2017).

In 2008, we had accumulated the necessary experience and resources to conduct a community-wide survey at the scale of a 100-ha plot, with metrics that could be compared to the handful of other studies that estimated space use and absolute abundance (Terborgh et al., 1990; Thiollay, 1994; Robinson et al., 2000b). Our objectives were to determine: species richness; density, biomass, and territory size of individual species; and the distribution of these metrics by foraging guild (Johnson et al., 2011).

The plot was within the gridded network at KM41, in an area that we considered representative *terra firme* forest. As is typical at the BDFFP, the topography included steep ascents and descents along old stream beds. One small stream passed through the plot. We sampled with mist nets from June to November 2008, with spot-mapping concentrated in June and July. Our methods for spot-mapping and interpretation of spot-map data were generally concordant with Terborgh et al. (1990). We improved abundance estimates for three of the most common species in the mist-net sample by estimating density of adults. Color-banded birds and radio-tagged birds helped us discover territory boundaries. Overall, we recorded 5,581 unique observations (sometimes of multiple individuals, as of a pair together or a monospecific flock) of 228 species and found community structure to be very similar to that at Cocha Cashu. Lower species richness compared to other Amazonian plots can be mostly attributed to the homogeneity of the surrounding landscape, which precluded the wandering birds that contribute to species richness without being part of the core resident avifauna (Johnson et al., 2011).

RESULTS FROM EXISTING PLOTS AND THEIR INFLUENCE ON THE LITERATURE

Community-Level Comparisons

The common theme across the Neotropical big plot studies was to characterize the species composition in the bird communities as fully as possible, and for most of them, to estimate plot-level densities of as many species as possible

using a variety of methods (**Supplementary Table 1**). We explored differences in community structure across the plots by compiling data on a suite of ecological traits including categorical (taxonomic family, migratory tendency and diet) and continuous variables (average body mass, population density; **Supplementary Table 2**). Population densities and body masses were unavailable for the Tiputini plots. To identify major differences across the plots in our categorical variables, we visually compared cross-plot differences using bar charts. For continuous variables, we compared distributions across the plots using pairwise Kolmogorov-Smirnov tests. We used a Bonferroni correction to generate adjusted p-values that corrected for multiple pairwise comparisons among the four plots. We used logarithmic transformations to normalize distributions of body masses and densities.

Across the six plots, fifty-four bird families were recorded. Cocha Cashu, Peru, had the highest species richness, followed by the two Tiputini plots, Nouragues, Limbo and Manaus (**Table 1**). Migrant species were minor components of most communities except for Limbo (**Figure 1**). Overall, plots were quite similar with respect to the number of species per family, with a few notable exceptions (**Supplementary Figure 1**). At Cocha Cashu, species richness of certain families was disproportionately high, including ovenbirds (Furnariidae), antbirds (Thamnophilidae), parrots (Psittacidae), and tinamous (Tinamidae). Conversely, at the only Central American plot (Limbo, Panama), species richness was disproportionately low for ovenbirds, antbirds and parrots. With respect to diet, Cocha Cashu had more granivores and insectivores than the other plots, whereas Panama had the most omnivorous species (**Supplementary Table 1**).

Population density distributions varied substantially among the plots (**Figure 2**). Limbo supported an average density of 7 versus 2.5 pairs/100 ha at the Amazonian plots. Total number of birds was estimated to be 3,230/100 ha at Limbo, nearly twice the densities in Amazonia. Yet, the total bird biomass was quite similar. Distributions of body masses across size categories were also remarkably similar across the four plots for which body mass data were available and pairwise comparisons did not reveal any significant differences among the plots (Kolmogorov-Smirnov tests, all $p > 0.05$; **Supplementary Figure 2**). Body size distributions favored many small insectivores at Limbo, versus larger granivores and frugivores in Amazonia. Collectively, the South American sites were most similar to one another while the structure of the Panama community exhibited lower richness and higher number of migratory species. Differences in species identities across the plots are obvious as are differences in richness and abundance values, suggesting different mechanisms structuring the communities. But, similarities in body mass distributions, in particular, suggest that some mechanisms determining community structure are common to all the plots. The biogeographic and human history of the Panamanian isthmus might be responsible for the differences between Limbo and the Amazonian plots owing to disturbances associated with changing sea levels and human alteration of forest structure and, to some extent, hunting of large-bodied birds. However, the small number of plots limits our ability to evaluate hypotheses rigorously. Furthermore, the degree to which potential errors in estimating densities may influence determination of community-level abundance patterns cannot yet be explored because of inherent limitations in survey methodologies.

