



Confronting Missing Ecological Data in the Age of Pandemic Lockdown

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Hossie TJ, Gobin J and Murray DL (2021) Confronting Missing Ecological Data in the Age of Pandemic Lockdown. Front. Ecol. Evol. 9:669477. doi: 10.3389/fevo.2021.669477 The COVID-19 pandemic profoundly affected research in ecology and evolution, with lockdowns resulting in the suspension of most research programs and creating gaps in many ecological datasets. Likewise, monitoring efforts directed either at tracking trends in natural systems or documenting the environmental impacts of anthropogenic activities were largely curtailed. In addition, lockdowns have affected human activity in natural environments in ways that impact the systems under investigation, rendering many widely used approaches for handling missing data (e.g., available case analysis, mean substitution) inadequate. Failure to properly address missing data will lead to bias and weak inference. Researchers and environmental monitors must ensure that lost data are handled robustly by diagnosing patterns and mechanisms of missingness and applying appropriate tools like multiple imputation, full-information maximum likelihood, or Bayesian approaches. The pandemic has altered many aspects of society and it is timely that we critically reassess how we treat missing data in ecological research and environmental monitoring, and plan future data collection to ensure robust inference when faced with missing data. These efforts will help ensure the integrity of inference derived from datasets spanning the COVID-19 lockdown and beyond.

Keywords: data missingness, data analysis, imputation, missingness mechanisms, data gap, full information maximum likelihood, Bayesian, COVID-19

INTRODUCTION

In ecology and environmental monitoring, studies conducted on free-living organisms or natural environments often span multiple field seasons. While lockdowns related to the COVID-19 pandemic have been necessary to ensure public safety, studies worldwide are now punctuated with missing data due to travel restrictions, laboratory closures, or suspended authorizations (Pennisi, 2020). Likewise, standard environmental monitoring programs relevant to public health and tracking impacts of industrial activity also have been curtailed or suspended (McIntosh, 2020; Patterson et al., 2020; Viglione, 2020; Conservation International, 2021). Restrictions on data collection in ecology have spanned more than a year and may continue to impact data collection for some time. Data have been (and continue to be) lost due to the pandemic. These losses will be globally pervasive, affecting an array of species, systems, and research or monitoring objectives. The community of data collectors and analysts have a responsibility to ensure that datasets with missing data are handled correctly to maintain the integrity of inferences derived from them. In practical terms, this requires improved commitment to employ appropriate statistical tools for dealing with data gaps, while also ensuring that future studies and data collections are robust to missing data.

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CHALLENGES IMPOSED BY MISSING DATA

Generally, data gaps affect estimated values, sample variability, detectable effect size, and statistical power, thereby challenging our ability to make robust inferences (Maxwell et al., 2008; Button et al., 2013a,b). In the context of the COVID-19 pandemic, the unexpectedness, extent, and synchronicity of data loss among ecology data sets is unprecedented. For example, studies tracking population size of endangered species will be missing critical data on population status, productivity, and survival. Cohorts or individuals being tracked through time will suffer from interrupted timelines. Active field experiments or studies requiring time-sensitive comparisons (e.g., Before-After-Control-Impact studies) will lack response measurements. Environmental monitoring programs could miss critical early warnings of contamination or industrial exceedance of approved standards. Long-term monitoring programs aimed at documenting trends or variability in species abundance or contaminant levels through time will be faced with uncertainty in their assessments and forecasts. Harvest quotas may need to be set without key data required to establish reliable sustainable yield targets. Beyond the sheer extent of data lost, lockdowns have also affected many of the systems under investigation, thereby impacting how data gaps should be addressed. The current and future extent of analytical challenges imposed by the pandemic therefore presents an important opportunity for statistical development and awareness.

Several widely used approaches for handling missing data can introduce bias or fail to effectively leverage existing data (Schafer and Graham, 2002; Nakagawa and Freckleton, 2008). Such approaches include: (1) Ignoring data gaps through listwise or pairwise deletion (i.e., complete-case and available-case analysis); (2) replacing missing data with an average value (i.e., mean substitution); and (3) interpolating missing data through loess regression or smoothing splines (McKnight et al., 2007; Enders, 2010; Table 1 and Figure 1). These approaches can compromise data integrity by exacerbating the effects of small sample size on statistical power (listwise deletion), artificially reducing sample variance (mean substitution, smoothing), biasing parameter estimates (listwise and pairwise deletion, smoothing), or synthesizing missing data based on spurious assumptions about data structure or distribution (smoothing) (Schafer and Graham, 2002; Nakagawa and Freckleton, 2008; Little and Rubin, 2019). Although such approaches may still have occasional utility (e.g., see Hughes et al., 2019), our goal is to motivate the uptake of more robust alternatives, where appropriate.

