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Planned missing data design: stronger inferences, increased research efficiency and improved animal welfare in ecology and evolution

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Abstract:

1. Ecological and evolutionary research questions are increasingly requiring the integration of research fields along with larger datasets to address fundamental local and global scale problems. Unfortunately, these agendas are often in conflict with limited funding and a need to balance animal welfare concerns.
2. Planned missing data design (PMDD), where data are randomly and deliberately missed during data collection, is a simple and effective strategy to working under greater research constraints while ensuring experiments have sufficient power to address fundamental research questions. Here, we review how PMDD can be incorporated into existing experimental designs by discussing alternative design approaches and evaluating how data imputation procedures work under PMDD situations.
3. Using realistic examples and simulations of multilevel data we show how a variety of research questions and data types, common in ecology and evolution, can be aided by utilizing a PMDD and data imputation procedures. More specifically, we show how PMDD can improve statistical power in detecting effects of interest even with high levels (50%) of missing data and moderate sample sizes. We also provide examples of how PMDD can facilitate improved animal welfare all the while reducing research costs and constraints that would make endeavours for integrative research challenging.
4. Planned missing data designs are still in their infancy and we discuss some of the difficulties in their implementation and provide tentative solutions. Nonetheless, data imputation procedures are becoming more sophisticated and more easily implemented and it is likely that PMDD will be an effective and powerful tool for a wide range of experimental designs, data types and problems common in ecology and evolution.

Keywords: data augmentation, multiple imputation, personality, quantitative genetics, mixed effects models, hierarchical models, multilevel modelling, refinement, reduction, multiple working hypotheses.

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Introduction

Missing data is a widespread problem in ecological and evolutionary research (Nakagawa & Freckleton 2010; Nakagawa & Freckleton 2011; Ellington *et al.* 2015; Nakagawa 2017), often resulting in the exclusion of a substantial amount of data. This contributes to a major reduction in statistical power and, if the nature of 'missingness' is not considered carefully, leads to biased parameter estimates (Enders 2001b; Graham 2009; Nakagawa & Freckleton 2010; Little *et al.* 2013). Theoretical frameworks for dealing with missing data, however, have received substantial attention and missing data theory is now a well-developed field of research grounded in solid statistical theory (Graham, Hofer & MacKinnon 1996; Enders 2001a; Little & Rubin 2002; Graham 2003; Graham 2009; van Buuren 2012; Little *et al.* 2013). Nonetheless, while social scientists have been at the forefront of applied missing data techniques, ecologists and evolutionary biologists have lagged behind (Nakagawa & Freckleton 2010).

Missing data is traditionally viewed by ecologists and evolutionary biologists with a sense of disdain and annoyance. But, what if including missing data in analyses could be advantageous? Indeed, social scientists have taken a rather different stance to missing data – instead embracing its power to help address fundamental research questions (Graham *et al.* 2006). Planned missing data design (PMDD) is an approach that involves deliberately planning to 'miss' data as an integral part of an experiment. In other words, deliberately not collecting data on certain variables or experimental subjects. While this seems like an odd thing to do, if missing data in the variables of interest is completely random or can be made random, existing statistical frameworks are known to do an excellent job at recovering parameter estimates and standard errors compared to complete case analyses (Schafer & Graham 2002; van Buuren 2012). The PMDD approach comes with a substantial number of benefits that have been largely ignored by ecologists and evolutionary biologists.

Here, we argue that PMDD can expand the scope, reduce the costs and improve animal welfare, facilitating higher impact research with more power. We begin our discussion by briefly introducing missing data theory and then describe a few core statistical tools that can be used to impute / augment (i.e., 'fill in') missing data. Using simulations, we show that, when missing data is 'completely' random (see next section), existing data imputation techniques can be excellent at recovering parameter estimates and their standard errors – even with hierarchically structured data that is common in ecological and evolutionary research (Enders, Mistler & Keller 2016; Quartagno & Carpenter 2016; Resche-

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Rigon & White 2016). We then describe PMDD, overviewing some of the different experimental approaches that can be implemented, what they involve and important design considerations. Following from this discussion, we overview the important benefits of utilizing a PMDD and end with a discussion on some of the challenges to their use – providing suggestions for how these can be rectified.

A brief introduction to missing data theory

Missing data patterns can generally be classified as falling into one of three different types – based on the different mechanisms generating missing data – missing completely at random (MCAR), missing at random (MAR) and missing not at random (MNAR) (Rubin 1976; Little & Rubin 2002; Graham 2009; Nakagawa & Freckleton 2010; van Buuren 2012; Nakagawa 2017). The distinction between these three missing data mechanisms is important to understanding the power of PMDD, which we will introduce below. Missing data (either in response or predictor variables) are considered to be MCAR when missingness is random with respect to both observed and unobserved (i.e., not collected in the study) variables (Enders 2001b; Nakagawa 2017). In other words, the observed data is simply a random sub-sample of complete data (Enders 2001b). In contrast, missing data are considered MAR when the missing values in a dataset depend on observed values of other variables in the dataset (Enders 2001b; Graham 2009). For example, if we were interested in understanding the correlation between survival to 1 year and mass at 6 months we would find that individuals that die before 6 months are missing data on mass, however, missing data on mass is correlated with their lifespan, which is known. Missing not at random (MNAR), however, occurs when missing values depend on unobserved variables that have not been quantified in the study, or on the variable itself. For example, we may be missing behavioural data on small sized animals within a population because they tend to be 'shy' and difficult to capture (e.g, Biro & Dingemanse 2009), in which case we would be missing both behavioural and morphological data. Under these situations, dealing with missing data is difficult (possibly even impossible) because statistical techniques for recovering missing information when data are MNAR are difficult to implement given the need to explicitly model the process of missingness (Schafer & Graham 2002).

These missing data mechanisms have different consequences on statistical results when missing data is excluded prior to analysis, as is often the case (i.e., referred to as 'complete case', 'pairwise deletion' or 'listwise deletion'). While MCAR results in a loss of power when missing data is excluded from an analysis, it does not bias parameter estimates

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(Enders 2001b; Schafer & Graham 2002; Graham 2009; Nakagawa & Freckleton 2010). In contrast, when missingness data is MAR or MNAR, excluding missing data will result in both a loss of power and biased parameter estimates (sometimes severly so; Enders 2001b; Schafer & Graham 2002; Graham 2009; Nakagawa & Freckleton 2010). To better appreciate the impact missing data can have on sample size (and thus statistical power), assume that we have 10 variables, each containing 5% missing data, and a total complete dataset of N = 1000. If we used all variables in a statistical model we may need to exclude as much as 500 observations, resulting in a substantial decrease in power and severely compromising our ability to detect significant effects (see '*Recovering power of experimental designs'* for more on this issue). Statistical techniques for dealing with missing data rely on the assumption of missing data being MCAR or MAR, and if this assumption is met, then both power and bias in parameter estimates can be recovered (Enders 2001b; Schafer & Graham 2002; Nakagawa & Freckleton 2010; van Buuren 2012; Ellington *et al.* 2015; Nakagawa 2017).

Statistical procedures for dealing with missing data

Planned missing data design hinges on the ability of researchers to make use of statistical procedures for handling missing data (Little & Rubin 2002; Graham *et al.* 2006; Enders 2010). It is therefore pertinent that we briefly review existing missing data techniques and provide some guidance on their implementation when data has been collected using a PMDD. We do not discuss these topics in great depth as there are a number of important, accessible reviews and books on these subjects already, which we direct the reader to for more details (Schafer 1997; Enders 2001b; Little & Rubin 2002; McKnight *et al.* 2007; Allison 2012; van Buuren 2012; Nakagawa 2017).

As mentioned above, imputation methods fall under two broad categories and we follow the general categorization of McKnight et al. (2007) in classifying them in to those implementing data augmentation (DA) techniques and those utilizing multiple imputation (MI) with the help of Rubin's rules (Rubin 1987; Enders 2010). Data augmentation procedures incorporate both observed and missing data into a single joint modelling approach that proceeds through the following steps: 1) the parameters of a model are estimated using observed data; 2) parameters estimated in step 1 are then used to augment missing data and 3) model parameters are re-assessed conditional on both the observed and imputed data (Figure 1a; Nakagawa 2017). These steps are re-iterated until the model converges (i.e., maximum likelihood or stable posterior distribution) (Figure 1a). Data augmentation is advantageous in

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that it is fast, easily implemented (under the assumption of multivariate normality) and results in robust parameter estimates and standard errors (McKnight *et al.* 2007).