Influence in the Scientific Literature

To explore influence of the plot studies on the scientific literature, we examined citation patterns by compiling all sources citing the bird plot papers from Web of Science and utilizing Vos Viewer (van Eck and Waltman, 2010) to conduct network analyses of keywords. For each citation, we also extracted metadata including the year of publication, whether or not the citing source collected data from the same geographic location (binary yes vs. no), the range of latitudes at which the study was conducted (temperate, tropical, subtropical, global), the research theme (biogeography, conservation, ecology, evolution, natural history), and the taxa studied.

Through the end of 2020, the six big bird plot papers had been cited 1,443 times (Google Scholar, accessed 18 Feb 2021). Overall, citations peaked between 2005 and 2010. Citations of the Cocha Cashu paper, in particular, have declined in recent years. The primary influences of the papers included topics focused on ornithology, ecological, biogeographical, and conservation-related themes. The papers have had extensive influence on specific topics ranging from forest fragmentation to community dynamics and from community organization to frugivory and dispersal (**Figure 3**). Studies citing the papers have focused almost exclusively on birds and been largely conducted at tropical latitudes. Most of the citing research took place at different study sites than the original studies. Importantly, most plots have also spawned numerous additional studies conducted on the plots, illustrating the value of providing logistical access for researchers and advancing scientific knowledge more broadly. Aside from the present collection of big bird plots being developed at least partly as a consequence of establishment of the Cocha Cashu plot, we did not see evidence that publication of the plot results has spawned production of additional tropical bird plots despite the strong citation rates. In addition, while most of the big bird plot publications interpret results in the context of findings from the other plots, no effort to analyze in depth the results across plots or coordinate development of additional plots has emerged. Therefore, looking to other plot networks, such as the global collection of tree plots, could provide a useful guide.

FOREST GLOBAL EARTH OBSERVATORY (FORESTGEO) FOREST DYNAMICS PLOTS AS A MODEL

A network of tropical tree plots began with the Hubbell-Foster 50-ha plot on Barro Colorado Island, Panama, in 1980. Motivations for creation of that plot were to generate horizontal life table data on tropical trees, quantify change so as to test competing equilibrium and non-equilibrium hypotheses for the creation and maintenance of species diversity, and to map individual trees to facilitate additional research by collaborators (Anderson-Teixeira et al., 2015; Davies et al., 2021). Within the

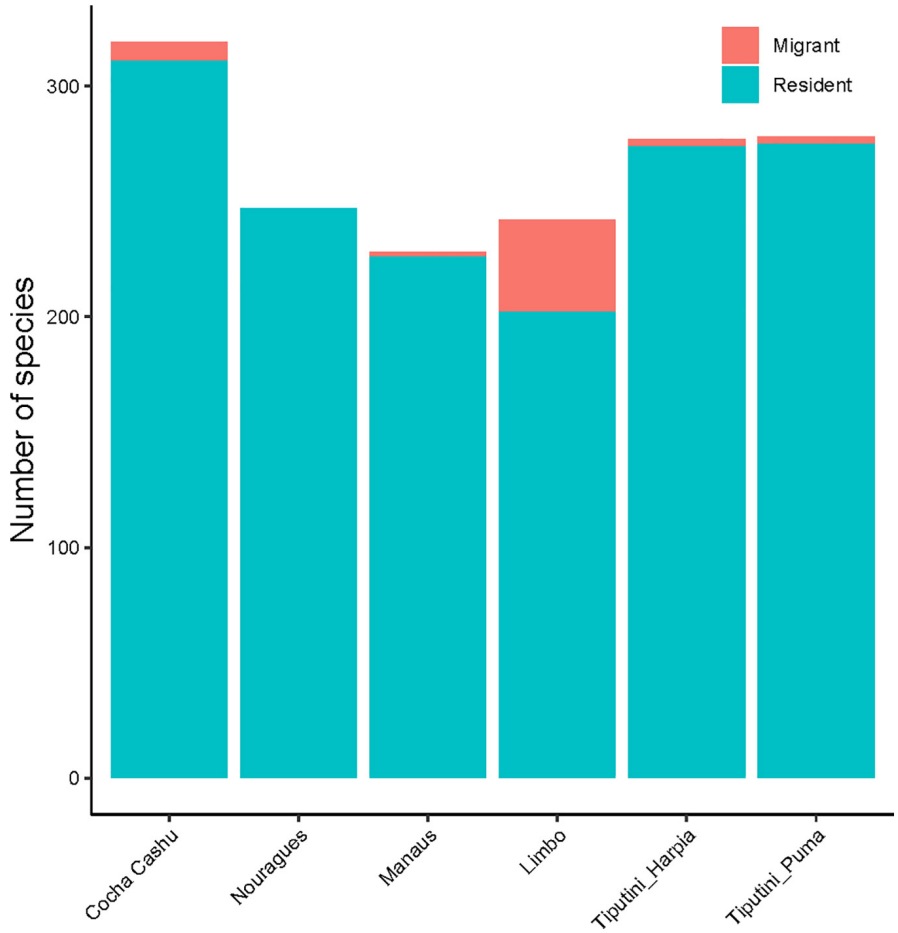

FIGURE 1 | Species richness of migrants and residents at six of the big Neotropical bird plots.