MECHANISMS OF MISSINGNESS AND THEIR IMPLICATIONS

It is tempting to assume that because a stochastic process led to the COVID-19 pandemic, data missingness is only problematic insofar as it reduces statistical power due to smaller sample size. Yet, there is an important distinction between scenarios where lockdowns are likely to have only affected data collection vs. those where lockdowns not only prohibited data collection but also directly or indirectly influenced the processes under investigation. In fact, we have already seen several indications that COVID-19 lockdowns altered human behavior in ways that could translate to unforeseen responses in natural environments. These include improvements in air quality, increased use of urban areas by wildlife, as well as possible increases in wildlife poaching and resource extraction (Buckley, 2020; Gardner, 2020; Haggert, 2020). Clearly, additional care must be taken to treat data missingness appropriately when this coincides with acute changes in the system of interest. For environmental monitoring in particular, assuming that industry behavior was unchanged during the lockdown when little/no monitoring and oversight were undertaken seems unwise (Gray and Shimshack, 2011). More generally, failing to critically examine patterns of missing data to infer mechanisms of missingness will promote poor statistical treatment and thereby impact inferences drawn from those datasets.

Statisticians recognize three "mechanisms of missingness": Missing completely at random, missing at random, and missing not at random (McKnight et al., 2007; Enders, 2010; Little and Rubin, 2019). The extent to which inadequate treatment of missing data leads to faulty inference, and how these gaps may be corrected, depends on the specific mechanism of missingness that has resulted in the missing data. For example, listwise deletion or complete-case analysis are only valid when data are "missing completely at random" (MCAR), meaning that there are no detectable patterns of missingness across any variables in the dataset (McKnight et al., 2007; Nakagawa and Freckleton, 2008). Assuming that data are MCAR means that researchers assume that data missingness is independent of both observed and unobserved data. This assumption is rarely tested despite this being relatively straightforward to do with current statistical techniques (McKnight et al., 2007; Nakagawa and Freckleton, 2008). One approach for evaluating whether data are MCAR, proposed by Little (1988), tests for differences between the means of different missingness patterns across the entire dataset and produces a single χ^2 statistic (see also McKnight et al., 2007; Enders, 2010). This test can be implemented using the mcar_test function from the "naniar" package in R (Tierney et al., 2021) but may be sensitive to departures from normality and small sample size (Little, 1988). Researchers can also create a binary dummy variable representing missing/not missing for the variable of interest, then examine whether missingness is related to the values of other variables in the dataset (McKnight et al., 2007; Nakagawa, 2015). The R package "mice" also provides tools for visualizing missing data to better understand patterns (van Buuren and Groothuis-Oudshoorn, 2011).

MCAR is a very strong assumption and rarely valid for empirical datasets, especially those derived from fieldwork in ecology where observer error, natural variation, collinearity, and serial autocorrelation can be substantive (Nakagawa and Freckleton, 2008; Nakagawa, 2015; Little and Rubin, 2019). Instead, data may be "*missing at random*" (MAR), where data missingness depends on one or more observed variables. In such cases, patterns of missingness within a specific dataset are associated with other observed variables and do not depend on

TABLE 1 | Methods used to treat missing data in ecology and evolution.