In contrast, multiple imputation proceeds by generating a set of *m* (usually m = 40–50 performs well under a variety of situations and has high efficiency; Nakagawa & de Villemereuil 2015; Nakagawa 2017) complete datasets where missing data is imputed using variables of interest. These *m* datasets can then be analysed normally (i.e., as if a complete dataset existed) and the results (i.e., parameter estimates and standard errors) pooled across the *m* datasets (Figure 1b; Schafer 1997; Schafer & Olsen 1998; Little & Rubin 2002; van Buuren & Groothuis-Oudshoorn 2011; van Buuren 2012; Nakagawa 2017). Multiple imputation provides a number of advantages over data-augmentation. First, it is extremely flexible, easily accommodating different distributions (i.e., Bernoulli, Poisson etc.), variables and model types if needed. Second, since MI creates *m* complete datasets it allows one to separate out the imputation step from the analysis step. In other words, we can have a set of auxiliary variables (see '*Auxiliary variables to aid in imputation*' below) that are used to impute missing values and then subsequently use only the variables of biological interest to run the analysis on *m* imputed datasets. This is particularly advantageous because including unnecessary variables in DA procedures can complicate the interpretation of model results (McKnight *et al.* 2007; Enders 2010). Lastly, MI procedures account better for imputation uncertainty as variation in parameter estimates across data sets can be explicitly incorporated in pooled estimates, protecting against type I errors (McKnight *et al.* 2007). Additionally, the effect of missing data on analysis results (i.e. efficiency) can be explicitly quantified and presented by deriving statistics summarising the variability in parameter estimates across imputed datasets (McKnight *et al.* 2007). Given the flexibility, ease of implementation and their general tendency to produce robust parameter estimates, it is unsurprising that Rubin (1996) recommends MI procedures over DA.

Auxiliary variables to aid imputation

Auxiliary variables are variables that are not necessarily of interest with respect to the biological question at hand, but that are correlated with other variables, or missing data itself, within the dataset (Collins, Schafer & Kam 2001; Graham 2003). Including auxiliary variables has been shown to improve the accuracy and stability of estimates and to reduce their standard error (Enders 2010; Allison 2012; von Hippel & Lynch 2013). The best auxiliary variables are those that are easy and cheap to collect and that are strongly correlated with a number of other variables within the data set (Collins, Schafer & Kam 2001; Graham

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2003; von Hippel & Lynch 2013). Collins et al. (2001) have shown that auxiliary variables can be particularly useful when the missing data is in the response variable, when they change the missing data mechanism from MNAR to MAR and when the correlation between auxiliary variables and response is high (r = 0.9). Adding even just 2–3 auxiliary variables can improve imputation procedures and for the most part, an inclusive analysis strategy where a large number of auxiliary variable are included in the analysis is recommended (Enders 2010 p.g. 128). However, this procedure can be slightly more complex than this in practice. Hardt et al. (2012) show that the inclusion of too many (> 10) can start to lead to a downward bias in regression coefficients and a decrease in precision. In addition, auxiliary variables will have little impact when the correlations between variables in the dataset are low (r = 0.10) (Hardt, Herke & Leonhart 2012). Therefore, we recommend including 36 auxiliary variables with moderate to high correlations (0.4–0.8) when utilizing imputation procedures where unplanned missing data might cause data to follow MNAR conditions.

Experiments in ecology and evolution often collect variables that are not necessarily of interest, but can be used as auxiliary variables. These variables can include body dimensions, sex, age, spatial data, or even researcher ID. These types of auxiliary variables can be included in imputation procedures (e.g., MI) with unplanned missing data to ensure that the MAR assumption is met, but then discarded when testing the biological questions and hypotheses of interest (Graham 2003). Considering these variables more carefully with respect to their potential correlations with other variables, and possibly with missing data itself, is an important aspect of imputation because it can change missing data from MNAR to MAR satisfying assumptions of imputation procedures. As an illustrative example, consider mark-recapture field studies that often collect spatial coordinates (i.e., UTM positions) of animals. While the spatial position may not be of interest to the question of interest, it may be the case that spatial positions are correlated with missing data. This might be the case, if for example, observations are missing for some animals because their territories are located in thick impenetrable forest or are on the boundaries of the study site. One way to use these spatial coordinates might be to generate a spatial covariance matrix between observations be (possibly using the SpatialTools package in R – French 2016) and decompose this matrix into a set of principle components (PCs). The PCs could be then included into a multiple imputation model (e.g. those using *mice* or *mi* – Table 2) to help recover missing data on individuals that were not observed on a given sampling occasion. Similar approaches have been developed that make use of phylogenetic covariance matrices

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(Nakagawa & de Villemereuil 2015) as well as the relatedness matrices (Hadfield 2008), and these have been shown to do an excellent job at recovering missing data.

Planned missing data designs and their application in ecology and evolution

Planned missing data designs (PMDD) allow researchers to collect incomplete data from subjects or observations of subjects on purpose by randomly assigning them to have missing measurements or measurement occasions (Graham *et al*. 2006; Rhemtulla & Little 2012; Little & Rhemtulla 2013). Researchers can then utilize data augmentation and multiple imputation techniques (discussed above) to fill in missing data such that the data contains complete information for all variables and experimental units within the dataset. Importantly, PMDD should always conform to the MCAR assumption because missing data is random by virtue of the experimental design making it ideal for use with imputation methods (Little & Rhemtulla 2013).

Subset Measurement Design

Planned missing data design was first developed for research utilising questionnaires or surveys to help deal with participant fatigue, and is particularly useful when there are also logistical and financial constraints to asking many different questions (Graham, Hofer & Piccinin 1994; Graham *et al*. 2006). For example, a common type of PMDD called the *multi-form design* (MFD) involves creating alternative questionnaires that each contain overlapping questions and a sample of new questions (Graham *et al.* 2006; Little & Rhemtulla 2013). Combining data on participants across the questionnaires, and then treating the questions participants were not given as missing information, allows missing data to be imputed based on the covariance between known answers (Graham *et al.* 2006).

In ecology and evolutionary biology, we often do not use questionnaires to collect data (aside from the field of ethnobiology; see Albuquerque *et al.* 2014), therefore, an analogous design is what we refer to as a *subset measurement design* (SMD) (Table 1a). Similar to the MFD, a SMD involves quantifying a common set of variables across all individuals (e.g., body size) and then randomly allocating subjects to be quantified on a subset of other variables (e.g., hormone concentrations, metabolism etc.) (Table 1a). Common variables can be those that are easily or cheaply quantified, such as body size indices (e.g., mass, body / wing length) or age (if this is known). In contrast, variables that are expensive or logistically challenging to quantify (e.g., gene expression, hormone

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concentrations) can be randomly sampled on a subset of subjects during the experiment. When using a SMD one should also consider, *a priori*, any potential interactions (Table 1a) of interest and whether the planned missingness provides sufficient power to test these interactions (Enders 2010).

Two-Method Design

The SMD can also be applied to situations where researchers have a choice between two variables that quantify similar constructs or have similar meaning, but where one is more easily and cheaply quantified but has large measurement error and the second is more logistically challenging but is considered the 'gold standard' (i.e., lower measurement error / more informative to the question). The latter design is referred to as a *two-method design* (TMD) in the social sciences (Little & Rhemtulla 2013), and can be a useful way at improving data quality, particularly when some measurement variables are recognized as being more powerful in addressing certain questions than others. For example, we may be interested in measuring 'metabolism' using both whole-organism resting metabolic rate and by quantifying a major metabolic hormone, thyroxine (T4) (Table 1a). Thyroxine is known to impact cell metabolism but is both costly and a more indirect measure of assessing metabolic rate because it is only a single hormone in a cascade of hormone signalling pathways that affect ATP turnover in a cell. As such, depending on our question we may actually measure more animals on whole-organism metabolic rate and fewer on T4, as it better represents whole-organism metabolic rate *per se* and is cheaper.