next decade similar plots were established in Malaysia and India. Today, the network includes 71 plots ranging in size from 4 to 120 ha (average of 26 ha) with every individual of more than 7 million trees mapped and identified to species or morphospecies. Two simple, but logistically challenging, innovations of Hubbell and Foster catalyzed rapid change in our understanding of tropical forest dynamics: (1) establishing plots sufficiently large to sample most of the tree species present and to contain reasonable sample sizes of most rare species; and (2) including individual trees down to 1 cm diameter at breast height. Previous tree plot studies were normally 1 ha or less and measured only trees 10 cm dbh or greater, missing a substantial portion of species diversity present and thereby inhibiting abilities to provide robust tests of hypotheses about species diversity and to quantify tree life table parameters.

The collaborative efforts of hundreds of scientists in the ForestGEO network have produced nearly 1,400 scientific publications, including dozens in top-tiered journals that have addressed fundamental ideas in ecology, global change, evolution, and forest management (Ashton et al., 1999; Losos and Leigh, 2004). To facilitate collaboration, standardization of protocols for data collection and management, metadata, data sharing agreements, as well as analyses through freely sharing R code, was implemented. These steps moved scientific knowledge gained from a collection of case studies, examining data from one plot at a time, to examination of emergent patterns across many sites. By creating the network, more generalizable conclusion about the drivers of species coexistence, creation and maintenance of species diversity, and factors influencing ecological function of tropical forests were within reach. The incorporation of data from many plots led to creation of new ideas such as neutral theory (Hubbell, 2001), the relative impacts of density-dependence on recruitment (Comita et al., 2010), the influence of pathogens on diversity and increasingly robust assessments of the intermediate disturbance hypothesis (Wills, 2006; LaManna et al., 2017). We suggest that the ForestGEO network should serve as a model for the development of a pantropical network of big bird plots, creating opportunities to discover mechanisms influencing the structure of tropical bird communities and to identify factors responsible for long-term drivers of diversity change (Blake and Loiselle, 2015; Stouffer et al., 2021).

Would the ForestGEO model be effective for birds? Trees stay where you leave them. The mobility of birds and the

