**Methods for testing publication bias in ecological and evolutionary meta-analyses**

Shinichi Nakagawa1\*, Malgorzata Lagisz1, Michael D. Jennions2, Julia Koricheva3, Daniel W.A. Noble2, Timothy H. Parker4, Alfredo Sánchez-Tójar5, Yefeng Yang1, & Rose E. O’Dea1

1 Evolution & Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia

2 Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT, Australia

3 Department of Biological Sciences, Royal Holloway University of London, Egham, Surrey, TW20 0EX, U.K.

4 Department of Biology, Whitman College, Walla Walla, WA 99362, U.S.A

5 Evolutionary Biology, Bielefeld University, Bielefeld, 33615, Germany

\* Correspondence: S. Nakagawa

e-mail: [s.nakagawa@unsw.edu.au](mailto:s.nakagawa@unsw.edu.au)

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**ORCID**

Shinichi Nakagawa: 0000-0002-7765-5182; Malgorzata Lagisz: 0000-0002-3993-6127; Michael D. Jennions: 0000-0001-9221-2788; Julia Koricheva: 0000-0002-9033-0171- Daniel W. A. Noble: 0000-0001-9460-8743; Timothy H. Parker: 0000-0003-2995-5284; Alfredo Sánchez-Tójar: 0000-0002-2886-0649; Yefeng Yang: 0000-0002-8610-4016; Rose E. O’Dea: 0000-0001-8177-5075

# Abstract

1. Publication bias threatens the validity of quantitative evidence from meta-analyses. Under publication bias, some findings are overrepresented in meta-analytic datasets because they are published more frequently or quickly (e.g., ‘positive’ results). Unfortunately, methods to test for the presence of publication bias, or assess its impact on meta-analytic results, are unsuitable for datasets with high heterogeneity and non-independence, as is common in ecology and evolutionary biology.
2. Here we aim to increase the use of appropriate publication bias tests in ecology and evolutionary biology. After defining basic statistical terminologies such as sampling variance, precision, and weight, we review both classic and emerging publication bias tests (e.g., funnel plots, Egger’s regression, cumulative meta-analysis, fail-safe *N*, trim-and-fill tests, *p*-curve and selection models), showing that some tests cannot handle heterogeneity, and, more importantly, none of the methods can deal with non-independence. For each method we estimate current usage in ecology and evolutionary biology, based on a representative sample of 102 meta-analyses published in last ten years.
3. Then, we propose a new method using multilevel meta-regression, which can model both heterogeneity and non-independence, by extending existing regression-based methods (i.e. Egger’s regression). We describe how our multilevel meta-regression can test not only publication bias, but also time-lag bias, and how it can be supplemented by residual funnel plots.
4. Overall, we provide ecologists and evolutionary biologist with practical recommendations on which methods are appropriate to employ under independent and non-independent effect sizes. No method is ideal, and more simulation studies are required to understand how false-negative and false-positive rates are impacted by complex data structures. Still, limitations of these methods do not justify ignoring publication bias in ecological and evolutionary meta-analyses.

**KEYWORDS:** Outcome reporting bias, p-hacking, multilevel meta-analysis, selection bias, radial plot, effective sample size; time-lag bias, decline effect

# 1 | INTRODUCTION

Evidence from meta-analyses often drives future research, and sometimes leads to changes in policy and practice (Nakagawa *et al.*, 2017; Gurevitch *et al.*, 2018). Therefore, it is essential for meta-analytic evidence to minimise bias. However, the validity of meta-analytic results can be compromised by publication bias (Marks-Anglin *et al.*, 2021). Publication bias occurs when a subset of research findings, such as statistically non-significant results, are less likely to be published (e.g., the file drawer problem; Rosenthal, 1979). In a wider sense, publication bias could encompass many different types of bias relating to dissemination of evidence (see Moller & Jennions, 2001; Jennions *et al.*, 2013; Marks-Anglin *et al.*, 2021). In this article, the following two types are most relevant: 1) outcome reporting bias, where selective reporting occurs within published studies (Marks-Anglin & Chen, 2020; 2021); and 2) time-lag bias, where positive results are published earlier than negative results (Trkalinos & Ioannidis, 2005; Koricheva, Jennions & Lau, 2013; Koricheva & Kulinskaya, 2019). Regardless of how publication bias occurs, if published findings are unrepresentative of all available evidence then meta-analytic results can be distorted.

Statisticians and methodologists have proposed numerous methods to test for publication bias. These tests can be broadly categorised into two types: those that detect publication bias, and those that also assess the impact of publication bias (Sutton, 2009). Both of these categories have been routinely used in meta-analyses in medical and social sciences (Rothstein, Sutton & Borenstein, 2005). However, in a survey of 100 meta-analyses in ecology and evolution, only 49% tested for publication bias, with just 22% conducting both types of tests (Nakagawa & Santos, 2012). In another survey, only 31% of 322 ecological meta-analyses reported at least one test of publication bias (Koricheva & Gurevitch, 2014). Low uptake might reflect that many currently available tests for publication bias are unsuitable for ecological and evolutionary meta-analyses (Nakagawa & Santos, 2012).

Two features common to meta-analytic datasets in ecology and evolution pose problems for publication bias tests: high levels of heterogeneity and non-independence. Importantly, many currently available tests for publication bias fail when there are high levels of heterogeneity (e.g., Macaskill, Walter & Irwig, 2001; Sterne, Egger & Smith, 2001; Moreno *et al.*, 2009). Furthermore, Nakagawa and Santos (2012) noted that, at the time, there were no statistical methods to test for publication bias that could explicitly account for non-independent effect sizes. Highly heterogeneous data are common in ecology and evolutionary biology, as research questions span many types of ecosystems and species. Non-independence is pervasive because many studies produce multiple effect sizes and, if a meta-analytic dataset includes multiple species, then effect sizes will also be correlated due to phylogenetic relatedness (Noble *et al.*, 2017). If a publication bias test were to be useful in ecology and evolution, we would need a test that could adequately handle both heterogeneity and non-independence (cf. Fernandez-Castilla *et al.*, 2019; Rodgers & Pustejovsky, 2020).