Wave Missingness Design

Longitudinal research questions, where repeated measurements on a set of independent individuals is of interest, can utilize a PMDD called *wave missingness* (Table 1b) such that a group of experimental subjects are assigned to a wave or set of measurement occasions randomly (Little & Rhemtulla 2013; Rhemtulla *et al.* 2014). Waves can be blocked such that some animals are measured at the beginning and end and others in the middle (i.e., pseudo-randomised missingness; Rhemtulla & Little 2012; Rhemtulla *et al.* 2014), or individuals can be randomized to a set number of waves were the measurement occasions are completely random (as in Table 1b). The specific design utilized will largely depend on the research question and the constraints faced in executing the study. For example, assume we are interested in understanding seasonal changes in individual hormone profiles and we would like to sample the same set of individuals at monthly intervals over the active season

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(6 months). If we have 60 wild animals (that we can regularly re-capture) we may decide to randomly allocate 10 individuals to one of 6 sampling waves. The first wave samples a set of 10 random individuals across all months, whereas the second wave samples a different 10 animals at months 2, 3, 5 and 6 (Table 1b). We can continue this such that any one animal in waves 2–6 is sampled a total of 3–4 times. Missing measurement occasions within waves are random, but animals in wave one are deliberately sampled on each occasion to ensure we can get a complete picture of hormone changes across time at least on a subset of animals. The full dataset would contain a total of 360 blood samples if each animal was sampled once. However, with our design (Table 1) we would have 240 blood samples and approximately 33% of the data would be missing. We could then impute missing data for subjects not measured on a given occasion.

Considerations for and General Performance of Missing Data Designs

We have overviewed three of the more common designs that can be applied to experimental systems, however, it is important to note that PMDD's can be diverse and are often not necessarily mutually exclusive of one another (Enders 2010; Rhemtulla & Little 2012; Little & Rhemtulla 2013; Rhemtulla *et al.* 2014). Combinations of the designs described above are probably necessary in many real research situations. Regardless of which PMDD is used, researchers should choose the variables that are most pertinent to the specific hypothesis being tested, or those that are likely to have small effect sizes (and lower power), as those being measured with as little missing data as possible (i.e., having complete measurements on these variables). This ensures that the most pertinent questions can be tested rigorously (Graham *et al.* 2006). In addition, researchers should also consider the hypothesized correlation between variables. More tightly correlated variables ($r > 0.50$) may allow for one to plan for a greater level of missing data then two variables that are weakly correlated.

While these designs can be powerful tools in aiding the testing of research questions, it is still unclear what designs work best at recovering parameter estimates and standard errors across various situations. Graham et al. (2006) and Enders (2010) (pg. 23–36) provide an excellent overview of the power of various PMDD's. Graham et al. (2006) showed that with moderate sample sizes (n = 200–300), subset measurement type designs can have >0.80 power in estimating even small effects in many situations (i.e., $d > 0.20$). Rhemtulla et al. (2014) also show that with reasonably large sample sizes (n = 300 for multi-form design and n = 500 for wave missing and hybrid designs) that parameter estimates and standard errors in

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latent growth models show little bias. In many situations, any loss in power resulting from missing data seems to be rather small relative to the gains in the number of questions that can be tested and the logistical and cost improvements for a given experiment (Enders 2010). PMDDs have, in some cases, even been shown to be more powerful than complete case designs under certain situations (See below for an example; Graham *et al.* 2006; Enders 2010).

Benefits of planned missing data design

The PMDDs outlined above provide a number of important advantages for ecologists and evolutionary biologists. Below we discuss these benefits more thoroughly utilizing realistic simulations and examples to support our arguments.

(i) Improved statistical power

We have already indicated above that missing data procedures can substantially increase the power of a given study by increasing the effective sample size. In the presence of missing data standard errors are estimated with less efficiency and thus the power to test the significance of an effect will decline (Little & Rhemtulla 2013). Studies in ecology and evolutionary biology are known to be under-powered in many cases (Møller & Jennions 2002; Jennions & Møller 2003) and so this has important consequences for the inferences drawn in a given study. This is particularly true for multi-level data that often require large sample sizes to achieve sufficiently high power (van de Pol 2012) or even in genotype–phenotyping mapping studies, such as GWAS (here using imputation can also improve power; e.g., Marchini & Howie 2010). Integrating a PMDD into one's experiment can recover the power lost after excluding missing data, facilitating the detection of small to moderate effects. To demonstrate how PMDD can improve inferences, even with multi-level hierarchical data, we conducted a couple simulations. In the first simulation, assume we are interested in estimating the between-individual level correlation between two traits (X_1 = "boldness" and X_2 = "time to emerge after predatory attack") using a multi-response model. We simulated three variables (X_1, X_2 and X_3) that follow a multi-variate normal distribution (MVN) with between (B) and within (W)-individual covariance matrices (assuming a standard deviation (SD) = 1) as follows:

$$\boldsymbol{X} \sim MVN\left(\begin{bmatrix} 2 \\ 4 \\ 10 \end{bmatrix}, \boldsymbol{B} + \boldsymbol{W}\right) \qquad \text{eqn. 1}$$

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$$\boldsymbol{B} = \begin{pmatrix} 1 & 0.40 & 0 \\ 0.40 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}, \ \boldsymbol{W} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

Note here that X_3 is not of interest, but if it was correlated with X_1, X_2 or missing data itself, it could be used as an auxiliary variable to improve the imputation process. For this example, we simulated 1500 datasets under varying sample sizes (100 – 1000) and levels of missing data (5–50%) and estimated the between– and within–individual covariance matrices using data augmentation (maximum likelihood approaches) in *ASReml–R* (vers. 3.4.1). Missing data was assumed to be MCAR throughout the data sets, as would be the case in a PMDD, and we evaluated how well imputation and complete case analyses performed in estimating the covariance between X_2 and X_1 under these varying situations (Figure 2). As expected, given our MCAR assumption, we did not see much impact at all on the point estimate (~0.40; Figure 2a & b). Imputation procedures actually tended to do slightly better at estimating the point estimate as there was a slight downward bias (although not significantly so) for the complete case analysis with small sample sizes and high levels of missing data in our simulation (Figure 2a & b). Despite this, we observed a major improvement in the estimation of standard errors when imputing data in comparison to the complete case analysis (Figure 2c & d), suggesting that imputation procedures, even with hierarchical data such as this, can lead to fairly substantial improvements in power. This is particularly important as many areas of research, such as quantitative genetics and behavioural ecology, are indeed interested variance partitioning methods such as this (Dingemanse & Dochtermann 2013; Brommer & Class 2017; Careau & Wilson 2017).

Ecological and evolutionary questions are often more complex than simply estimating variance components. Experiments will often combine experimental manipulations of independent individuals and repeatedly measure these individuals across their life. To understand the benefits of PMDD at improving statistical inferences when both fixed and random effects might be of interest we conducted a second simulation. Assume we have manipulated the early thermal environment of a sample of lizard eggs – a common approach in lizard research (e.g., Noble, Stenhouse & Schwanz 2017). We might be interested in understanding how these thermal environments affect the growth curves of animals within each treatment (Figure 3). We simulated data assuming that individuals follow a linear growth trajectory (random regression model), at least over the period in which we measured their weight, according to the following model:

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$$m_{ij} = (\beta_0 + \alpha_i) + \beta_1 T_{ij} + (\beta_2 + s_j)A_{ij} + \beta_3(A_{ij} \cdot T_{ij}) + \varepsilon_{ij} \qquad \text{eqn. 2}$$

where m_{ij} is the mass of individual *i* for observation *j*, T_{ij} is a dummy variable ('0' or '1') indicating whether individual *i* belongs to the control group (23ºC) or the treatment group (26ºC), A_{ij} is the age of individual *i* at observation *j*, β_1 is the contrast between the control group mean at age 0 (β_0) and the treatment mean at age 0, β_2 is the effect of age on mass, and β_3 is the interaction effect between change and mass across age and treatment group. α_i and s_j are individual level random effects assumed to follow an $\sim MVN\left(\begin{bmatrix}0\\0\end{bmatrix}, \begin{bmatrix}0.5 & 0.25\\0.25 & 0.4\end{bmatrix}\right)$ and ε_{ij} observation random effect (i.e., residual variance) assumed to follow an $\sim N(0,1)$. An example of the simulated data along with parameters are shown in Figure 3. If we were only limited in the total amount of sampling, say 100 observations, then we can design an experiment where 10 animals (5 / treatment) were each measured 10 times (*Scenario 1*) or we could use a PMDD whereby we increase the number of total individuals to n = 20 (10 / treatment), but instead measure each individual randomly only five times, instead of 10 (*Scenario 2*) (Figure 3). Under these two scenarios we simulated 1500 datasets and used *ASReml–R* to estimate the parameters and their standard errors in eqn. 2. Overall, scenario 2 had a substantial number of benefits both with respect to estimating fixed and random effects performing nearly as well a complete data (i.e., 20 individuals measured 10 times – *Scenario 3*). For all fixed effect estimates, there was between ~10–28% reduction in the standard errors with standard errors for the slopes and treatment interactions receiving a substantial boost. Most interestingly, we also see a greater ability to estimate random slopes more precisely (Table 2). Overall, our simulations suggest that PMDDs can provide power benefits under realistic experimental situations that are common in ecology and evolution.

(ii) Improved animal welfare: considering the three 'R's'

Central to research in ecology and evolutionary biology, particularly with respect to research on vertebrate animals, are issues surrounding animal welfare (Stamp Dawkins 2006; Barnard 2007; Cuthill 2007; Stamp Dawkins 2008; McMahon *et al.* 2012). Biological research on vertebrates, and indeed any study species, should strive to alleviate pain, suffering and distress caused by experimentation (Cuthill 2007). Nonetheless, some pain and distress is acceptable should the research being undertaken be justified and sufficient to advance

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knowledge. Critical to this point is the ability of research to '*advance knowledge*', as this requires researchers to strike a balance between experimentation that involves large samples of independent animals (reducing type II errors and allowing one to detect an effect of interest) and the stress inflicted on experimental subjects (Cuthill 2007). The ladder two points are often in conflict with each other, particularly when the experimentation involves invasive procedures, and we look to the three R's (Refinement, Replacement and Reduction) to strike a balance between these important points (Cuthill 2007). Research in ecology and evolutionary biology often requires animal subjects to address empirical questions, either in the lab or the field, and so, replacement in many cases is not a viable option. Therefore, empiricists primarily try to design experiments with Refinement and Reduction in mind.

Planned missing data design can be an important tool during the experimental planning stages that directly targets two of the three R's (Refinement and Reduction) to improve animal welfare. Often, PMDD can concurrently target both Refinement and Reduction at the same time by utilizing less invasive procedures and allowing data to be collected on fewer experimental subjects (or less often on a given subject). For example, we could use a subset measurement design to randomly assign the measurement of different physiological traits (e.g., metabolism, hormones) to a subset of subjects, reducing the number of subjects quantified on a given physiological measure. Additionally, fewer repeated physiological measurements can be done on a given subject by randomly assigning a different temporal sequence of measurements to individuals, refining the experimental design to reduce stress inflicted by repeated handling. Refinement can further occur by adopting a two-method design where different physiological assays measuring the same construct, say 'innate immunity', can be done in such a way that no one individual has both measurements, but rather is randomly assigned to the cheaper less invasive measure. In summary, PMDD can improve animal welfare without compromising our ability to effectively answer a given question by designing an experiment that has too little power.

(iii) Reduced research costs

One critical benefit of implementing PMDD is the ability to get a 'bigger bang for your buck' in terms of the research cost to outcome ratio. The cost savings when using a PMDD design can be substantial, particularly for experiments involving expensive biochemical, proteomic, metabolomic and genomic work. For example, take our example of measuring hormone profiles for individuals across a six-month activity period (Table 1b). Using this PMDD design we were able to reduce our sample size from n = 360 to n = 240 individual samples

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(i.e., using a subset measurement PMDD). If we assume that the cost of individual reactions to run assays, in addition to labour costs, was $8 per sample, then we would save $900 for this experiment. These additional savings could be used to run an additional follow up experiment or even go towards assaying a sub-sample of individuals on a second hormone that is known to interact with the first. Alternatively, we may be interested in quantifying telomere length to understand cellular senescence using either flow cytometry (estimated cost / sample = $68) or qPCR (estimated cost / sample = $13) methods (Nussey *et al.* 2014) to understand patterns of telomere attrition over the season in relation to hormones. In this example, using a PMDD would save $8160 for flow cytometry methods or $1560 using qPCR methods (assuming we really had money to do all animals). It also demonstrates how a two-method design can also save costs. Flow cytometry has been identified as a promising method for accurate, high throughput quantification of telomere length (Nussey *et al.* 2014). However, it is quite expensive compared to qPCR methods. A PMDD where a sample of animals are quantified both on qPCR and flow cytometry, and those missing flow cytometry data are then imputed would lead to a fairly substantial cost savings and allow one to verify the utility of both methods. We therefore view PMDD as a promising approach to improving cost efficiency.

(iv) Stronger inferences and testing predictions from multiple working hypotheses

Strong inference involves devising alternative hypotheses and then running an experiment or set of experiments to test alternative predictions generated from these hypotheses (Platt 1964; Chamberlain 1965). Identifying and testing among alternative hypotheses is the hallmark of rapid scientific progress, indeed Platt (1964) suggested that this was one of the primary reasons for the rapid advancement of molecular biology through the 1960s. Nonetheless, it is clear that ecological and evolutionary studies rarely test predictions from multiple working hypotheses (Betini, Avgar & Fryxell 2017). Betini et al. (2017) suggest a number of intellectual and practical barriers impeding the use of multiple working hypotheses, but particularly relevant for our argument are the barriers limiting one's ability to "execute" investigations involving multiple working hypotheses. Designing fully factorial experiments to disentangle predictions from alternative hypotheses is a major hurdle, referred to as the "*fallacy of the factorial design*" (Betini, Avgar & Fryxell 2017), whereby the addition of every new working hypothesis requires a new treatment or set of measurement variables leading to a geometric increase in number of required replicates. Including variables or experimental manipulations that test predictions from alternative hypotheses into a PMDD,

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can off-set the need for prohibitively large sample sizes and time-consuming and costly measurements. These approaches may maximize the information gained from an experiment to facilitate more rapid scientific progress compared to testing single working hypotheses. Additionally, predictions from hypotheses can be tested using the same sample of experimental subjects, reducing the spatial and temporal differences between experiments.

(v) Greater integration between research fields and disciplines

Understanding ecological and evolutionary processes requires an integrative, multidisciplinary approach to tackling research questions (Wake 2009). Integrative research includes assimilating researchers with diverse expertise to identify problems and articulate their solutions. Often it requires holistic approaches to address fundamental questions, including the use of observational, experimental and theoretical modelling (Wake 2009). Questions involving integrative research cut across traditional boundaries, often making use of novel, and sometimes expensive techniques to help facilitate an understanding of a system or process. Despite its benefits, it can often be challenging to implement in practice given the different norms in various research fields, along with the costs and logistical constraints in doing integrative work. Nonetheless, PMDD may help facilitate more multidisciplinary research efforts as it alleviates these constraints.

To demonstrate the potential of PMDD in facilitating integrative research, assume that we are interested in testing Pace-of-life syndrome (POLS) theory (Reale *et al.* 2010). POLS theory explicitly predicts covariation between physiological, behavioural and life-history traits (Biro & Stamps 2008; Careau *et al.* 2010; Reale *et al.* 2010). Integration of suites of behavioural traits can lead to consistent individual differences in behaviour (i.e. personality - Reale *et al.* 2010; Stamps & Groothuis 2010) that can form behavioural syndromes (Sih & Bell 2008; Stamps & Groothuis 2010; Sih & Del Guidice 2012). Importantly, physiological mechanisms are thought to be the primary mechanisms (e.g. hormones, metabolism; Biro & Stamps 2008; Biro & Stamps 2010; Careau *et al.* 2010; Reale *et al.* 2010) underpinning both behavioural and life-history variation in populations.

