

How to critically read ecological meta-analyses.

Christopher J. Lortie¹, Gavin Stewart², Hannah Rothstein³, and Joseph Lau⁴

1. Department of Biology, York University. 4700 Keele St. Toronto, ON, Canada. M3J1P3.

PH: 416.736.2100 FAX: 416.736.5698 lortie@yorku.ca & chris@christopherlortie.info

2. Centre for Reviews and Dissemination, University of York, UK. Tel: 01904 321068. Fax:

01904 321041 gavin.stewart@york.ac.uk

3. Department of Management, Department of Management, Baruch College—CUNY. 1

Bernard Baruch Way, New York, NY 10010, USA Hannah.Rothstein@baruch.cuny.edu

4. Center for Clinical Evidence Synthesis, Tufts Medical Center. 800 Washington Street, Box 63,

Boston, MA 02111. Tel: 617-636-7670 Fax: 617-636-8628 joseph_lau@brown.edu

Brief note on reading ecological meta-analyses.

Abstract

Meta-analysis offers ecologists a powerful tool for knowledge synthesis. Albeit a form of review, it also shares many similarities with primary empirical research. Consequently, critical reading of meta-analyses incorporates criteria from both sets of approaches particularly because ecology is a discipline that embraces heterogeneity and broad methodologies. The most important issues in critically assessing a meta-analysis initially include transparency, replicability, and clear statement of purpose by the authors. Specific to ecology more so than other disciplines, tests of the same hypothesis are generally conducted at different study sites, have variable ecological contexts (i.e., seasonality), and use very different methods. Clear reporting and careful examination of heterogeneity in ecological meta-analyses is thus crucial. Ecologists often also test similar hypotheses with different species, and in these meta-analyses, the reader should expect exploration of phylogenetic dependencies. Finally, observational studies not only provide the substrate for potential current manipulative experiments in this discipline but also form an important body of literature historically for synthesis. Sensitivity analyses of observational versus manipulative experiments when aggregated in the same ecological meta-analysis are also frequent and appropriate. This brief conceptual review is not intended as an instrument to rate meta-analyses for ecologists but does provide the appropriate framing for those purposes and directs the reader to ongoing developments in this direction in other disciplines.

Keywords: ecology, criteria, guidelines, interpretation, meta-analysis, reading, and synthesis.

Introduction

We propose that progress in ecology is best promoted by using a range of non-exclusive methods including data synthesis based on formal meta-analyses, systematic reviews, conceptual and theoretical papers, and combinations of large and small experimental studies. ‘Conceptual evolution’ can occur through detailed experimental tests that refine and hone hypotheses thereby increasing the depth of our understanding of a natural system or set of ideas describing patterns or predicting processes (Paine 2002). Synthesis in ecology can however also be achieved via the integration of different concepts (Ford 2000; Ford and Ishii 2001) and quantitative syntheses of studies testing related ideas or hypotheses (Arnquist and Wooster 1995). It may seem obvious that science can progress effectively through both avenues, but the majority of ecological research in recent decades has focused on experimental tests of hypotheses. Similar to other mature/maturing disciplines, ecology is now in a position to capitalize on these intensive efforts because the literature has accrued adequate capacity in breadth and depth on many dominant ideas (Cadotte *et al.* 2012; Castellanos and Verdu 2012; Hampton *et al.* 2013; Jennions *et al.* 2013a). Ecology is thus ripe for a profound shift in focus to use reviews as a means to develop ideas, explore conceptual evolution, seek general conclusions, and assess validity. This paradigm shift is not a new idea in evidence-based medicine (Higgins and Green 2006) nor in ecology (Arnquist and Wooster 1995; Carpenter *et al.* 2009; Gurevitch and Hedges 1993; Gurevitch *et al.* 1992; Hampton *et al.* 2013; Sidlauskas *et al.* 2009), but the reviews in ecology are shifting from narrative descriptions to systematic reviews and meta-analyses (Cadotte *et al.* 2012; Chaudhary *et al.* 2010; Pullin and Stewart 2006; Vetter *et al.* 2013). These forms of synthesis provide quantitative, replicable insights that can accelerate progress within ecology by

providing the reader with a general framework of research completed to date and ideally insights into future directions.