Our aim for this article is two-fold. First, we review classic and emerging methods for publication bias by conducting a new survey using 102 meta-analyses in ecology and evolution. Second, we introduce a method that both detects and adjusts for publication bias, while dealing with heterogeneity and non-independence among effect sizes. To make our article widely accessible, we start by revising key statistical concepts in meta-analysis such as sampling variance, weights, and heterogeneity (readers who are familiar with these concepts can, therefore, skip the next section).

# 2 | KEY STATSTICAL CONCEPTS

## 2.1 | Sampling variance, standard error, precision and weight

In ecology and evolution, three standardised effect statistics are most common (Nakagawa & Santos, 2012; Koricheva & Gurevitch, 2014). The first effect statistic is the standardised mean difference, SMD (also known as Cohen’s *d* or Hedges’ *g*), whose point estimate and sampling variance can be written as (Hedges & Olkin, 1985):

where the *i*th effect size (SMD) and sampling variance (Var) are a function of the means (), standard deviations (SD of sample) and sample size (*n*) of the two groups (1 and 2). Second, the logarithm of response ratio (Hedges, Gurevitch & Curtis, 1999; also known as the ratio of means; Friedrich, Adhikari & Beyene, 2008) can be written as:

where the notations are the same as above (see also Lajeunesse, 2015; Senior, Viechtbauer & Nakagawa, 2020). Finally, Fisher’s transformation of the correlation coefficient, *Zr* (unbounded and normally distributed), can be written as (Hedges & Olkin, 1985):

where *ni*is the *i*th sample size used to obtain the correlation coefficient, *ri*. Incidentally, the variance of the correlation coefficient is: , although a meta-analysis using *r*, which is bounded at -1 and 1, is not recommended (see a relevant point in Section 4.2).

Sampling variance is the heart of meta-analysis as this quantity, which is always a function of sample size, indicates (un)certainty for the point estimate of each effect size (see equations above). It is important to note that sampling variance, (sampling) standard error, precision, and weight are often used interchangeably in the meta-analytic literature; for example, a point estimate with high certainty has low standard error and variance, but high precision and weight (Figure 1).

## 2.2 | Heterogeneity

Ecologists and evolutionary biologists predominately use a ‘random-effects model’ of meta-analysis rather than a ‘fixed-effect model’ (Nakagawa & Santos, 2012; Koricheva & Gurevitch, 2014). A fixed-effect model assumes that a common overall mean exists among the population of effect sizes (i.e. homogeneity). A random-effects model and its extensions, on the other hand, assume each study has its own mean estimate (for an extension, see Section 4.1; Nakagawa & Santos, 2012; see also Figure 4 in Nakagawa *et al.*, 2017). A random-effects model can be written as:

where *s*i is the between-study (effect-size) effect for the *i*th effect size, normally distributed with a mean of zero and a variance of (which is more commonly referred to as ; note when = 0, this model reduces to a fixed-effect model), and *mi*is the sampling error for the *i*th effect size, distributed with the *i*th sampling variance (note that *i* = 1, 2, …, *Neffect-size*, the number of effect sizes; when *Neffect-size* = *Nstudy*, the number of studies, effect sizes are usually independent). The proportion of against the total variance is often quantified as where is referred to as the ‘typical’ within-study (sampling) variance, which can be considered as a mean value of (Higgins & Thompson, 2002). In ecological and evolutionary meta-analyses, *I*2 is around 90%, on average, meaning only ~10% of variation among effect sizes is due to sampling variance (Senior *et al.*, 2016). Therefore, publication bias tests assuming homogeneity (*I*2 or = 0) are unlikely to be useful for ecology and evolution.

# 3 | PUBLICATION BIAS TESTS

The primary goal of this section is to provide a non-exhaustive but up-to-date overview of publication bias tests, both classic and emerging, especially for ecologists and evolutionary biologists (cf. Moller & Jennions, 2001; Jennions *et al.*, 2013; for thorough and technical reviews, see Rothstein, Sutton & Borenstein, 2005; Vevea, Coburn & Sutton, 2019; Marks-Anglin & Chen, 2020; Marks-Anglin *et al.*, 2021). Therefore, we summarise different methods of testing for the presence of publication bias and assessing its impact on meta-analytic findings, and describe which methods are suitable for datasets with high heterogeneity and non-independence. Our recent survey of publication bias tests used in 102 ecology and evolutionary meta-analyses indicates many of these methods will be unfamiliar to ecologists and evolutionary biologists; Figure 2 shows the results of the survey (for the details of survey procedure see Supporting Information, Appendix S1).

Following Sutton (2009) (see also Vevea, Coburn & Sutton, 2019), we categorise publication bias tests into two types: 1) detecting publication bias (e.g., funnel plots, Egger’s regression; Section 3.1), and 2) assessing the impact of publication bias (e.g., Fail-safe *N*, trim-and-fill method, and selection models; Section 3.2). Publication bias, including outcome reporting bias, creates patterns of missing data (known as ‘funnel asymmetry’; see the next section). Commonly, the magnitude of the overall effect is exaggerated because statistically non-significant effect sizes are less likely to be published, especially when they are based on small sample sizes. For time-lag bias, statistical significance mediates publication year more than publication outcome, so that this bias requires different tests from publication and outcome reporting bias (see Section 3.1.3).

## 3.1 | Detecting publication bias

### 3.1.1 | Funnel plots

In the absence of publication bias and heterogeneity, plotting effect sizes against a measure of certainty (or uncertainty; see Figure 1) should produce a symmetrical funnel shape around the overall effect, referred to as funnel plots. These graphs are the most popular method for detecting publication bias among ecological and evolutionary meta-analyses (Figure 2). Funnel plots are also the most preferred graphical tool to detect publication bias in medical and social sciences (Sterne, Becker & Egger, 2005; Sutton, 2009; Vevea, Coburn & Sutton, 2019; Marks-Anglin & Chen, 2020), even though many other graphical methods have been proposed such as weighted histograms and normal quantile plots of effect sizes (as in Figure 2; for other graphical methods, see Rothstein, Sutton & Borenstein, 2005; Marks-Anglin & Chen, 2020).