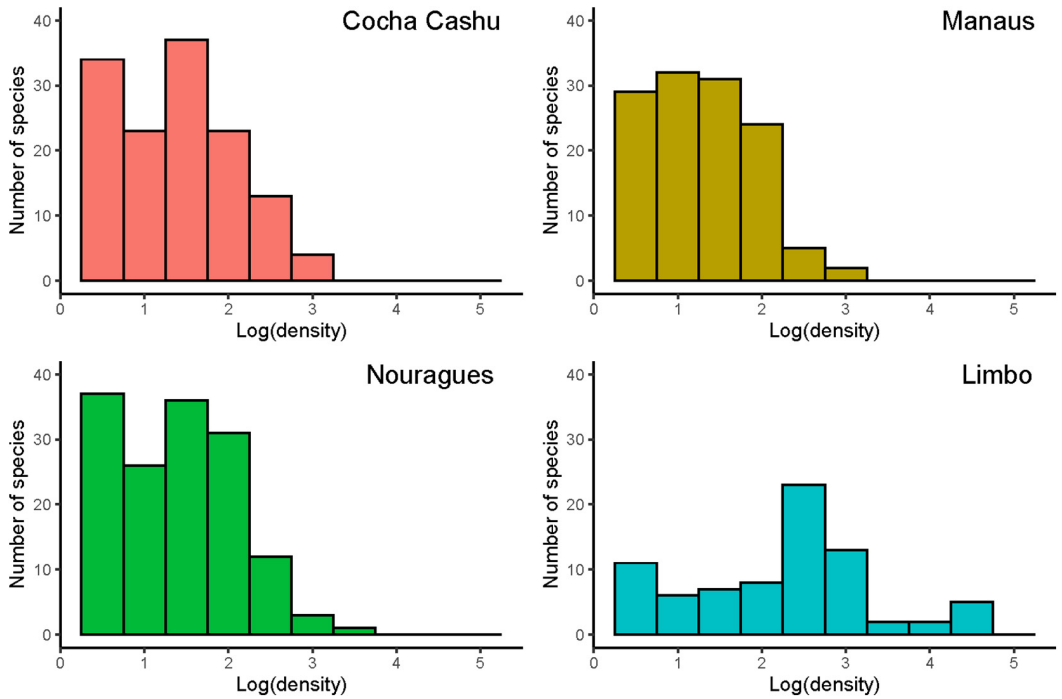

FIGURE 2 | Population density (log 2 intervals) across species in the four Neotropical bird communities where density was estimated. The South American sites (Cocha Cashu, Manaus, and Nourages) are largely similar but quite different from the Central American site (Limbo).

challenges of detecting and attempting to count them accurately can be formidable (Robinson et al., 2018). Nevertheless, we argue that creation of a network of big bird plots, which expands beyond the existing six plots, could provide new insights into tropical, avian and conservation ecology. Formally establishing collaborative agreements so that birds can be sampled on the existing ForestGEO plots could quickly produce ecological insights regarding the roles of birds in tropical forests, especially the linkages between bird and plant diversity. Because plants are identified and mapped, and in many locations their phenological patterns, herbivorous consumers and fruit production patterns are quantified, opportunities to measure roles of birds as seed dispersers and in control of herbivorous insects are unprecedented. Two potential challenges might reduce collaborative opportunities. First, any disturbance of plants by ornithological activities, particularly mist-netting that involves clearing of plants or trampling by repeatedly walking the same routes across plots, could negatively influence plant communities, but carefully constructed collaborative agreements should minimize such challenges. Second, most ForestGEO plots are 50 ha or less (averaging 26 ha), thus are smaller than the current notion of an ideal size for sampling tropical birds (100 ha). However, no critical analysis of optimal tropical bird plot sizes has yet to be conducted. Depending on the questions of interest, more 50 ha plots might be better solutions than fewer 100 ha plots. Current data do indicate that bigger plots sample more species and provide more opportunities to characterize space use (territory sizes) for a greater percentage of the bird community. If, however, primary goals are to benchmark smaller fractions of each community for tracking change in numbers through time, to link bird and plant community data, and to connect bird diversity data with other forms of habitat data (e.g., satellite observations) and predict distributions across geographic space, then smaller plots can be useful. As the ForestGEO network has demonstrated, many research questions can be addressed within single large plots, whereas other questions may be addressed with data gathered from collections of much smaller plots (Condit et al., 2012). Thus, the questions drive and are influenced by the details of sampling designs.

The success of the existing six big bird plots indicates that creation of an expanded network like the ForestGEO forest dynamics plots would produce new insights. For example, even with the small sample of plots currently, clear differences in richness, abundance patterns and biomasses appear across the plots. Amazonian sites tend to be similar but show obvious variability in abundances even within the same species. Such geographic variation in abundances as well as life history traits remains largely undocumented (Wolfe et al., 2014). The Panama community hosts a larger proportion of migrants than the Amazonian sites. The Amazonian sites have many more large granivores. How do all of these observed differences translate to meaningful ecological dynamics? Without more coordinated studies across more plots, particularly addition of more Central American sites, it is challenging to draw robust conclusion. Finally, beyond advancement of scientific knowledge *per se*, plots can also build capacity for local researchers to develop and share expertise, contribute data, and publish results. The successful development of a big bird plot network, or