Method	Approach	Comments	References
Listwise deletion	Ignore missing data	Also called <i>complete-case analysis</i> . Excludes all cases with missing data from at least one of the selected variables. May give unbiased results under some conditions, and may be suitable when amount of missing data is small (\sim 5%). Estimates relate only to observed data. Generally, underestimates sample variability and produces biased results when data are MAR or MNAR. Loss of statistical power from reduced sample size.	McKnight et al., 2007, Ch. 7 ; Enders, 2010, Ch. 2 ; Dong and Peng, 2013; Jakobsen et al., 2017; Hughes et al., 2019; Little and Rubin, 2019, Ch. 3
Pairwise deletion	Ignore missing data	Correlation coefficients in the same correlation matrix are created using different subsets of subjects. This may cause problems when data are not MCAR or if the amount of missing data is large (>5%). Analyses such as multiple regression require a positive-definite correlation matrix, and this approach may produce correlations matrices that are not positive-definite, requiring additional <i>ad hoc</i> modifications.	Enders, 2010, Ch. 2 ; Little and Rubin, 2019, Ch. 3
Mean imputation	Imputation	Also called <i>mean substitution</i> . Uses conditional or unconditional mean to fill in data gaps. Can considerably change value of correlations among variables in a dataset. Underestimates sample variability and inappropriately narrows CI around parameter estimates. Decreases in variation are proportional to amount of missing data (i.e., bias worsens with more missing data).	Enders, 2010, Ch. 2 ; Little and Rubin, 2019, Ch. 4
Neighbor interpolation, loess regression, and related approaches	Imputation	Uses neighboring data points on each side of missing data to estimate missing value. Preserves local nature of time series data when the number of neighbors used is small. Neighbor interpolation provides a better estimate than cubic spline interpolation when multiple values are missing. Methods tend to underestimate natural variability.	Enders, 2010, Ch. 2
Smoothing spline (e.g., cubic spline interpolation)	Imputation	A form of interpolation used for longitudinal data where a piecewise continuous curve is fitted to the entire dataset. Can be used to adjust outliers or fill in missing values. Neighbor interpolation provides a better estimate when missing multiple values. Method underestimates natural variability and is sensitive to missing data at the end of a time series.	Daniels and Hogan, 2008, Ch. 2; He et al., 2011; Lepot et al., 2017
Weighting Methods (e.g., inverse probability weighting)	Reweighting	Complete cases are weighted by the inverse of the probability of being a complete case. Weighting the analysis is an attempt to make complete cases representative of all cases. Useful under MAR, and can be superior to multiple imputation when misspecification of an imputation model is likely. Extensions to this approach have been developed, including augmented inverse probability weighting.	Tsiatis, 2006; Li et al., 2013; Seaman and White, 2013; Mansournia and Altman, 2016; Perkins et al., 2018; Hughes et al., 2019; Little and Rubin, 2019, Ch. 3
Resampling	Resampling/ imputation	A variety of approaches that use resampling of the observed data (e.g., via bootstrap or jackknife) to account for uncertainty resulting from missing data. Imputation and analysis of several versions of the resampled dataset can help identify uncertainty of point estimates resulting from missing data. Assumes large sample size. Fails to account for patterns in dataset. May not address bias in parameter estimates which arise as a result of missing data (i.e., under MAR or MNAR).	Little and Rubin, 2019, Ch. 5
Single imputation	Imputation	Various approaches that fill-in gaps with draws based on a predictive distribution or based on explicit models. Use of a single imputed value fails to account for uncertainty in imputed values, meaning that all single imputation methods underestimate standard errors.	McKnight et al., 2007, Ch. 9; Little and Rubin, 2019, Ch. 4
Multiple imputation	Imputation	Simple to use and can accommodate categorical variables. Appropriately captures uncertainty in parameter estimates, and avoids systematic bias in parameter estimates. May be susceptible to high levels of missingness and the number of imputations employed. Valid results depend on careful construction of the imputation model.	Allison, 2005; McKnight et al., 2007, Ch. 10; Enders, 2010, Ch. 7–9; Dong and Peng, 2013; Jakobsen et al., 2017; Little and Rubin, 2019, Ch. 10; van Ginkel et al., 2020
Data augmentation (e.g., via markov chain monte carlo)	Model-based	Analogous to multiple imputation with more imputations and a feedback process between missing data and parameter estimation. Whereas in MI users see the replaced values, this process is internalized in data augmentation. Appropriate under MAR. Suitable when sample size is small. Can treat missing values in multilevel datasets appropriately, and is preferrable to MI in such cases. Whereas multiple imputation can use auxiliary variables when accounting for missing values, data augmentation can only use variables included in the model, which may limit its value in some cases.	Tanner and Wong, 1987; McKnight et al., 2007, Ch. 8 ; Nakagawa and Freckleton, 2008; Nakagawa, 2015; Little and Rubin, 2019, Ch. 10

(Continued)