Pace-of-life theory is therefore highly integrative, requiring concurrent measurements of physiological, behavioural and life-history traits to understand their covariance. It often requires laborious, time intensive, and sometimes costly measurements of the same individuals over time. Repeated measurements of the same animals over long periods of time pose major hurdles in obtaining the high quality longitudinal data required to rigorously test POL's theory. Additionally, physiological measures (e.g. metabolism, hormones, ROS,

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immunity) can be costly to obtain (as indicated above) thus limiting the number of samples that can be collected. Nonetheless, the importance of taking many physiological measurements has been emphasized in different disciplines (Adamo 2004). Differences in the approaches and typical sample sizes of collaborators on might also be quite different and in conflict for such integrative projects.

Challenges in implementing planned missing data designs

As with any new research philosophy, there will be challenges, particularly in establishing the relevant and most suitable approaches that work across a wide diversity of different research questions and experimental designs in ecology and evolution. Given that PMDD is still new, stimulating interest in them will be the first step to identifying, solving and implementing solutions to some of the challenges that crop up. Below we discuss some of the hurdles we see to implementing PMDD and suggests some tentative solutions.

Unplanned missing data

Of course, as with any experiment, unplanned missing data will creep into PMDD designs. Often these data may be random, such as when a piece of equipment malfunctions during a set of measurements or when recording errors are identified and so the data are considered to be missing. Random instances of missing data, even if unplanned, will not affect the imputation process or the utility of PMDD unless missing data levels begin to get quite high. However, our simulations show, as well as others', that imputation procedures perform quite well even with large amounts of missing data (~50% – see Figure 1). Nonetheless, there are real situations where unplanned missing data can be MNAR and this will affect any experiment regardless of whether a PMDD is implemented or not. We have outlined above how data can be made MAR though the use of auxiliary variables, and these unplanned missing data, can then be imputed normally along with any planned missing data using the same statistical methods. We therefore advise colleagues to collect possible auxiliary variables where possible to counter unplanned missing data.

Imputation with generalised linear mixed effect models

Multiple imputation and DA both work well with normally distributed data, however, in reality variables often are non-normally distributed. While DA is limited to multivariate normality, MI procedures can also work with non-normal data fit using generalised linear

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mixed effect models (e.g., Poisson GLMMs) (Schafer 1997). However, implementation in the context of GLMMs is still under active development, and in many cases, is restricted to simple random effect structures (van Buuren & Groothuis-Oudshoorn 2011; Enders, Mistler & Keller 2016; Quartagno & Carpenter 2016; Audigier & Resche-Rigon 2017). Nonetheless, two-level random regression models can be run in a number of existing packages (e.g., *mice*) and we believe that the capacity to run more sophisticated models will grow in the near future.

To re-assure readers that imputation can and does work with non-normal distributions, we provide a simulated example along with a sample of R code to demonstrate MI procedures with GLMMs in Box 1 (all R code for simulations are provided as supplementary material). For our hypothetical example, assume we are interested in provisioning rates (the number of feeding visits by a parent) in a bird species, the fictitious Missing Capped Warbler (*Sylvia absenscapilla*). We would like to understand the costs of female provisioning by experimentally manipulating brood sizes (n = 6 chicks) in a random sample of birds compared to a control group, which has normal brood sizes (n = 3 chicks) (Liebl, Browning & Russell 2016). The Missing Capped Warbler is notoriously difficult to observe as it is found in thick scrub, and so, we placed cameras at random nests during the first two weeks of the breeding season to observe provisioning rates in the two treatments over a 5-hour period. Provisioning rates are known to change as the chicks develop (Khwaja *et al.* 2017), and so, the cameras were on each nest for a total of 20 days to understand how the demands of chicks change, and whether females can keep up with these demands. Unfortunately, it is a laborious process to observe all the resulting video for 40 birds measured over 20 days (a total of 4000 hours of video!). We therefore decided to implement a planned missing data design, where we randomly sampled a set of videos (n = 536 out of 800 videos; ~ 33% missing data) where provisioning rates can be quantified (cutting the total hours of video watching to 2680 hours). After a long and laborious field season, we were able to collect data that shows a tendency for experimentally elevated clutch sizes to have higher rates of provisioning that increase over the care period (Figure 2). The planned missing data can be imputed, for example, using the *mice* package (see Box 1). We see that the imputed data matches well with the complete simulated dataset, with nearly identical results (see table in Box 1).

Overcoming psychological barriers to missing data

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One of the biggest challenges to implementing PMDDs probably involves the need for researchers to over-come the 'psychological taboos' around missing data, and the suspicion of techniques for handling these missing data (Enders 2010). We can re-assure readers that missing data practices are now very well established (Graham *et al.* 2006; Nakagawa 2017), and are rather painlessly implemented in many commonly used statistical software such as R, *SAS*, *SPSS*, and *MPlus* (See Table 3 for an overview). In fact, many techniques are implemented by default when missing data is included as response variables in models for a number of mixed modelling packages (e.g. data augmentation procedures in '*MCMCglmm*' and '*ASReml*'). While statistical algorithms vary across these platforms, fairly sophisticated and versatile ones are now implemented in packages for some of the most widely used platforms (e.g. '*mice*', '*mi*', '*multimput*' and '*Amelia*' in the R environment – Table 3) that implement MI algorithms known to perform well under a wide variety of situations (Schafer & Graham 2002; van Buuren 2012; Enders, Mistler & Keller 2016; Quartagno & Carpenter 2016; Resche-Rigon & White 2016; Audigier *et al.* 2017). These techniques are under active development (e.g., the *mice* package in R), and so we envisage the breadth of problems these tools can tackle to increase and be even easier to apply in the future. Nonetheless, caution is still needed in their implementation as it is unclear whether imputation procedures perform well under all circumstances (Nakagawa 2017). Statistical procedures for missing data are still rarely taught in undergraduate and graduate level courses, so part of the solution will be to begin educating students and practitioners about how to perform imputation procedures, explicitly highlighting some of the challenges and caveats that need to be considered. Nonetheless, there are now excellent resources that provide an in depth look at imputation procedures (Gelman & Hill 2002; Schafer & Graham 2002; Graham 2009; Enders 2010; Su *et al.* 2011; van Buuren & Groothuis-Oudshoorn 2011; Nakagawa 2017).

Uncertainties surrounding the best PMDD

One challenge in implementing PMDD is the uncertainty around what the most appropriate missing data design is for a given experiment. This is particularly true in ecology and evolutionary biology because different questions, experimental systems, data structure and measurement variables may require creative combinations of different PMDD's that we discuss in our paper, or possibly even new ones! While we argue that the benefits of PMDD can be substantial it will still likely be important to test the robustness of any given design – possibly through simulations to test the power of different types of missing data designs. With some very simple simulated data based on effect sizes and experimental designs

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relevant to the question at hand, the power of different PMDD's can be thoroughly tested during the design stage of an experiment (Enders 2010). Enders (2010 p.g. 30) provide a nice introduction on how to conduct power analysis with PMDD's using simulations, and we provide all our R code which we hope can act as a skeleton for readers to familiarize themselves with simulations to help them sort out the best PMDD for their particular situation. Additionally, new multi-level simulation packages, such as SQuID, allow for researchers to simulate hierarchical data easily (Allegue *et al.* 2017). Data can be downloaded and a missing data introduced to evaluate the power of different PMDD's. While definitive guidelines will depend on the experimental design, question and covariance between traits, we believe that up to ~30% missing data overall across a wide variety of different designs will likely not compromise performance of imputation procedures.