The original funnel plot used sample size as the measure of uncertainty (Light & Pillemer, 1984; Figure 3a). Yet, more recent recommendations are to use either SE, precision, variance or the inverse of variance (Figure 1; Sterne, Becker & Egger, 2005; but for why sample size may often be preferred, see Section 4.3). For these four quantities, unlike for sample size, we can draw 95% confidence intervals (based on the *y*-axis; 1.96 x SE) that create a funnel, showing the degree of heterogeneity among effect sizes (if data are homogeneous, most dots will be inside the 95% confidence interval region, e.g., Figure 3b & c). This confidence region also makes it easier to see funnel asymmetry caused by the lack of statistically non-significant effect sizes with high uncertainties (see Figure 3b & c). In a similar vein, a contour-enhanced funnel plot shows different statistical significance regions (around 0) to help detect asymmetry (Peters *et al.*, 2008; Figure 3c). Lastly, Kossmeier, and colleagues (2020) have recently proposed a sunset funnel plot, a type of contour-enhanced plot, which adds visual indicators of statistical power (Figure 3d).

One of limitations of funnel plots is that funnel asymmetry can be caused by something other than publication bias (as in Figure 3b, missing large effect sizes of high uncertainties; see also Terrin, Schmid & Lau, 2005). The most important source is heterogeneity among effect sizes, which can create asymmetries of many kinds (Figure 3c & g); the other sources of asymmetry are data irregularities (e.g., mistakes, frauds, unique observations; cf. Nakagawa & Lagisz, 2016), artefacts (see Section 4.3), and chance (Egger *et al.*, 1997). As mentioned above, high heterogeneity is common in ecological and evolutionary meta-analyses (Senior *et al.*, 2016). Therefore, a standard funnel plot is unlikely to be informative about publication bias. To account for some of the heterogeneity, several researchers recommended plotting residuals from a meta-regression model (Figure 3e; e.g., Roberts & Stanley, 2005). In practice, however, no meta-regression model would explain all the heterogeneity. The remaining heterogeneity could still generate asymmetry in a residual funnel plot. The funnel plot should, therefore, be seen as a tool to explore small-study effects where effect sizes based on small sample sizes tend to be larger. Small-study effects may indicate publication bias, but not necessarily (Sterne, Becker & Egger, 2005). Although extensive work exists on funnel plots and heterogeneity, no systematic studies exist asking how funnel plots would perform under the scenario where effect sizes are correlated (but see Section 4.1).

Before moving to the next section where we introduce inferential tests of funnel asymmetry (or small-study effects), the radial plot proposed by Galbraith (1988) is worth mentioning, even though our survey found no use of these plots. The idea of a radial plot is similar to that of a funnel plot. The radial plot shows effect sizes divided by their SEs (essentially, *z* scores) on the *y*-axis and corresponding precisions on the x-axis. The plot, as in Figure 3f, has a slope with a zero intercept (solid line) and its 95% confidence interval based on lines drawn from ±1.96 values (dashed lines) with the steepness of the slope representing the overall mean. The radial plot is useful for visually detecting heterogeneity because data are completely homogeneous when all the data are inside this rectangle (analogous to a funnel shape in funnel plots). These axes of the radial plot (not those of the funnel plot) help us better understand the original inferential test for observed funnel asymmetry, the so-called Egger’s regression (Egger *et al.*, 1997), which is our next topic.

### 3.1.2 | Regression- and correlation-based methods

Egger’s or Egger regression in its original form can be written as:

where *zi*is the *i*th *z* score obtained from dividing an effect size by its SE (*yi/sei*), is the intercept, is the slope for the precision (*prec* or 1/ *se*) and *e* is residuals, normally distributed with a variance of . When (not ) is significantly different from zero, then we statistically detected funnel asymmetry (Figure 4a); the more deviates from zero, the more severe the asymmetry.

Although Egger’s regression checks for asymmetry in a funnel plot, Equation 8 does not have effect sizes as a variable, while a funnel plot does (Figure 3). We intuitively like to draw a regression line ( and ) not just the line of the intercept () in a funnel plot, but doing so is incorrect. However, it is possible to reformulate Egger’s regression (Equation 8) into a weighted regression, as follows (Thompson & Sharp, 1999):

where *yi* is the *i*th effect size and is the residuals, normally distributed with a variance of , which is sampling variance (*v*) and the multiplicative parameter () estimated in the weighted regression (in a meta-regression, is set to be 1, which assumes that *vi* is the exact sampling variance; see the next equation and also cf. Equation 7). Notably, Equation 8’s is identical to Equation 9’s and also Equation 8’s is identical to Equation 9’s (we demonstrate this in Supplementary Information, Appendix S2). Therefore, we can now look at the statistical significance of the slope of SE (*sei*in Equation 9), whose magnitude indicates the severity of asymmetry, and we are also able to put a regression line through a funnel plot (Figure 4b).

Given that Equation 9 is very similar to a meta-regression, later versions of Egger’s regression variants have taken the same form as a meta-regression (Moreno *et al.*, 2009), for example:

which is the same as Equations 7 (the random-effects model) plus the slope of SE () (note that different variants have precision, variance o the inverse of variance instead of SE; Moreno *et al.*, 2009).

According to simulation studies (Macaskill, Walter & Irwig, 2001; Sterne, Egger & Smith, 2001; Moreno *et al.*, 2009), Egger’s regression and its variants suffer from low power and poor performance when there are fewer than 20 effect sizes, or when the effect is large. However, meta-analyses in ecology and evolution often include over 20 effect sizes and our overall effect is usually small (Senior *et al.*, 2016). Therefore, the regression-based method for publication bias is likely to be of use, at least to detect small-study effects. Furthermore, in this meta-regression formulation, two things are possible: 1) adding moderators to absorb some heterogeneity, and 2) using multilevel meta-analysis to account for non-independence among effect sizes. We expand these possibilities in Section 4.