TABLE 1 | Continued

Method	Approach	Comments	References
Maximum Likelihood methods (e.g., full Information maximum likelihood)	Model-based	Various approaches based on statistical models and the associated likelihood function. Yields narrow confidence intervals and high statistical power. Can be used for hypothesis testing. Unbiased for MAR or MCAR as long as multivariate normality assumption holds. Consider data augmentation as an alternative when sample size is small.	Nakagawa and Freckleton, 2008; Enders, 2010, Ch. 3–5; Dong and Peng, 2013; Little and Rubin, 2019, Ch. 6–9
Selection models and PATTERN-mixture models	Model-based	Both methods can be used under MNAR. Models are constructed based on specific assumptions about the nature of (unobserved) missing data. If assumptions are incorrect the model will perform poorly. Alternatively, MAR approaches combined with best-case/worst-case sensitivity analysis can help diagnose the impact of MNAR.	Allison, 2002; Molenberghs and Kenward, 2007; Daniels and Hogan, 2008, Ch. 5, 8, 9; Enders, 2010, Ch. 10; Little and Rubin, 2019, Ch. 15

Notably, multiple imputation and maximum likelihood methods are the most commonly used, but the larger list is provided for generality. Pattern of data missingness: Missing completely at random (MCAR), missing not at random (MNAR) and missing at random (MAR).

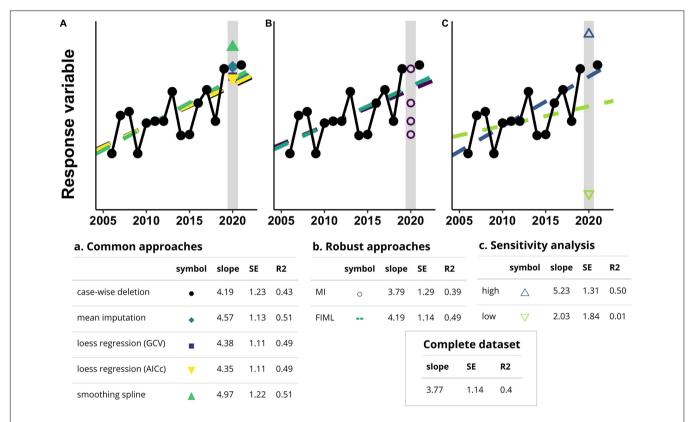


FIGURE 1 | Simulated results from the analysis of a hypothetical dataset based on missing data from the pandemic lockdown. The annual time series has a simple linear trend, where a single data point representing the year 2020 is missing (highlighted by the gray bar). Parameter estimates (slope), and measures of variance (SE) and fit (R2) for the complete dataset are shown in the box for comparison. (A) Common approaches for dealing with missing data yield biased parameter estimates, often underestimate variance, and overestimate fit, as can be seen by the comparison to the complete dataset. (B) For data that are missing at random (MAR) or missing completely at random (MCAR), using multiple imputation (MI) or maximum likelihood-based approaches (e.g., full information maximum likelihood—FIML) help minimize these issues by accounting for uncertainty in the missing data. (C) If data are missing not at random (MNAR), best/worst case sensitivity analyses (e.g., via an evaluation using extreme values as depicted here) can be used to evaluate the effects that the full range of potential values might have on subsequent analyses. Source code available here: https://github.com/JenileeGobin/confronting-missing-data.

the value of the missing observations themselves (Schafer and Graham, 2002; Dong and Peng, 2013). If pandemic restrictions only limited data collection, and were unlikely to affect the system itself, such data would be MAR so long as missingness in the response variable depends on other explanatory variables that were observed/recorded. A third possibility is that data are "*missing not at random*" (MNAR), which occurs when the

probability of missingness depends on the value of the missing observation, or on an unobserved variable (Schafer and Graham, 2002; Nakagawa and Freckleton, 2008; Dong and Peng, 2013). Specifically, if missing data resulting from a lockdown coincides with acute changes in the system under investigation and/or prohibited the collection of suitable auxiliary data, such data would be MNAR. These acute changes would include those imposed by the researcher (e.g., as part of a Before-After Control-Impact experiment), or those arising as a direct/indirect effect of the lockdowns themselves (e.g., increased use of urban areas by wildlife). Other plausible scenarios where data may be MNAR as a result of lockdowns include changes in emissions or wildlife harvest due to reduced human activity or lapses in oversight during the lockdown period.