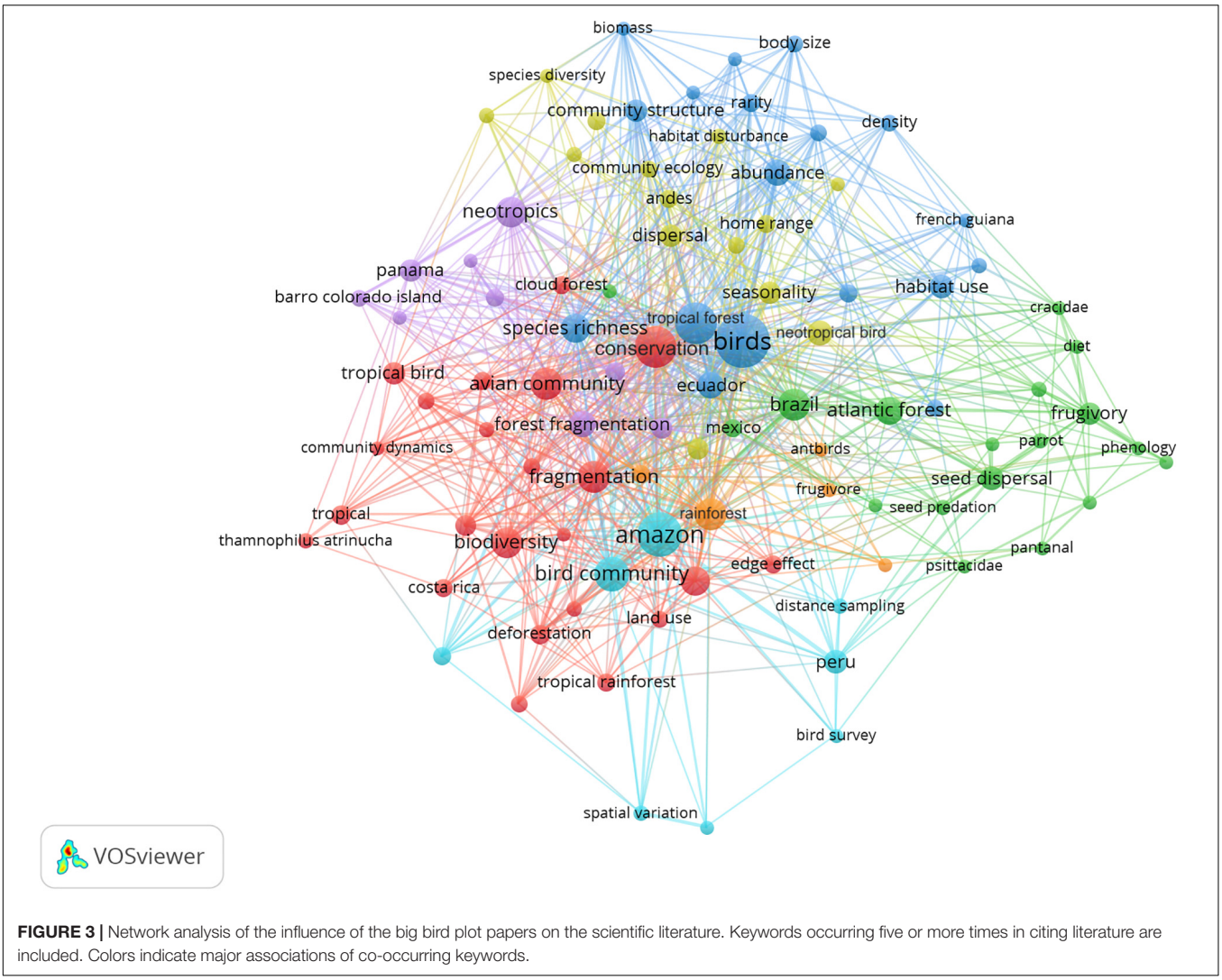

FIGURE 3 | Network analysis of the influence of the big bird plot papers on the scientific literature. Keywords occurring five or more times in citing literature are included. Colors indicate major associations of co-occurring keywords.

extension of collaborations with the existing ForestGEO plots, should involve collaborations with local experts as foundational aspects of the work.

REASONS FOR BUILDING A LARGER NETWORK OF BIG BIRD PLOTS

Plots can play a major role in developing methodologies for larger scale efforts to facilitate long-term monitoring of community dynamics as well as quantifying community structure and how it varies according to relative influences of biotic and abiotic processes (Kraft et al., 2008; Gómez et al., 2020). Knowledge of long-term population trends is essential for effective evaluation of potential regional and global changes on bird populations. Large-scale plots can facilitate field-intensive techniques such as spot-mapping or radio telemetry to determine territory sizes and the number of territories for a subsample of species representing a broad range of traits (e.g., body size). By establishing known density estimates for a number of species, these plots can then be used to calibrate abundance estimates derived from point counts, which can be more easily replicated consistently across larger temporal and spatial scales, also then providing opportunities to precisely repeat surveys and monitor temporal changes. Historically, point counts have been limited by difficulties detecting and estimating abundances of social species and rare species, in particular (Robinson et al., 2018). The differences in estimates between spot-mapping and point counts can be used to calibrate the detection probabilities used in statistical models. Furthermore, with the development of N-mixture models which take into account both probabilities of detection and abundance (Gómez et al., 2018), assumptions can be built into the models which will allow for the estimation of densities of rarely detected species. If a goal is reliable estimation of population densities for all or nearly all species occurring on plots, then utilizing combinations of methods, and those currently under development, will improve quantity and quality of data available for analyses.