Similar to regression-based publication bias tests, correlation-based methods also statistically test for a relationship between effect sizes and corresponding uncertainties (e.g. sampling variance). All the correlation methods are based on a version of the rank correlation test first proposed by Begg and Mazumdar (1994). This method essentially calculates a Kendall’s rank correlation between effect sizes and their sampling variance (or other uncertainty measures, including sample size); a statistically significant correlation can indicate a small-study effect. Thus, it is very simple to implement, but it seems that the rank correlation is not as powerful as Egger’s regression under many circumstances (Macaskill, Walter & Irwig, 2001). Also, a recent simulation shows that the rank correlation methods, using both sampling variance and sampling size, had severely inflated Type I error rates when effect sizes are correlated (Fernandez-Castilla *et al.*, 2019). Therefore, we recommend meta-analysis authors and peer-reviewers preference regression-based methods over correlation-based methods to test for publication bias (in our survey, these methods were roughly equally popular, being reporting in around 10% of papers; Figure 2).

### 3.1.3 | Time-lag bias tests

Time-lag bias occurs when larger or significant effects are published more quickly than smaller or non-significant effects, and can manifest as a decline in the magnitude of the overall effect over time (i.e., a decline effect; Koricheva & Kulinskaya, 2019). According to our survey (Figure 2), fewer than 5% of meta-analyses in ecology and evolution tested for this type of publication bias. This is concerning, as time-lag bias is likely to be prevalent in ecology and evolution (Jennions & Moller, 2002; Sanchez-Tojar *et al.*, 2018). To test for time-lag bias, we caution against using correlation-based methods, because this approach does not account for different precisions of effect sizes (e.g., quantifying a rank correlation between effect size and publication year; Barto & Rillig, 2012). Instead, there are two recommended ways to investigate time-lag bias (or a decline effect): 1) using a cumulative meta-analysis, and 2) using a regression-based method (see Trkalinos & Ioannidis, 2005; Koricheva, Jennions & Lau, 2013; Koricheva & Kulinskaya, 2019).

Cumulative meta-analysis is where a meta-analytic model (e.g., random-effects model) is applied to a set of effect sizes, which is increased by one effect size at a time iteratively (starting from the oldest effect size). Then, the results are displayed as a forest plot (see Figure 4c). One can easily see when significance or magnitude of the overall effect size changes over time. When multiple effect sizes are obtained from each study, adding one study (one or more effect sizes) rather than one effect size is more practical. For complex data structures (see Section 4.1), limited sample sizes might prevent models from running in the early years of the dataset.

The second method is based on regression and is easy to fit, for example (cf. Equation 10):

where *yeari* is the publication year for the *i*th study (effect size). As with Equation 8, this method can accommodate other moderators (i.e. potential confounding variables) and also can be extendable to model non-independent effect sizes (see Section 4.2).

## 3.2 | Assessing the impact of publication bias

### 3.2.1 | Fail-safe *N*

We now move to the methods that can assess the impact of publication bias rather than merely detecting publication bias. Fail-safe *N* (also known as the ‘file-drawer number’) represents the number of negative unpublished results needed to make a statistically significant overall effect non-significant (e.g., Rosenthal, 1979; Rosenberg, 2005) or negligible (e.g., Owrin, 1983). The original fail-safe approach by Rosenthal (1979) is the oldest publication bias method and probably the simplest:

where *zi* is the *i*th *z* value (*yi/sei*) as in Equation 7 and 1.645 is the *z* value for α = 0.05 (the one-tailed test). The method by Owrin (1983) relies on the magnitude of the effect size rather than statistical significance; one version of this method can be written as:

where is the overall mean (i.e. an estimate from a fixed-effect model) and *yn* is the effect size value that is considered to be small or negligible. Although Rosenthal’s and Orwin’s fail-safe numbers ignore sample sizes (uncertainty) of effect sizes in the dataset, the method proposed by Rosenberg (2005) explicitly includes such information. An equation that assumes a fixed-effect model can be written as:

where *wi* is the inverse of sampling variance (1/*vi*; note that *wi* can be modified for a random-effects model) and denotes the *t* value with the α level of 0.05 with the number of studies (effect sizes) as the degrees of freedom, DF (for the use of a different DF, see Rosenberg, 2005).

Although fail-safe approaches are the most popular method after the funnel plot in our survey (14.1%), Becker (2005) has called for abandoning all the fail-safe approaches, given the other methods for publication bias available. She has argued that the fail-safe *N* is difficult to interpret (e.g., no criterion on what constitutes a small or large *N)*, and also that depending on the exact method, a variety of fail-safe numbers can be obtained for the same data set. For example, the *R* package *metafor* implements the three methods above (Viechtbauer, 2010); its example dataset shows *NRosenthal* = 598, *NOwrin* = 13, and *NRosenberg* = 370 (for details, see Supporting Information, Appendix S3). Unfortunately, none of the proposed methods adequately control for heterogeneity (e.g., by incorporating moderators) nor non-independence among effect sizes. Furthermore, none of the methods of fail-safe *N* are inferential.

### 3.2.2 | Trim-and-fill tests

The trim-and-fill test provides a non-parametric method that can visualize potentially missing data, and statistically both detects and corrects for funnel asymmetry (Duval & Tweedie, 2000b; Duval & Tweedie, 2000a). A recent survey showed that the number of studies using the trim-and-fill method is increasing every year (in 2018, over 2000 meta-analyses used this method; Shi & Lin, 2019), and this method is not exceedingly rare in ecology and evolution (7.5% of the meta-analyses in our survey). In short, this method uses an iterative process to determine how many effect sizes are missing (say, *Nmissing*) from a funnel, using an initial overall estimate and one of three estimators (*R*0, *L*0, & *Q*0; see an accessible account, see Duval, 2005). Then, it ‘trims’ off *Nmissing* effect sizes to suppress funnel asymmetry, and estimates a new overall mean to see whether it can trim more effect sizes until the value *Nmissing* stabilizes. Subsequently, *Nmissing* effect sizes are ‘filled’ as mirror images (Figure 4e & f). Finally, an overall effect is re-estimated including the filled values. We note that Duval (2005) has recommended the use of *R*0 and *L*0, and that the estimator *R*0 can provide a significance test for whether the number of missing values is zero or not.