Diagnosing the mechanism of missingness (sensu Little and Rubin, 1987, 2019) is an important step to guide subsequent data analysis and should be prioritized for all datasets with missing data (Schafer and Graham, 2002; McKnight et al., 2007). It would be risky, especially for regulators, to assume that missing data owing to the lockdown are MCAR. In fact, even when diagnostic tests identify missing data as MCAR, it is generally safer to presume MAR when dealing with missing values in ecological studies (Nakagawa and Freckleton, 2008; Nakagawa, 2015, but see Chen and Ibrahim, 2013). In so doing the researcher acknowledges that, in practice, MAR is a more realistic assumption and that missing data may be MAR or MNAR even when tests fail to detect departures from MCAR (Nakagawa and Freckleton, 2011; Nakagawa, 2015). When data are MAR, bias in parameter estimates can be avoided by leveraging information about the non-random associations between data missingness and other observed variables in the dataset, but this is not the case when data are MNAR (Schafer and Graham, 2002; Little and Rubin, 2019). Differentiating between MAR or MNAR is difficult, in part because it relies on fundamentally untestable assumptions related to unobserved data, however, a number of conceptual, numerical, and graphical methods can be helpful (see McKnight et al., 2007; Enders, 2010). Detectable patterns of missingness that cannot be explained by other variables in the dataset also indicate MNAR. Ultimately, researchers must rely primarily on their understanding of the system, and a correct interpretation of missingness mechanisms, to determine whether data are likely to be MAR or MNAR (Bhaskaran and Smeeth, 2014; Nakagawa, 2015) before moving on to determine how to handle gaps in their datasets.

REMEDIES FOR MISSING DATA

Once the mechanism of missingness has been determined, researchers should adopt statistically robust procedures for dealing with missing data, of which there are several options that differ in their statistical philosophy, complexity, and underlying assumptions (Table 1). Here, we focus on the most relevant and straightforward approaches that should meet the needs of the majority of professionals in ecology (Nakagawa and Freckleton, 2008; Nakagawa, 2015). When missing data are MAR, multiple imputation (MI) and full information maximum likelihood (FIML) are reliable options (Nakagawa and Freckleton, 2008; Jakobsen et al., 2017; Little and Rubin, 2019; Figure 1). In MI, missing data are replaced with a set of random draws from the observed data distribution, and this process is repeated to create several new full datasets. Each full dataset is then analyzed separately, and the results are combined (McKnight et al., 2007; Enders, 2010). The number of imputations required

depends in part on how much data are missing. As few as 3-20 imputed datasets may be sufficient to generate unbiased estimates (Dong and Peng, 2013; Jakobsen et al., 2017), but 30-40 imputations are often required (Nakagawa, 2017; Nakagawa and de Villemereuil, 2019). FIML does not replace missing values, but instead uses observed data distributions to infer the characteristics of the complete dataset. The approach uses an iterative process of implicit replacement and analysis with feedbacks, most commonly using the expectation-maximization algorithm (Enders, 2010; Dong and Peng, 2013; Little and Rubin, 2019). Whereas FIML permits the use of likelihood ratio tests for comparing nested models and generally yields smaller standard errors, MI can deal with categorical variables (Dong and Peng, 2013, see also Allison, 2005). Both MI and FIML maintain statistical power, preserve patterns of natural variability in data, and generally lead to unbiased estimates when assumptions are met and data are MAR (Donders et al., 2006; Nakagawa and Freckleton, 2008). Reporting guidelines for missing data analysis can be found in Enders (2010) and van Buuren (2018).

The above approaches may not be helpful when the proportion of missing data are large (e.g., $\sim 40\%$), only missing from the dependent variable and suitable auxiliary variables are not identified, or when the assumptions of MNAR are plausible (Dong and Peng, 2013; Jakobsen et al., 2017). Importantly, even when < 5% of data are missing it may not be "ignorable" (i.e., MCAR or MAR). When missing data is "non-ignorable" (i.e., MNAR), analysis requires that the researcher make specific assumptions about how the unobserved data are missing and build a model to fill in missing values. Methods for dealing with MNAR include selection models and pattern-mixture models (see Enders, 2010; Little and Rubin, 2019). Assumptions about unobserved data are fundamentally untestable, and these models will perform poorly if the assumptions are incorrect. Supplementary analyses (e.g., worst/best case sensitivity analyses) should be employed to quantify the potential impacts of missing data on key outcomes (Enders, 2010; Jakobsen et al., 2017), and efforts should be made to clearly articulate uncertainly in the outcome. Given that pure forms of MCAR, MAR, and MNAR are unlikely in ecological datasets, employing MAR approaches (e.g., multiple imputation) may still be helpful when dealing with datasets where the mechanism of missingness is, in part, MNAR, because it will help mitigate problems caused by the MAR component (Nakagawa, 2015; van Ginkel et al., 2020).