For most questions addressing patterns of community structure or species diversity, plots must be sufficiently large

to include most of the species locally present. Many forest species have large home ranges, patchy distributions, and are rare, being present in densities of less than five individuals per 100 ha. In addition, the intrinsic scale of many forms of disturbance, which will affect species composition on the local scale, such as treefall gaps, occurs at the spatial extent of 0.5–5 ha. Thus, plots of 100 ha or more in size are likely to contain multiple individuals of most species and should be less likely to have patterns of species composition driven mostly by local disturbance dynamics. Therefore, large plots increase the chances to more fully characterize local communities while reducing chances that any especially local effects, such as smaller-scale past disturbances, strongly influence community structure. Even if 100 ha plots are too small to include sufficient numbers of most rare species for detection of statistically significant trends over time, alternative methods of community composition analysis can characterize patterns of rare species loss. Plot-based studies still face challenges of how to estimate numbers of the most mobile species, such as parrots and many raptors, colonial species that utilize plots but may breed elsewhere, and the most cryptic components of the avifauna that elude detection with traditional methods.

If tracking temporal trends in bird numbers is an objective, precise repeatability of sampling methods is important. Stationary, or point, counts provide a high degree of repeatability because the same locations can be monitored, even repeating surveys on the same date and time of day in future years (Robinson and Curtis, 2020). Large plots also provide, obviously, a larger number of points, again improving sample sizes and increasing statistical power to detect trends. Even as new analytical methods for estimating abundances or population densities are developed, the simple elegance of a design where qualified surveyors or automatic recording units are deployed at exactly the same locations through time creates opportunities to accurately measure temporal change, establishing the benchmarking value of large plots (Robinson and Curtis, 2020).

Another potential value of large observational plots is that they are less likely to be affected by unpredictable land use, especially if those plots attract other scientists to study various aspects of the site's biology. Well-constructed plots (for example, with trail networks permitting easier access) allow for coordinated research with scientists addressing a broader array of ecological questions. Such plots also may serve as a focal point for building social capital by engaging local inhabitants in plot construction, maintenance, and data collection, as well as building scientific literacy. Ideally, plots could and should be established by in-country residents who know the landscapes and their avifauna the best. The involvement of regional scientists as project leaders can contribute to advancing equity in ecological sciences and open more efficient avenues for educating local residents of the value of characterizing dynamics of native biodiversity (Seidler et al., 2021). At the same time, establishment of trail networks near human settlements may have the detrimental effect of elevating harvest for food, particularly cracids and other game species, and for the pet trade (Peres and Lake, 2003; Peres et al., 2006; Ferreguetti et al., 2018). Such consequences might be evaluated by establishing plots so that some are in undisturbed sites isolated from easy human access whereas others are less isolated. This approach might also permit detection of global-change-driven temporal changes versus changes influenced mostly by local landscape effects (e.g., hunting, introduced species).

IMPEDIMENTS TO OVERCOME

The academic culture of ecological science normally values testing of period-specific hypotheses and publications in peer-reviewed journals. Although we have clearly argued that a network of big bird plots can align with such values, we also note that establishment of such plots with a goal of tracking changes in bird populations over long time periods can be perceived by some as having lower value. Yet, a tension certainly exists between testing modern ecological ideas, addressing pressing conservation issues in landscapes with the world's most diverse bird communities, and the continuing challenge of filling information needs concerning basic natural history of species, proper taxonomic identification and the iterative development and improvement of reliable sampling methods. We consider that the effect of certain academic philosophies is well-illustrated by the demise of the former, very extensive, North American network of bird spot-mapping plots (e.g., Short, 1979). After several decades of surveys in the 1900s with results being published in ornithological journals, the effort was deemed too unimportant and unproductive to take up valuable journal space. The disappearance of journal support eroded the volunteer base and the network died.

Despite the current academic cultural emphasis on ecological publications in high-impact journals as the yardstick by which "success" and "importance" are measured, some new methods for publishing and archiving big plot data have arisen. For example, electronic archiving of metadata so that they remain available through open access is becoming increasingly common. The opportunities for publishing "data papers" where extensive community inventory observations may be made available also continue to increase. Such opportunities may promote the proliferation of further academic specialization where people skilled at identifying and counting birds but uninterested or unable to publish hypothesis-focused papers can share information publicly and concomitantly receive credit for their expertise. Emphasizing the benchmark value of bird population and community studies, additional progress toward recognizing the value of *Transgenerational Collaborations*, where current community members establish a well-designed survey so that it can be precisely repeated in the future, needs to be made. The insights provided by several famous studies and their recent re-surveys such as the Grinnell project (Tingley et al., 2009) and Forbes's early 19th century of Illinois birds (Walk et al., 2010) demonstrate the value of well-executed benchmark studies. Expanding opportunities to develop such benchmark datasets outside the traditional academic realm offers to increase inclusivity and build social capital, particularly in

human communities with less opportunities for advanced academic training.