The problem with the trim-and-fill test is that the original method assumes homogeneity (i.e. a true mean for all effect sizes). In practice, the trim-and-fill method seems to tolerate some heterogeneity, but performs worse as heterogeneity increases (Peters *et al.*, 2007; Moreno *et al.*, 2009). Although trim-and-fill tests have been extended for meta-regressions (Weinhandl & Duval, 2012), the implementation of this extension is currently limited to one moderator. Further, recent simulation work by Rogers and Pustejovksy (2020) shows that ignoring non-independence and fitting a trim-and-fill method (using *R*0) increases Type I error rates, especially when a large effect exists.

### 3.2.3 | *P*-value-based methods and selection models

Ecologists and evolutionary biologists have hardly used the available methods based on *p*-values and selection models (*p*-value-based: 1.4%, selection models: 0%, Figure 2), even although both types of methods can provide adjusted overall means. The *p*-curve method was introduced by the same researchers who popularized the terms ‘researcher degrees of freedom’ (Simmons, Nelson & Simonsohn, 2011) and ‘*p*-hacking’ (Simonsohn, Nelson & Simmons, 2014). The *p*-curve method relies on the distribution of statistically significant *p* values of effect sizes in a dataset (Figure 5a). The *p*-uniform method is a similar method, which also exploits the distribution of *p* values (van Assen, van Aert & Wicherts, 2015). Interestingly, McShane et al. (2016) has pointed out that both *p*-curve and *p*-uniform tests are versions of a selection model first suggested by Hedges (1984); all of these methods, unfortunately, do not perform well with heterogeneity as they assume one true effect (see also, van Aert, Wicherts & van Assen, 2016). Clearly, in ecology and evolution where high levels of heterogeneity are commonplace (Senior et al. 2016), these methods may be of limited use, especially compared to more advanced selection models.

Selection model-based methods represent the most sophisticated and complex class of publication bias methods (reviewed in Rothstein, Sutton & Borenstein, 2005; Vevea, Coburn & Sutton, 2019; Marks-Anglin & Chen, 2020), yet there are probably as many selection models as all other methods combined (Marks-Anglin & Chen, 2020). One property common to all selection models is that they model how effect sizes are missing (or selected to be published), based on, for example, *p* values, effect sizes and/or sampling variance (e.g., Preston, Ashby & Smyth, 2004; Carter *et al.*, 2019; Rodgers & Pustejovsky, 2020;Figure 5b-c). Importantly, selection models can tolerate and model heterogeneity. Indeed, the recent model by Citkowicz and Vevea (2017) can statistically test for publication bias, incorporate moderators, tolerate substantial heterogeneity, provide an adjusted overall effect, and even correct estimates for small sample sizes. Yet, no selection methods are implemented for non-independent effect sizes, and as far as we are aware, such implementation is extremely challenging.

# 4 | METHODS FOR DEPENDENT EFFECT SIZES

In this section, we first define a multilevel model that explicitly incorporates non-independence among effect sizes. Next, we consider how to best visualize such datasets as a funnel plot. Then, we build upon a regression-based method introduced above to propose a new publication bias method. This new method can both detect and correct for funnel asymmetry or small-study effects, while modelling heterogeneity and complex non-independence involving both correlation and variance-covariance matrices.

## 4.1 | A multilevel meta-analysis and funnel plots

The simplest multilevel meta-analytic model can be written as (Nakagawa & Santos, 2012):

Where: is the overall estimate (or meta-analytic mean); *sj* is the between-study effect for the *j*th study, normally distributed with the variance of ; *ui* is the between-effect-size effect, or within-study effect, for the *i*th effect size, distributed with a mean of zero and the variance of ; and *mi* is as in Equation 7 (but note that *j* = 1, 2, …, *Nstudy*, the number of studies, and *i* = 1, 2, …, *Neffect-size*, the number of effect sizes; *Neffect-size* > *Nstudy*). Equation 15 explicitly models multiple effect sizes per study. Also, in Equation 7, the term is the only source of heterogeneity, while in Equation 15, both and are each contributing to effect size heterogeneity.

Now we can easily extend this to a meta-regression model. For example, a meta-regression with two moderators can be written as:

where is the slope for *x*1, a study-level moderator (characteristics of different studies, *j*;e.g.,experimental *vs.* observational) and is the slope for *x*2, an effect-size-level moderator (characteristics of effect sizes, *i*; different measurements or sexes). We have mentioned that we can draw a funnel plot with residuals rather than the observed effect sizes (Figure 6a). A complication is that, given Equation 15, we can extract at least 3 different residuals, which are:

where *residm* represents marginal residuals (subtracting only fixed effects from the observations; Figure 6b), whereas *residc*1 and *residc*2 are conditional residuals (Figure 6c & d; Nobre & Singer, 2007). As shown in Figure 6a-d, marginal residuals still show the patterns due to study origin (i.e. sample sizes are the same or similar). Contrastingly, conditional residuals no longer show such obvious patterns as we took out a clustering factor (*sj*), meaning that these residuals are independent, at least with respect to this clustering factor. Thus, funnel plots with conditional residuals (Figure 6c-d) seem like a useful exploratory tool for publication bias when effect sizes are correlated, in addition to using marginal residuals (Figure 6b).