In many cases MI and FIML will be appropriate, however fully Bayesian approaches are among the most powerful for dealing with missing data. Here, missing data are treated like any other unknown random variable that is represented by a probability distribution (i.e., the posterior distribution) and can be obtained by assigning priors to the unknown parameters. When data are MCAR or MAR, missingness does not depend on the unobserved response variable, meaning that any inference would be equivalent to that from a model for the observed data alone (Rubin, 1976; Seaman et al., 2013). However, construction of a joint model that accounts for the mechanism of missingness is required when data are MNAR, and this can take a variety of forms (e.g., selection, pattern mixture, shared parameter models) (Little and Rubin, 2019). This process may still involve making strong and unverifiable assumptions. The strengths of this approach are that: (1) Assumptions are made clear and explicit through the specification of priors as part of the model, and (2) missing data can be accommodated within a Bayesian framework and applied to both missing response and covariate data distributions without a need for new techniques for inference (Ibrahim et al., 2002, 2005). Understanding that Bayesian statistics are not yet widespread among ecologists, whichever approach is selected must be appropriate given the extent and type of data missingness.

The pandemic presents a unique situation where research and monitoring activities that vary widely in their goals and data collection techniques have all been affected by the same problem; the solutions to which may ultimately take similar forms. Missing data theory is an active area of research, and a growing number of techniques are available which offer useful or promising routes for robust inference in the face of missing data. It is not possible for us to review them all here, although we provide a summary of the most prominent approaches in **Table 1**. For additional coverage of the topic, we direct readers to McKnight et al. (2007), Daniels and Hogan (2008), Enders (2010); Nakagawa (2015), and Little and Rubin (2019).

AVOIDING AND PLANNING FOR MISSING DATA

The pandemic should prompt researchers and monitors to consider how they might mitigate the missing data problem in the future. Indeed, in specific cases the lockdown may motivate development and use of automated or remote data collection techniques that limit reliance on human site attendance. Many of these tools are already available and are rapidly becoming less costly (e.g., Wickert, 2014). However, validation of many techniques is still forthcoming, some data collection simply cannot be automated, and prohibitive costs of automation may preclude widespread adoption. Nonetheless, researchers can prepare for missing data at the study design and data collection stage by identifying variables most likely to suffer from data loss, as well as auxiliary variables that can be collected more reliably. These adjustments will ensure that even with missingness, data will meet the assumption of MAR rather than being relegated to MNAR (Nakagawa, 2015; Noble and Nakagawa, 2018). Automating the collection of auxiliary variables may increase its availability for dealing with missing data. Researchers interested in this approach should explore Two-method Measurement Design and Planned Missing Data Designs, both of which are active areas of research (Enders, 2010; Nakagawa, 2015; Noble and Nakagawa, 2018). Once lockdowns are rescinded and data collection has fully resumed, we may also see an increase in the use of backcasting methods which predict unobserved values that might have existed in the years where data collection was on hold, given the observed data (e.g., Le et al., 2008; Saghafian et al., 2018; Contreras-Reyes and Idrovo-Aguirre, 2020). These methods are distinct from others discussed in this paper and could serve as an effective alternative in data restoration for robust inference in the future.

DISCUSSION

Missing data are now prevalent across virtually all longitudinal datasets in ecology, and similar gaps could arise in the future. COVID-19 lockdowns should therefore motivate researchers and environmental monitors to critically reassess how data collection is planned and missing data are dealt with. Failing to do so will surely lead to future instances of weak or faulty inference, with potentially severe impacts on academic research, environmental monitoring and management. The statistical tools discussed here are not new, but novel approaches continue to become available (Table 1). Multiple imputation and other model-based methods already have R libraries available and will be appropriate for many missing data scenarios encountered in ecology (Nakagawa and Freckleton, 2008; Nakagawa, 2015). More broadly, missing data theory and corrective measures should be covered more extensively in graduate statistics training in ecology and environmental science. We will also benefit from efforts to make approaches for handling missing data more accessible (e.g., via development of R packages and tutorials), and greater collaboration with statisticians. In light of the ongoing global environmental crisis it is increasingly evident that longitudinal data in ecology will play a critical role in understanding, anticipating, and possibly mitigating environmental changes. To meet these challenges, we have a responsibility to employ procedures that ensure reliable insight from our research and monitoring efforts.

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://github.com/ JenileeGobin/confronting-missing-data.

AUTHOR CONTRIBUTIONS

TH, JG, and DM contributed equally to the study conception. TH wrote the initial manuscript draft, with JG and DM contributing significantly to the manuscript writing. All authors contributed to the article and approved the submitted version.

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