RECOMMENDATIONS

Without long-term, high-quality data, we have no reliable way to identify mechanisms of population or community change through time and across geography in the world's richest bird communities. Recent declines of some insectivorous species have occurred in bird communities of mature forests with no evidence of direct impacts from anthropogenic activities (Blake and Loiselle, 2015; Stouffer et al., 2021). Presumably, declines in insectivores, particularly terrestrial species, may have some link to subtle shifts in regional precipitation or climatic conditions, but the mechanisms cannot be reliably identified yet and cannot even be separated from simple stochastic processes that play out over long periods of time. Even basic information on tracking relevant insect populations is generally lacking (Lamarre et al., 2020; Montgomery et al., 2021). In short, the paucity of basic data on bird populations from the world's richest locations is a glaring deficiency in our abilities to understand drivers of change, community structuring mechanisms and the importance of birds as interactants on their ecological stage.

We recommend two major steps in using tropical big bird plots to enhance knowledge of tropical avian ecology. First, assemble metadata and data from existing plots and make them publicly accessible to facilitate future re-surveys. Many options for storage of metadata now exist. The ForestPlots.Net group database could provide a useful model (ForestPlots.net et al., 2021) if big bird plot data were to be managed as a stand-alone resource. Addition of a Neotropical node in Avian Knowledge Network would be appropriate (Robinson and Curtis, 2020) and has the advantage that the diversity of methods used to survey birds are already included. With current efforts to resurvey some plots, now is an appropriate time to establish data archival sites. The Cocha Cashu plot has recently been resurveyed and a planned re-survey of the Limbo plot was postponed by the coronavirus pandemic. Existing plots have the immediate advantage of the initial surveys to facilitate analysis of temporal change. During re-surveys, adopting new methodologies, such as deployment of automated recording units, and utilizing methods that improve precise repeatability of surveys (stationary counts) and help adjust for detectability issues when estimating density are important. Additionally, quantifying other aspects of plot characteristics, such as environmental conditions at the time of surveys, habitat patchiness owing to treefall gaps and other disturbances, and even insect sampling would provide opportunities to link changes in bird numbers or richness with potential mechanisms influencing change. Training local technicians to contribute can add temporal continuity and social value to re-survey efforts.

Second, we recommend expanding the size of the network of plots. This could be done by linking with the ForestGEO network, through development of collaborative agreements to add bird surveys where such data would be logistically feasible and mutually beneficial and building on that network's standing support infrastructure to provide opportunities to link with existing data on plant communities. Most ForestGEO plots are smaller than 100 ha, so expansion by adding bird surveys around those plots in buffers would be necessary (Robinson and Curtis, 2020). Alternatively, ForestGEO could be invited to expand their network to incorporate portions of big bird plots. Addition of plots in other locations should also be relatively straightforward if the main goal is to benchmark current bird populations in a manner that uses highly repeatable survey methods (Robinson and Curtis, 2020). In that case, labor-intensive methods such as mist-netting, which sample portions of tropical communities that may be difficult to accurately measure with auditory surveys, are potentially de-emphasized in favor of efficient and more precisely repeatable sampling methods. If exhaustive surveys of communities are desired, then multiple methods extending beyond the grids of stationary counts and collections of transects advocated by Robinson and Curtis (2020) will need to be employed as we discussed earlier.

Estimated costs of surveying big plots for birds are important to consider. Decisions about intensiveness of survey effort, whether or not plots are already existing or will be established at new locations, plot size and costs of labor and travel will influence estimated costs per plot. The existing big plots were largely established and surveyed by foreign scientists, elevating travel and labor costs above potential costs if local talent were available. A probable key to long-term success of a big bird plot network would be to enhance local human capacity to establish and monitor plots. Despite potential large variability in costs, it is probably worth suggesting that surveying Neotropical bird plots may not cost much relative to the estimated costs of surveying tree plots. For example, the ForestPlots.Net assemblage of 1105 small plots (normally averaging about 1 ha in size) costs about 27,000 USD to install a plot (ForestPlots.net et al., 2021). Those costs are high because each individual tree is mapped and identified. Subsequent resurveys of plots have been estimated to cost less, about 18,000 USD or around 30 USD per tree. Because we argue large plots, approximately 100 ha in size, are necessary for adequate surveys of forest bird communities, costs could be prohibitively high per plot if costs of surveying birds were as high as they are for trees. However, the spatial precision with which birds can be mapped, given their mobility, is much lower, the total number of birds per plot is much smaller than that of trees, and the taxonomy is much better known so identifications are not as time-consuming. If the advice of Robinson and Curtis (2020) is heeded to create a simple benchmark survey designed to measure species richness accurately and estimate abundances of most species in a community, they estimated the effort could be accomplished in 4 weeks. Assuming two skilled surveyors are involved, as they recommended, and the plot has already been established, such as with a large ForestGEO plot, the costs for surveying a single plot in one year could be approximately 4,800 USD (320 h times 15 USD/h) notwithstanding consideration of travel and lodging costs. If new plots were established, we estimate at least 4 weeks to create the basic trail system with measured stops along transect routes, which could double initial costs. The costs assume surveyors are already sufficiently