Conclusions and future directions

Our goal was to put planned missing data design on the radar of ecologists and evolutionary biologists given the substantial number of ethical, logistical and cost saving benefits it affords. We have provided some guidance on possible PMDD that can be implemented in research programs and shown with simulations that even with hierarchical / multilevel data imputation procedures can perform quite well. While it is still unclear whether imputation procedures in a multilevel framework will work under all circumstances there is increasing awareness of the need to develop such techniques with hierarchical data and in many cases existing methods will likely perform well (Drechsler 2015; Quartagno & Carpenter 2016; Audigier & Resche-Rigon 2017; Audigier *et al.* 2017). We encourage colleagues to begin thinking about PMDD's and their utility in their research both to improve research quality and to promote integrative, cost effective research projects in ecology and evolutionary biology.

Acknowledgements

We would like to thank the I-DEEL lab (Joel Pick, Rose O'Dea, Malgorzata Lagisz, and Fonti Kar) and Diego Barneche for feedback on previous versions of our manuscript, along with anonymous reviewers for constructive comments that greatly improved our manuscript. DWAN was supported by an ARC Discovery Early Career Research Award (DE150101774) and SN an ARC Future Fellowship (FT130100268). The authors declare no conflicts of interest.

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Author's contributions

SN and DN conceived the research. DN and SN conducted the simulations. DN wrote the manuscript, with input from SN. SN contributed critically and constructively to the revisions of the manuscript.

Data Accessibility

All R code and simulation results used in the manuscript were provided upon submission.

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Tables and Figures

Table 1 – Two types of planned missing data designs relevant for ecological and evolutionary research. a) Subset measurement designs randomize a set of variables to be measured on a sample of individuals. Body mass (BM) is strongly correlated with all three other variables and so is measured on all individuals in the study (complete cases). In contrast, molecular determination of sex is needed with our species, and along with thyroxine (T4) can be costly to quantify so these traits are measured on a sample of individuals. Metabolism is also time consuming to measure and so is also only quantified on a sub-sample of animals. While we can estimate all main effects (i.e., single variables of interest) with this design, if interactions are of interest then one should have a PMDD that ensures there is enough data to effectively estimate the interaction parameters. b) Wave missing design can be applied to longitudinal data. Each wave contains a set of 10 random individuals and their measurement occasions across six months are indicated with '1'. Forty individuals are measured each month, but a different sub-sample of the 60 total through the experiment. Abbreviations: BM = body mass, S = Sex, M = metabolism; TH = thyroxine (T4).

a) Subset Measurement Design

Variable	**Variables**				**Interactions**	
subset	*BM*	*S*	*M*	*TH (T4)*	*M*S*	*M*BM*
S1	1	–	1	1	–	1
S2	1	1	–	1	–	–
S3	1	1	1	–	1	1
S4	1	–	–	1	–	–
S5	1	1	1	1	1	1

b) Wave Missing Design

Measurement	**Month**					
Wave	**1**	**2**	**3**	**4**	**5**	**6**
1	1	1	1	1	1	1
2	–	1	1	–	1	1
3	1	–	–	1	1	1
4	–	1	1	1	–	–
5	1	–	–	1	1	–
6	1	1	1	–	–	1

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Table 2 – Effect of two scenarios on the ability to estimate parameters of a mixed model. Average parameter estimates and their standard errors for both fixed and random effects over 1500 simulated datasets. Example data is shown in figure 2 and a description of the two scenarios are provided in the text. Abbreviations and symbols as follows: ρ: correlation between intercept (α) and slope (s); σ_α^2 = variance estimate for random intercept; σ_s^2 = variance estimate for random slope; trt = treatment; age = change in mass across age. % SE Decrease = the percent decrease in standard error from Scenario 1 compared to Scenario 2. "True" are the true parameters that the simulation was based on. Scenario 3 is also provided for comparison. In scenario 3, this is the full data set (i.e., 20 animals, measured 10 times).

	Scenario 1		**Scenario 2**		**Scenario 3**		**% SE Decrease**	**True**
Fixed Effects								
	Est.	***SE***	***Est.***	***SE***	***Est.***	***SE***		
intercept	1.21	0.45	1.22	0.40	1.19	0.31	9.83	1.20
trt	2.01	0.63	1.98	0.57	2.00	0.44	9.82	2
age	1.79	0.28	1.80	0.21	1.79	0.20	27.06	1.8
trt*age	1.61	0.40	1.60	0.29	1.60	0.28	27.06	1.6
Random Effects								
	Est.	***SE***	***Est.***	***SE***	***Est.***	***SE***		
σ_α^2	0.57	0.55	0.62	0.59	0.51	0.34	-6.96	0.50
$\rho(s,\alpha)$	0.24	0.25	0.23	0.21	0.24	0.16	16	0.25
σ_s^2	0.41	0.21	0.41	0.15	0.40	0.14	31.26	0.40

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Table 3 – Examples of common packages and statistical programs that can be used to deal with missing data. Abbreviations are as follows: SA = stand-alone program; MI = multiple imputation; DA = data augmentation; B = both, R = response, P = predictors. Y = yes.

Package	Prog.	Algorithms	Response/ Predictor	Multi-level	Reference / link
mi	R	MI	B	Y	Su et al. (2011)
mice	R	MI	B	Y	Van Buuren & Groothuis-Oudshoorn (2011)
micemd	R	MI	B	Y	Audigier & Resche-Rigon (2017)
jomo	R	MI	B	Y	Quartagno & Carpenter (2016)
Amelia	R	MI	B	Y	Honaker et al. (2011)
multimp	R	MI	B	Y	https://github.com/inbo/multimput
MCMCglmm	R	DA	R	Y	Hadfield (2010)
ASReml	R/SA	DA	B	Y	Butler (2009)
SAS	SA	DA/MI	B	Y	https://stats.idre.ucla.edu/sas/seminars/multiple-imputation-in-sas/mi_new_1/
SPSS	SA	DA/MI	B	?	https://www.ibm.com/ms-en/marketplace/spss-missing-values
MPlus	SA	DA	B	Y	https://www.statmodel.com/index.shtml