Meta-analysis is an effective tool for synthesizing independent research efforts, comparing the relative success of treatments associated with groups of studies, testing whether mean treatment effects are significantly different than zero, and testing whether the effects are homogeneous or heterogeneous among and within groups or categories of studies. Unfortunately in ecology, the semantics of synthesis, particularly use of the term meta-analysis, varies significantly (Vetter *et al.* 2013). Herein, we define an ecological meta-analysis as a review that includes statistical analysis of strength of evidence within and between the studies summarized (Koricheva and Gurevitch 2013; Vetter *et al.* 2013). In other disciplines, systematic reviews frequently include not only a summary of the population of studies included in the review and analyses of the literature but statistical analyses of the strength of evidence. The emerging trend in ecology is to decouple use of the term systematic review from meta-analysis and reserve the latter for reviews that include effect sizes and statistic analysis of within-study evidence. This is an appropriate and convenient distinction for ecology we would like to further propagate for readers. Meta-analyses thus offer readers in ecology additional capacity to assess attributes of a hypothesis such as testability, generality, consistency, accuracy, and bias in the studies published on a topic at the time of analysis. In contrast to experimental hypothesis testing, the only attribute of a hypothesis perhaps not best tested via meta-analysis is direct falsification. Meta-analyses can be used to summarize evidence for or against an ecological hypothesis. However, when the evidence in a meta-analysis fails to support a hypothesis this does not necessarily constitute rejection of the hypothesis as useful or predictive in ecology *per se* given that there are a wide range of

hypotheses in this discipline ranging from purely descriptive to more formally falsifiable when associated with sets of manipulative experiments (i.e. trials are uncommon in ecology). A meta-analysis is not a test of the hypothesis, the respective experiments summarized are often so however, and failure to support may be a product of the set of studies testing the general proposition. Nonetheless, this synthesis tool is an important shift towards 'effective thinking' in weighing the strength of ecological ideas, i.e. considering effect sizes, (Jennions *et al.* 2013a) and this ongoing shift away from p-values is an important disciplinary transition (Sterne and Smith 2001). In summary, a meta-analysis is not an experiment and does not test hypotheses but is a means to explore the strength of evidence associated with hypotheses. This is a critical clarification for ecology because there are often large collections of studies documenting only pattern, randomized controlled trials are not used, and even the exact replication of experiments or general protocols is unfortunately relatively infrequent (Kelly 2006). This trajectory is not unlike progress made in other disciplines such as evidence-based medicine, but ecologists may face unique or at least more frequent subsets of challenges specific to the study of diverse natural systems.

The effective execution of meta-analyses is comprehensively described in a recent handbook for ecologists (Koricheva *et al.* 2013). A recent special issue in the journal 'Evolutionary Ecology' also details trends in publications including a meta-analysis of meta-analyses (Castellanos and Verdu 2012), and the demonstration that meta-analyses are increasing in both frequency and complexity in ecology (Cadotte *et al.* 2012). However, a simple guide or outline for readers is lacking for ecologists. Only a handful of summaries are available along these lines but for clinicians (Leucht *et al.* 2009; Ried 2006) or medical practitioners in general (Russo 2007), and

these deal primarily with synthesis of randomized controlled trials. Epistemologically and empirically, ecology is applying this approach to very different forms of experiments and datasets. Herein, we provide a broad-stroke summary of the general principles/philosophy for the reader to aid in assessing a meta-analysis and summarize a few of the emerging issues specific to ecology. The goal is to provide a general heuristic for the ecological practitioner that has not yet done a meta-analysis.

Reader guidelines

General principles

Literature & scope

In reading ecological meta-analyses, the form of evidence used in the synthesis should be assessed to ensure that the review possesses the capacity to describe the ecological process or hypothesis of interest. Surrogate measures of fitness, stress, population regulation, performance, and sometimes-even diversity are common in primary-research ecological studies. This does not weaken the discipline in any way. It is simply a product of the wide-ranging patterns and processes associated with the study of complex and diverse study systems that include different species and categories of drivers. Ecological synthesis efforts include similar decisions. A clear statement of purpose by the authors is necessary and defines the scope of the review and the datasets aggregated. A meta-analysis is still a review and must tell a story (Humphrey 2011). In spite of its statistics, it is nonetheless a simplification of primary research, and in a discipline that rarely replicates experiments and infrequently uses exact methodologies (Vetter *et al.* 2013), ecological review stories have considerable potential as an explanatory tool but can also be very easy to misinterpret when the natural history of a system is neglected or as the degree of