experienced to reliably detect, identify and count birds. Building capacity of local surveyors is also an important cost and contribution (Magnusson et al., 2013). Teaching bird surveying skills to local scientists would add to the time and funds required in the short-term but would save costs in the long-term and also contribute to the development of human capacity for ecological research. We consider our estimate to be at the low end of a potentially large range in costs influenced by local labor costs, terrain, lodging, and many other considerations including annual plot maintenance and data management.

How many plots should be added and where should they be located? This remains an open question that might be addressed with modeling experiments or considerations of specific scientific goals. If questions comparing broad patterns in community organization require less detailed data from each plot, then larger numbers of smaller plots sampled less intensively may be suitable (Rosa et al., 2021). On the other hand, highly detailed data from fewer big plots might be needed to assess geographic variation in patterns of community structure, species rank-abundance profiles, and beta diversity, and determine if those patterns are parallel in trees and birds, or at least correlated. Data from the ForestGEO plots might be used to predict the quantity and distribution of big bird plots that could sufficiently characterize Neotropical forest bird communities. From our simple comparisons of community structure among the existing forest bird plots, it is clear that Limbo, Panama, stands out as being quite different. Adding plots in Central America should, therefore, be a priority to better understand differences between Central and South American bird communities. At this point in history, any plot at any location would be a welcome addition and would promise to expand perspective on geographic variation in community structure.

We also recommend a few specific priorities for addition of new plots. Add plots in a wider diversity of "undisturbed" forest types and across elevational gradients. Add plots in sites recovering from disturbance to provide opportunities to characterize long-term successional effects on bird communities. Locate plots in threatened habitats that may be most likely to change in the near future, either within the plots themselves or in the surrounding landscapes, to provide before-after perspectives on local and landscape-level influences on communities, such as the role of mass effects on plot-level diversity (Condit et al., 2012). Use as many methods as possible to characterize entire communities as not all guilds or functional groups are equally responsive to each potential driver of change. Beyond enumeration of richness and density, inclusion of new approaches to sample diets and genetic and genomic diversity can help identify interactions of birds with other forms of biodiversity (Garcia and Robinson, 2021). Finally, as the sample size of well-surveyed plots is increased and data are associated with environmental aspects of each plot, the information may be connected with the growing effort to use satellites to track and predict global biomass and diversity, offering opportunities to model patterns across huge geographic extents (Quegan et al., 2019; Tang et al., 2019; Dubayah et al., 2020).

Of the practical aspects affecting creation of a network of big bird plots, establishment of detailed best practices for survey methodology is still necessary. The existing big plots were all surveyed with multiple methods, yet no specific coordination of common methods was attempted. Given the complexities of tropical bird communities, especially those in forests where many species are cryptic and/or range widely, further attention to survey methodology strengths and weaknesses is still required. It may be possible that cross-plot comparisons of densities or biomass may be reliable for only certain subsets of communities that can be surveyed with precisely repeatable methods. Yet, exhaustive surveys using multiple methods might still permit useful general comparisons of community organization like we have summarized here. Overall, more advances are needed to establish the most reliable set of survey methodologies for consistent and standardized data collection to benchmark such diverse species communities.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

WR and HP conceived the study. DE, HP, and F-YS organized the database. HP and F-YS performed the statistical analysis. WR, DE, HP, AM, JB, and PS wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

ACKNOWLEDGMENTS

We thank the many technicians and field biologists who helped gathering the data on each of the big bird plots, as well as the relevant authorities for permitting such surveys.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.697511/full#supplementary-material

Supplementary Figure 1 | Number of species per taxonomic family across the six big Neotropical bird plots.

Supplementary Figure 2 | Distribution of species richness across body mass categories at four of the big Neotropical bird plots. Body mass data were unavailable from the two Tiputini plots.

Supplementary Table 1 | Additional characteristics of survey effort on the ~100-ha plots and the landscape context in which each plot occurs.

Supplementary Table 2 | Data from the six big Neotropical bird plots.

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