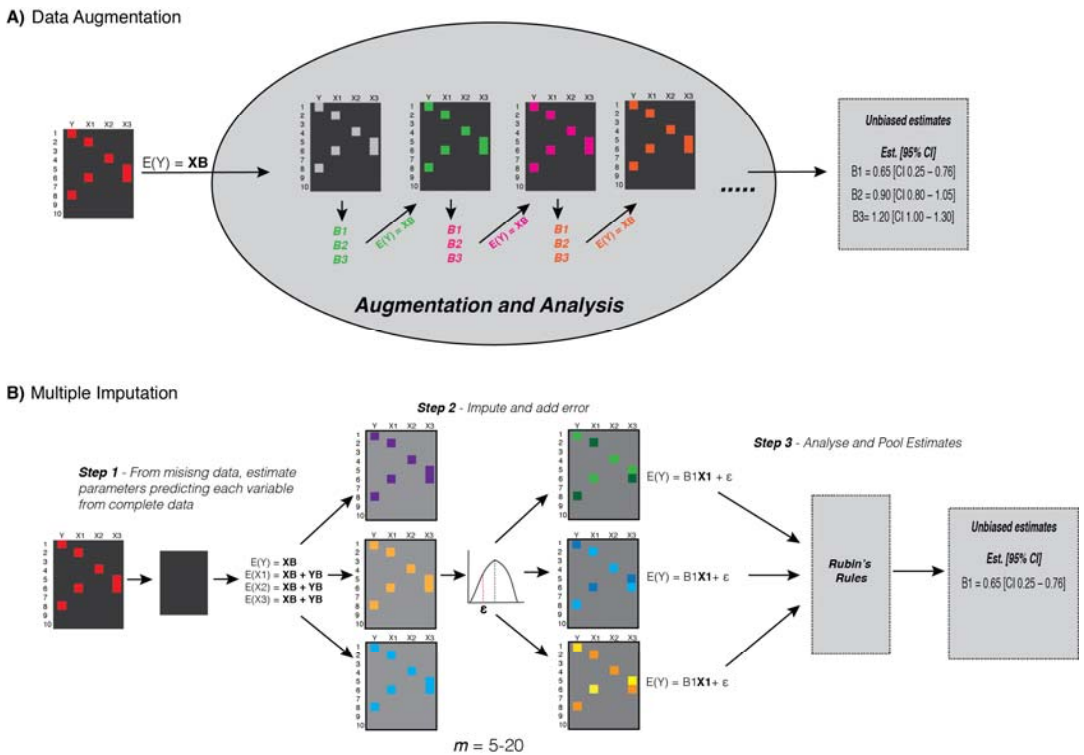

Figure 1 – Two major types of imputation procedures A) data augmentation (e.g., full information maximum likelihood, expectation maximization) and B) multiple imputation. Each large square represents a dataset containing four variables (Y, X1, X2, X3) and n = 10 observations. Small red squares represent missing data and black squares complete data. Data augmentation procedures (A) take both observed and missing data in the analysis under a pre-specified model [E(Y) = **XB**)], augment missing data, estimate parameter estimates (B1, B2, B3) and then re-iterate this process with updated parameters [different coloured B1, B2, B3 and E(Y) = XB] until the model converges on a set of unbiased parameter estimates. Multiple imputation (B) uses complete data and often (but not always) uses other variables within the data as predictors of a specific variable. It then imputes using regression equations plausible values of missing data for *m* complete datasets. To prevent biased estimates residual error is added to each of the imputed data points (checkered small squares in step 2). These *m* datasets are then analysed with a given model, which can be different from the ones used to impute and, using Rubin's rules (Rubin 1987), pool the parameter estimates across datasets (in this case B1). Abbreviations are as follows: E = expectation of variable, or mean estimate of variable; X = design matrix; B = vector of parameter estimates (e.g., B1, B2, B3); ε = residual effect or random error.

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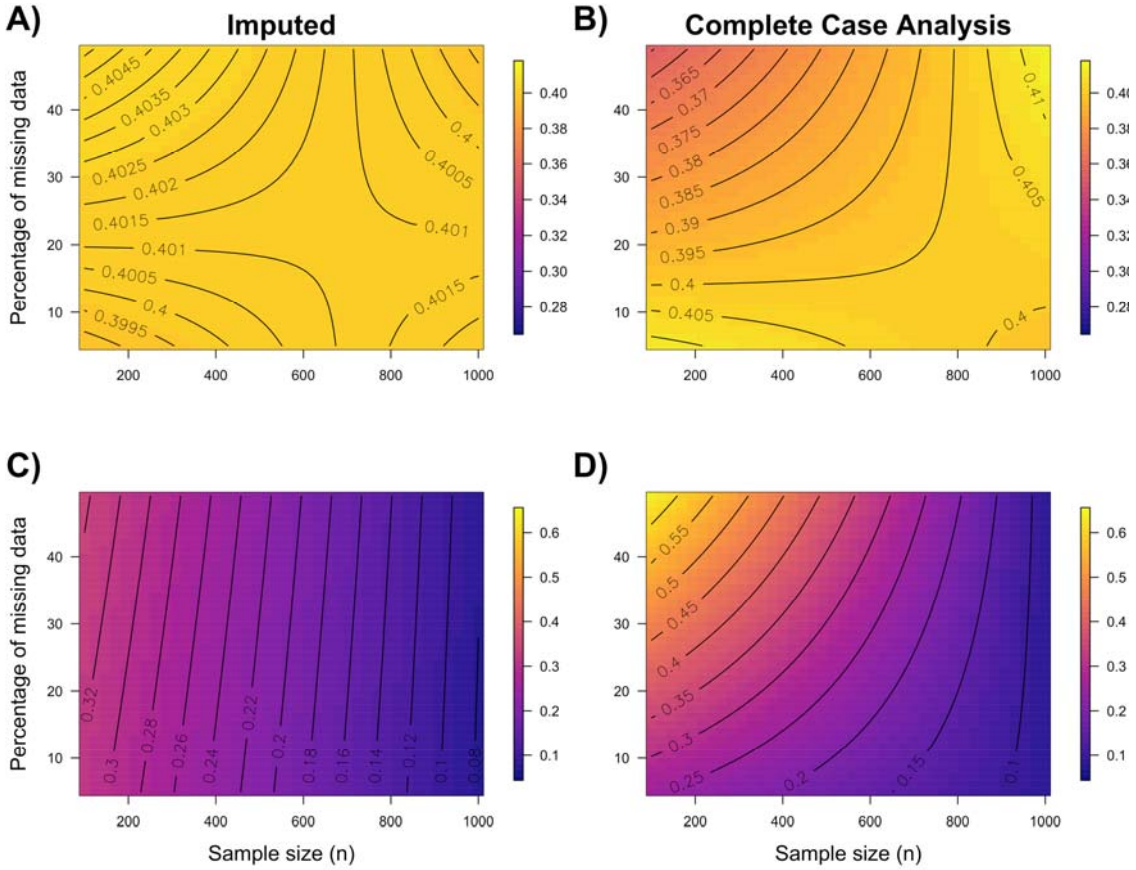

Figure 2 – Simulation results of the estimation of a between–individual correlation for two traits [$(X_1, X_2) = 0.40$] under varying levels of missing data (5–50%) and sample sizes (n = 100–1000). Plots show average point estimates (a & b) and their corresponding standard errors (c & d) from 1500 randomly generated datasets when imputing missing data (a & c) or running a complete case analysis (excluding missing data – b & d). Note that convergence problems are more prevalent with small samples and high levels of missing data. Parameter estimates and their precisions are therefore summarised on simulations in which models did converge.

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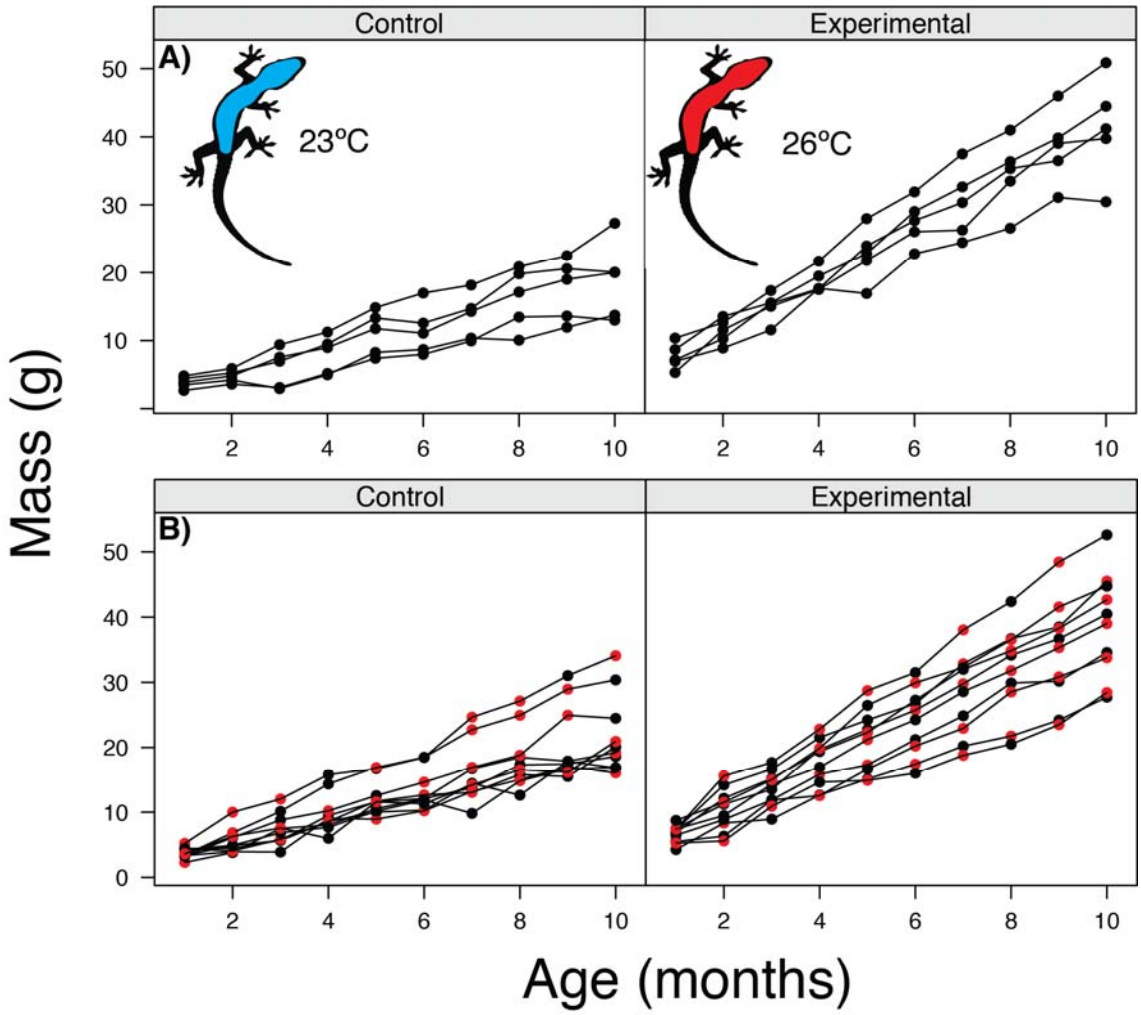