As the conditional residuals are supposed to be independent, Nakagawa and Santos (2012) suggested using conditional residuals along with corresponding sampling variance or standard error (*vi* or *sei*) in publication bias tests (e.g., the original Egger’s regression and trim-and-fill tests). However, this approach is limited by some assumptions. First, all such residual analyses assume that sampling SE (*sei*) does not covary with moderators in meta-regression (e.g., *x*1 and *x*2 in Equation; see Freckleton, 2002). Second, sampling SE is assumed to be the same as the SE of the residuals (which are shown in Figures 6b-d), but they are not the same, although they are strongly correlated (see Doleman *et al.*, 2020). Finally, in the presence of non-independent data, Equation 15’s sampling variances are often correlated; that is, where **M** is a variance-covariance matrix. For example, when *Neffect-size* = 3 and the first two effect sizes’ sampling variance are correlated, then we can write **M** as:

where is the correlation between the sampling effects of the first two effect sizes ( is the covariance). Whenever sampling (error) effects are correlated, neither *residc*1 nor *residc*2 are independent. Then, none of publication bias tests, reviewed in Section 3, should be used. Incidentally, we note that the robust variance estimator originally proposal by Hedges et al (2010) can circumvent modelling the variance-covariance matrix **M** even when sampling errors are correlated. This is because covariances are estimated from the data and the associated errors are incorporated into models with robust variance estimation(cf. Rodgers & Pustejovsky, 2020).

## 4.2 | Multilevel meta-regression and Egger’s regression

As an alternative to using residual analysis, we can directly model sampling SE in Equation 15 (cf. Equation 10; Fernandez-Castilla *et al.*, 2019; Rodgers & Pustejovsky, 2020):

By examining Equation 21, we may realise that represents a conditional estimate of an overall effect when SE is 0, which means, theoretically, there is no uncertainty (Figure 5e). Then, does provide an adjusted estimate of an overall effect, when is statistically significant (i.e., detaching a small-study effect)? This question has been examined by Stanley and Doucouliagos (2012; 2014). They have shown that, with significant , provides an adjusted estimate that is downwardly biased, when a true positive or a null effect exists (which is illustrate in Figure 5e; note that they state that with non-significant , provides the best estimate of an adjusted mean). If the slope of SE () is statistically significant, fitting sampling variance instead of SE, as follows:

This is equivalent to fitting , which is a quadratic term. Stanley and Doucouliagos (2012; 2014) have shown that in Equation 22 is still downwardly biased, but much less so, although Equation 21 is more powerful (i.e. an adjustment tends to underestimate) when there is a positive (or no) effect (cf. Figure 5f). While this two-step approach may seem simplistic (see also Stanley, 2017; Stanley, Doucouliagos & Ioannidis, 2017), it provides an easy-to-implement publication bias test which explicitly models non-independent data.

Further, this regression approach can be used to test time-lag bias (a decline effect) by modelling the publication year (*yearj*):

When heterogeneity exists, it is best to combine Equation 21 and 23 with moderators, for example:

where is the slope for the *k*th moderator (*k* = 3, 4,…, *Nmod*; the number of moderators), the other parameters are as above. However, simulation studies have shown Egger’s regression variants with sampling standard error as a moderator (e.g., Equations 10 & 21) perform poorly, even when adequately powered (Macaskill, Walter & Irwig, 2001; Deeks, Macaskill & Irwig, 2005). This is especially true under two scenarios: 1) when there is a relationship between effect size and sampling SE, and 2) when SE is not estimated accurately.

## 4.3 | Multilevel meta-regression using sample size

To understand how a correlation between effect size and SE can come about, and when SE can be estimated inaccurately, we now go back to comparing sampling variance among the three commonly used effect sizes (Equations 2, 4 and 6). The SMD’s variance has the square of the point estimate (i.e. SMD; Equations 2). This can lead to a correlation between SMDs and sampling SE, resulting in ‘artefactual’ funnel asymmetry (Section 3.2). Further, we also notice that in Equation 4 (i.e. lnRR’s variance), when sample sizes (*n*1 and *n*2) are small, (sample mean) and especially SD (sample standard deviation) will be poorly estimated, resulting in an unreliable estimate of sampling variance (this is also the case for Equation 2). These issues do not affect the sampling variance of *Zr*, which is a function only of sample size (*n*; Equation 6). Therefore, the sample size (*n*1 + *n*2) has been suggested as a moderator instead of SE (e.g., Equation 19) when we use effect size statistics such as SMD and lnRR (also correlation, *r*; see Section 2.1); this approach is known as the funnel plot test (Macaskill, Walter & Irwig, 2001). Simulations suggest using the sample size as a moderator outperforms SE with close to nominal Type 1 error rates in the cases of both independent (Macaskill, Walter & Irwig, 2001; Deeks, Macaskill & Irwig, 2005), and non-independent effect sizes (Fernandez-Castilla *et al.*, 2019).

Instead of the sample size (*n*1 + *n*2), however, for a meta-analysis of SMD or lnRR we propose using the ‘effective sample size’ () because it accounts for unbalanced sampling. The effective sample size is given by (Bakbergenuly, Hoaglin & Kulinskaya, 2020b; 2020a; also see; Deeks, Macaskill & Irwig, 2005; Bakbergenuly, Hoaglin & Kulinskaya, 2020c):

When *n* = *n*1 = *n*2, the formula reduces to 2*n*. Indeed, the inverse of is a part of sampling variance in both SMD and lnRR (Equations 4 & 6):

where the middle part of the formula corresponds to Equation 2 when setting SMD = 0, while the righthand side corresponds to Equation 4 when setting CV (SD/) = 1. This means that the use of is comparable to that of sampling variance after taking out uncertain elements.

Taken together, we can rewrite Equations 21 and 22, respectively, as (Deeks, Macaskill & Irwig, 2005):

where is a replacement of *sei* in Equation 21, and is a replacement of *vi* in Equation 22 (note that, at the intercept, is infinitely large). We recommend using Equation 27 to check the statistical significance of funnel asymmetry (small-study effects) because it has greater statistical power than Equation 28. Equation 27 can also be used to obtain an adjusted mean when is not significant. When is significant in Equation 27, we recommend can use Equation 28 (less biased) to obtain an overall estimate adjusted for publication bias, because it is less biased. Note that these recommendations are for the effect sizes SMD and lnRR (with *Zr*, we should use Equations 21 and 22). This adjusted estimate should not be taken as a true estimate, however. We should treat it as a possible overall estimate as a part of sensitivity analysis in which we run alternative statistical models to test the robustness of results from the original analysis (Noble *et al.*, 2017).