abstraction is overextended (Lortie and Callaway 2006). The ecological reader should thus initially confirm that the meta-analysis includes the salient elements needed for any good study such as transparency, replicability, and a clear statement of purpose by the authors (Text box 1). The savvy reader should look for scope of the search, choice of relevant studies, and reporting of the methods used to combine studies (Text box 1a). There are formal evaluation tools available for syntheses of clinical research such as QUOROM (Moher *et al.* 1999) and MOOSE (Stroup *et al.* 2000), and a quality standard checklist for ecological meta-analysts as well (Rothstein *et al.* 2013). These instruments need not be used by the reader in any/every instance, but PRISMA (Moher *et al.* 2009) style tables or visualizations showing how the final population of studies included in the meta-analysis was decided should be provided to the reader. Inclusion of this aspect of the synthesis workflow provides a degree of transparency (studies were not selected at random or preferentially), the opportunity for replication (the reader could repeat the search on Web of Knowledge or Scopus to confirm that a similar set of studies is generated), and evidence for the purpose of the meta-analysis (excluded studies were outside the statement of scope provided by the authors). The extent to which a meta-analysis can generate robust results thus depends not only on the quality of the synthesis but also on the scope and quality of the included studies. Publication bias is discussed at great length in the technical literature associated with meta-analysis (Koricheva 2003; Leimu and Koricheva 2004; Moller and Jennions 2001; Peters *et al.* 2007; Tomkins and Kotiaho 2004), and the reader should expect at least some examination of the studies such as a funnel plot, fail-safe number, or sensitivity analysis to ensure that the studies included were not unduly skewed to only those that reported positive findings (Jennions *et al.* 2013b). The capacity for a reader to assess the reported statistical evidence will be facilitated by consideration of these three general criteria (transparency, replicability, and

purpose) and will reduce the likelihood that the story becomes an oversimplification of the underlying ecology associated with the synthesis effort.

It is useful to consider two elements of study validity when interpreting the purpose proposed for ecological meta-analyses. The first element, internal validity, relates to whether a study answers its research question using methods that are free from bias (Gates 2002; Juni *et al.* 1999; Treadwell *et al.* 2007). The reader could consider the experimental design or sampling accuracy of the studies included in the meta-analysis (Text box 1a). Tight experimental control tends to result in studies with high internal validity. The second element, external validity, relates to the generalizability of the research question. Studies with high external validity (i.e. have a broad scope in spatial scale or are not very taxon specific) have high generalizability, thus the use of appropriate spatial and temporal scales may be important elements of external validity (Text box 1a). Ecology often involves tradeoffs between internal and external validity with reductionist approaches having high internal validity and holistic approaches increasing external validity but decreasing internal validity. These tradeoffs are not specific to synthesis.

Formal study inclusion criteria have been proposed and discussed extensively for meta-analysis due to its quantitative nature relative to narrative reviews (Moher *et al.* 1995; Moher *et al.* 1996) and for ecology (Cote *et al.* 2013; Gates 2002). The primary purpose of inclusion criteria is to ensure that the patterns detected within a meta-analysis are representative of the ecological processes. Effective interpretation of a meta-analysis should include a brief inspection of the studies listed. Similar to primary research studies, most readers acquaint herself/himself with the study species or geography of the study to place the findings reported into the appropriate