Figure 3 – Example of experimental data on growth rates across age for two simulated scenarios. A) Mass of n = 10 lizards for a control group incubated at 23ºC and experimental group incubated at 26ºC. In this scenario, each animal was measured 10 times across the first 10 months of age. B) Mass of n = 20 lizards for a control group 23ºC and experimental group incubated at 26ºC was measured five times with 50% of the mass data on each of the 20 animals considered missing ('red' points). In both scenarios, there were main effects of treatment and an interaction between growth across ages and treatment. Individual lizards varied in both their intercept and slope (see text for more details). Data was simulated according to eqn. 2 with the following parameters: β_0= 1.2; β_1= 2; β_2= 1.8; β_3 = 1.6. In scenario B, missing data was imputed using likelihood based approaches in ASReml-R.

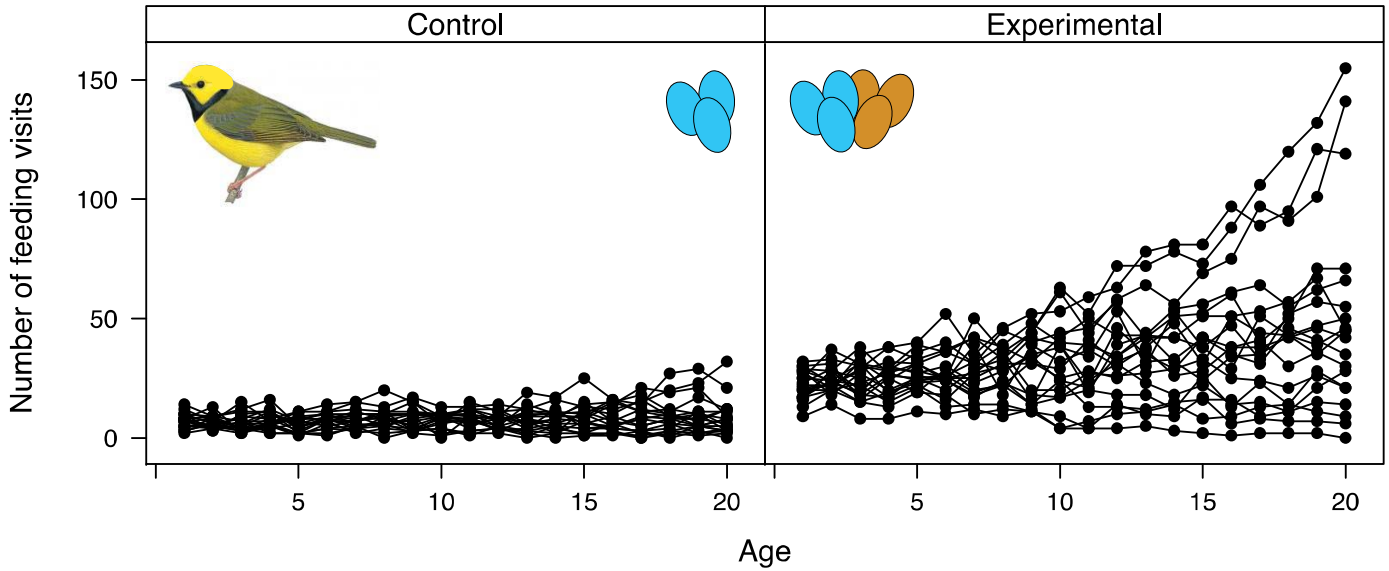

Figure 4– Example data showing the provisioning rates (number of feeding visits within a 5-hour period) for control (3 eggs) and experimentally elevated (6 eggs) brood sizes in the fictitious Missing Capped Warbler across the first 20 days of chick age. Provisions were simulated assuming a Poisson error distribution using the following model: $n_{ij} = (\beta_0 + \alpha_i) + \beta_1 T_{ij} + (\beta_2 + s_j)A_{ij} + \varepsilon_{ij}$ where Provisions = $\log(n_{ij})$. $\beta_0 = 2$; $\beta_1 = 1$; $\beta_2 = 0.01$ with a random intercept and slope variance and covariance matrix as: $\sim MVN\left(\begin{bmatrix}0\\0\end{bmatrix}, \begin{bmatrix}0.01 & 0\\ 0 & 0.0025\end{bmatrix}\right)$.

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BOX 1

Multiple imputation (MI) for a few different generalised family types can be implemented in the *mice* package (van Buuren & Groothuis-Oudshoorn 2011). Given that our data (number of visits in 5 hours) is Poisson distributed (or nearly so), and hierarchical in nature, we will need to impute using a new add-on developed in the *countimp* package (Kleinke & Reinecke 2013), which imputes Poisson random regressions. It can be installed, along with other needed packages in R as follows:

```
> link <- "http://www.uni-bielefeld.de/soz/kds/software/countimp_1.0.tar.gz"
> install.packages(link, repos=NULL, type="source")
> library(countimp)
> library(mice)
> library(glmmADMB)
```

Our data, including the missing data is set up as follows:

```
> head(data)
  ind age trt     trt_name provision
1   1   1   1 Experimental        NA
2   1   2   1 Experimental        22
3   1   3   1 Experimental        29
4   1   4   1 Experimental        38
5   1   5   1 Experimental        33
6   1   6   1 Experimental        37
```

In total, we have approximately 33% missing data at the individual level. To impute these missing data, we need to first set up the predictor matrix to define what variables are class (random effect groups) and what are to be used as random and fixed effects:

```
> data$ind <- as.integer(data$ind) # Need to keep class variable as integer
> imp <- mice(data, maxint = 0, printFlag = FALSE) # Do quick run of mice to set up pred matrix
> pred <- imp$pred # Extract the pred matrix
```

Now that we have the predictor matrix, to run a multi-level imputation we need to change the predictor matrix row for provision to define the class variable (i.e., random effect group – set as '–2' – only one level can be included currently), the variable included as both a fixed and random effect (i.e., age – set as '2' because we have a random regression model) and we will set our 'trt' as a fixed effect only (i.e., set as '1') as follows:

```
> pred["provision", ] <- c(-2,2,1,0,0)
```

The '0' is used to tell mice not to include these variables as predictors in the imputation. Now that this is set up we can run multiple imputation telling mice that the 'provision' variable is a 2-level Poisson variable:

```
> imp <- mice(data, m = 20, meth = c("","", "","","","2l.poisson"), pred = pred)
```

This will impute missing information in the 'provision' variable creating m = 20 'filled in' datasets for which estimates and standard errors can be pooled as follows:

```
> fit <- do.mira(imp = imp, DV = "provision", fixedeff = "trt+age", randeff = "1 + age",
grp = "ind", id = "ind", fam = "poisson")
> summary(fit)
```

We can compare the overall pooled estimates with estimates from our 'real complete dataset' (assuming we watched all videos):

Full Dataset			**Imputed Dataset**	
Fixed Effects			*Pooled Fixed Effects*	
	Estimate	**SE**	**Estimate**	**SE**
Intercept	1.93	0.05	1.93	0.06
trt	1.13	0.06	1.13	0.07
age	0.01	0.01	0.01	0.01
Random Effects			*Pooled Random Effects*	
	Estimate		**Estimate**	
Intercept	0.011		0.015	
Age	0.0025		0.0023	

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