In practice, multilevel meta-analytic models are often more complex. For example, Nakagawa and Santos (2012) have proposed a phylogenetic multilevel model with a phylogenetic random factor and a non-phylogenetic random factor as a theoretically sound model when effect sizes are obtained from different species (see also Hadfield & Nakagawa, 2010). The major benefit of our proposed meta-regression approach for publication bias tests is that we can easily extend these models to incorporate other sources of heterogeneity. An example of a meta-regression model testing publication bias and time-lag bias that also includes phylogenetic and non-phylogenetic random effects can be written as:

Where: *ah* is the phylogenetic effect for the *h*th species, considered multivariate normally distributed with a covariance of (**A** is a correlation matrix derived from a phylogeny); *qh* is the between-effect-size effect, or within-study effect, for the *h*th species, distributed with the variance of (*h* = 1, 2, …, *Nspecies*, the number of species; ); and the other notations are the same as above. Relevantly, when using SMD or lnRR, we may be better off using along with residuals for drawing funnel plots (see Section 4.1; Doleman *et al.*, 2020) rather than SE, precision, or variance. In the Supporting Information we use two datasets and the three effect sizes to illustrate these proposed methods (Appendix S4).

## 4.4 | Alternative approaches: averaging or sampling

Many of the methods we introduced in Section 3 are still useful, even in the presence of non-independent data, if we aggregate effect sizes per study or sample one effect size per study. When sampling variances are correlated (i.e. **M** as in Equation 29), ‘average’ sampling variance needs to be calculated by using the following formula (not by simple weighted averaging as for the mean; Borenstein, 2009):

where *yg* is the *g*th effect size in a study (*g* = 1, …, *Nwithin* and *l* = 1, …, *Nwithin* where *Nwithin* is the number of effect size within a paper or a species to be combined), and are the sampling error variances for *yg* and *yl*, and *rgl* is the correlation between the sampling errors of *yg* and *yl*.

Overall means will generally not be biased using aggregated or single sample/study effect sizes (Song *et al.*, 2020). Also, Rodgers and Pustejovsky (2020) showed that when averaging effect sizes within studies, all Egger’s regression (similar to Equation 10), the trim-and-fill test (using *R*0 estimator) and the three-parameter selection model (as in Vevea & Hedges, 1995) had the appropriate level of Type 1 error, although the three-parameter selection model was noticeably more powerful than the others. However, averaging or sampling is not a general solution when we have a phylogenetic signal ( > 0; Equation 29). In such a case, averaging or sampling per species will not eliminate non-independence as effect sizes are still correlated via phylogeny (i.e. **A** in Equation 29). Furthermore, even when there is no phylogenetic signal ( = 0), or we do not have the species-level structure in a dataset, these alternative approaches could be problematic. For example, if we average effect sizes, we will lose all effect-size-level moderators (e.g., one cannot average categorical moderators such as types of measurements, evaluation methods or sex). Although iteratively sampling one effect size per study could capture moderating effects, this approach also reduces the information content of the dataset. Despite these limitations, under some circumstances, averaging and sampling could be useful (examples and implementations for the trim-and-fill test and a selection model in Supporting Information, Appendix S5).

# 5 | CONCLUSIONS

Given high levels of heterogeneity and non-independence in ecological and evolutionary meta-analytic datasets, our choice of suitable tests for publication bias is limited. We have described the main methods for testing publication bias alongside our recommendations, as summarised in Figure 7. Our proposed multilevel regression method appears to be the only practical method that could fulfil statistical assumptions under most circumstances. Although using averaging or sampling are not a universal solution, they may be useful in supplementing our regression-based method. This is because all publication bias tests should be seen as a part of sensitivity analysis (Noble *et al.*, 2017), meaning that we should run more than one publication bias test.

Few simulation studies exist explicitly investigating the performance of publication bias tests with non-independent data. Two studies that we are aware of supported similar models to the multilevel-regression method we proposed here (Fernandez-Castilla *et al.*, 2019; Rodgers & Pustejovsky, 2020). In addition, a general point to take from these two simulation studies is that most methods are prone to Type 2 error, with a possible exception of some selection models, even when the methods have nominal Type 1 error rates. Therefore, not detecting publication bias in a publication bias test should not be taken as a proof of no publication bias, including for multilevel regression. Clearly, we need more methodological and simulation-based work in the future.

Finally, we repeat that the results of publication bias tests should always be cautiously interpreted because no methods could ever verify the actual number of missing effect sizes. By way of example, a recent study compared the results of 15 meta-analyses and pre-registered replication projects on the same topics (Kvarven, Stromland & Johannesson, 2020). The overall effects from the replication projects are smaller than those of the meta-analyses. More importantly, the replication projects’ estimates are, in general, also smaller than adjusted effects from the trim-and-fill method, the three-parameter selection model and the 2-step regression model (the method by Stanley & Doucouliagos, 2012; 2014). Nonetheless, as long as we acknowledge the limitations and assumptions of these methods, publication bias tests are an essential part of meta-analysis. All future meta-analyses in ecology and evolution should test for publication bias, and try to identify related biases.

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# AUTHORS’ CONTRIBUTIONS

Conceptualization: SN & REO; Data curation REO, AST, & YY; Formal Analysis, REO, AST, YY & SN; Validation: REO & DWAN; Investigation: SN, ML, MDJ, JK, DWAN, THP, & REO; Visualization: SN, ML & REO; Methodology: SN; Writing – original draft: SN; Project administration: SN & REO; Writing - review & editing: all authors. We note that the supplementary information (Appendices S1-S5) was put together by REO, AST, YY & SN.

# DATA AVAILABILITY

We have relevant data and code available at the GitHub repository (https://github.com/itchyshin/publication\_bias).

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# FIGURE LEGENDS

**FIGURE 1.** A schematic showing the relationship among standard error (SE), sampling variance, precision (the inverse of SE) and weight (the inverse of variance). Note that the inverse of variance is the weight for a fixed-effect model (the weight for a random-effect model is the inverse of the sum of sampling variance and between-study variance). In the statistical literature, the inverse of variance is also referred to as precision. Importantly, ‘standard error’ (SE) can be referred to as ‘standard deviation’ (SD), which is not incorrect because standard error is ‘standard deviation of a statistic’ – not to be confounded with ‘standard deviation of a sample’.

**FIGURE 2.** Frequencies of the usages of different publication bias tests in our survey of 102 meta-analyses in ecology and evolution. Note that only one paper employed a method (a weighted histogram) belonging to a category that was not pre-specified (including ‘None reported’; the labels for items A-K match the labels used in our survey). For the details of the survey, see Supporting Information, Appendix S1.

**FIGURE 3.** Examples of funnel plots and a radial plot using the same dataset (*Neffect-size* = *Nstudy* = 100): a) a funnel plot with sample size as a measure of uncertainty; b) a funnel plot with precision (1/SE) as a measure of uncertainty, red dots representing ‘expected’ missing data under publication bias, and blue dots representing ‘unexpected’ missing data; c) a counter enhanced funnel plot with SE as a measure of uncertainty; d) a sunset plot showing statistical power of data as the overall effect estimate as a true effect; e) a residual funnel plot (one moderator removed); and f) a radial plot. We used the *R* packages *metafor* (panels a-c & e; Viechtbauer, 2010), *metaviz* (panel d; Kossmeier, Tran & Voracek, 2020) and *meta* (panel f; Schwarzer, Carpenter & Rücker, 2015) for visualizations.

**FIGURE 4.** Examples of various plots (using the same dataset as Figure 3b minus 25 red datapoints, therefore *Neffect-size* = 75): a) a scatter plot with the height of the solid line representing the degree of funnel asymmetry (cf. the radial plot at Figure 3 f); b) a scatter plot with the steepness of the slope representing the degree of funnel asymmetry; c) a forest plot showing results of cumulate meta-analyses, where only a portion of the dataset (*Neffect-size* = 15) was used; d) a bubble plot showing a ‘decline effect’ over time, where only a portion of the dataset (*Neffect-size* = 15) was used; e) a funnel plot with precision (1/SE) and with a trim-and-fill method filling missing data (red circles; using the *R*0 estimator); and f) the same as panel e but with SE as a measure of uncertainty. We used the *R* packages *gglot2* (panels a, b & d; Wickham, 2009) and *metafor* (panel ; Viechtbauer, 2010) for visualizations.

**FIGURE 5.**  Example plots for *p*-curves and selection models (using the same dataset as in Figure 4; *Neffect-size* = 75): a) a line plot showing the distribution of statistically significant *p* values under 3 scenarios: 1) with the observed *p* values (blue solid line), 2) when there is no effect (red dotted line), and 3) when there is an effect (i.e. an observed overall effect as a true effect) with 33% statistical power (note that if a blue line increases at the α level of 0.05, this is a sign of *p*-hacking; for more details of this plot, see www.p-curve.com); b) a plot showing 4 different weight functions that model, based on the data, the likelihood of effect sizes being selected for publication: 1) a half-normal function based on *p* values (black solid line), 2) the same function but based both on *p* values and precisions (black dotted line), 3) a logistic function based on *p* values (red solid line), and 4) the same function but based both on *p* values and precisions (red dotted line; these functions are based on Preston, Ashby & Smyth, 2004); and c) a plot showing two different ‘step’ weight function based on: 1) three cut-points (α = 0.05, 0.1, 0.5) and 2) one cut-point (α = 0.05; this model is sometimes referred to as a 3 parameter selection model, PSM with the 3 parameters being an overall mean, the between-study variance, and an index determining the likelihood of selection; e.g., Carter *et al.*, 2019; Rodgers & Pustejovsky, 2020). We used the *R* packages *dmetar* (panel a; Harrer *et al.*, 2019) and *metafor* (panel b & c; Viechtbauer, 2010) for visualizations.

**FIGURE 6.**  Examples of funnel plots from a dataset with lnRR (*Nstudy* = 70; *Neffect-size* = 271) and a different dataset with *Zr* (*Nstudy* = 48; *Neffect-size* = 104): a) a funnel plot of raw data (the same colour indicating effect sizes from the same studies); b) a funnel plot of marginal residuals with the fixed effects removed (as in Equation 17); c) a funnel plot of conditional residuals with fixed effects and the between-study effect removed (as in Equation 18); and d) a funnel plot of conditional residuals with all effects apart from sampling errors removed (as in Equation 19); e) a scatterplot showing a meta-regression on SE (black line; the red line is the same line as in panel f). Note that an overall mean is set to be 0 in this simulated dataset along missing effect sizes imitating publication bias; and f) a scatterplot showing a meta-regression on sampling variance (red line, the same line as in panel ‘e’). Both red lines showing to intersect the zero effect size at the intercept. We used the *R* packages *metafor* (panels a-d; Viechtbauer, 2010) and *ggplot2* (panels e-f; Wickham, 2009) for visualizations.

**FIGURE 7.** A summary of main publication bias tests reviewed in this article, and our recommendations under two different conditions (effect sizes are independent or non-independent). Superscript notes: 1) for funnel plots, residuals from a meta-regression can be plotted instead of raw effect sizes, and using sample sizes instead of standard errors may be a good option for lnRR and SMD; 2) for non-multilevel regression methods, precision and sampling variance (or and ) can be used; 3) technically, fail-safe *N* methods do not provide an adjusted overall mean, but the numbers indicate how many non-significant studies (null effect sizes) would render the overall effect zero (or a particular small effect size value); 4) for trim-and-fill methods, although some heterogeneity can be tolerated the ability to model moderators is limited; alternatively, residuals along with their corresponding variances could be used.

## FIGURE 1

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## FIGURE 2

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## FIGURE 3

**Chart

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## FIGURE 4

**Chart, scatter chart

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## FIGURE 5

Chart

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## FIGURE 6

Chart

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## FIGURE 7

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