context. Because studies are the substrate of meta-analyses, the same principle applies; the reader should ensure that the synthetic ecosystem is appropriate. This does not imply that authors should not seek to aggregate studies from different ecosystems or use studies with relatively small sample sizes but that several general principles are relevant for ecologists. The sole criteria for the interpretation of a good ecological study should not be its p-value but rather an assessment of whether a given study has the capacity to detect the treatment effect or ecological process of interest. In meta-analyses, the capacity of the primary study to test for an effect is called the pre-study odds that a true effect can be detected (Wacholder *et al.* 2004), and this is a judgment made by the author. If possible, the reader should consider whether this decision corresponds with their judgment. Importantly, it has been shown that for ecology there is a strong tendency to cite primary research papers with larger effect sizes regardless of data quality (Barto and Rillig 2012). Cumulatively adding single studies does little to increase the capacity of a meta-analysis to detect treatment effects (Ioannidis *et al.* 1998; Ioannidis and Lau 2001), but a major strength of meta-analysis is nonetheless that many small or even low-quality studies can be combined to provide a comprehensive overview of a hypothesis when each independent experimental test may be equivocal. The reader should be prepared for such surprises but also ensure that the studies used match the scope and scale of the ecological process.

Results

Interpreting the magnitude of effect sizes differs from the interpretation of significance levels of statistical tests within a given study. The purpose of any meta-analysis is to compare standardized data across studies, but the interpretation of relevance of the meta-analysis takes

place in a much larger context (Walker *et al.* 2008). The reader needs a frame of reference to assess the outcome of a meta-analysis and several options are available to generate context. These include comparison to other meta-analyses, translation of effect sizes to other metrics, or direct contrasts to contexts or specific groups that readers can comprehend readily (Lipsey and Wilson 2001). For example, a very general delineation was made from 300 meta-analyses of small vs. large effect sizes in the social sciences (in terms of standardized mean difference) with ‘small’ being less than 0.2 (i.e., the means of the experimental and control groups differ by 0.2 standard deviations) and ‘large’ being 0.8 or greater (Lipsey and Wilson 2001) similar to the original levels proposed for individual studies (Cohen 1992). A similar exercise was conducted for ecology and evolutionary biology, and it generated 44 published examples (that met particular criteria including reporting effect sizes etc.) with a mean number of data sets of 5.3 +/- 1.0 (Jennions and Moller 2002), and mean effect sizes ranging from Pearson $r = 0.180$ to 0.193 and Hedges’ $d = 0.631$ to 0.721 (Moller and Jennions 2002). As a starting point, this is an excellent example of an opportunity to both calibrate and estimate the efficacy of sets of ecological approaches. Other researchers reported in a summary note that most effect sizes in ecology and evolution are less than $d = 0.3$ (Kotiaho and Tomkins 2002). In general however, interpretation of the magnitude of mean effect sizes in ecology, at least at this point in time, conforms to the coarse benchmarks for other disciplines with $d = 0.2$ as small, 0.5 moderate, and 0.8 large, and as such we propose that readers adopt a similar perspective on assigning relevance. Nonetheless, meta-analytical statistical relevance does not necessarily map directly onto biological significance, and smaller mean effect sizes may be highly relevant and ecologically important in complex, diffuse natural systems (Text box 1b). Given the increasing frequency of meta-analyses (Cadotte *et al.* 2012; Chaudhary *et al.* 2010) and reporting of effect sizes in

primary studies in addition to significance tests, readers can nonetheless now begin to calibrate strength of evidence frameworks for ecology

Finally, the reader should also consider the breadth of confidence intervals when considering the magnitude of mean effect size because variation is such an important aspect of ecology in general. For instance, $d = 0.8$ is a large effect but if its 95% confidence interval varies from 0.1 to 1.5, one or two extra studies can change its statistical significance from ‘different from 0’ to ‘not significantly different’. The reader may wish to consider the following questions: is the number of studies very low, is there an important source of variation which has been ignored in the analysis, is there partial reporting of important covariates in some studies and not others which could be added to the meta-analysis, and is there another response variable available to assess whether the patterns of variability associated with the confidence interval is a property of this particular biological system or the response variable selected (Text box 1b). The reader should also assess whether the authors explored potential dependencies between moderators provided more than one is available, i.e., the importance of latitude in plant community studies or plant size in interaction studies. In many respects, ecology is about variation. Hence, confidence intervals and moderators provide the reader an opportunity to not only more soundly infer the state of research but to assess whether additional studies are needed and if the primary research studies are best categorized into the groupings commonly adopted.

Particularly relevant issues for ecology

Heterogeneity

The more diverse the designs used in a meta-analysis, the more likely the synthetic conclusions are to be false (Ioannidis 2005). Ecologists frequently use very different methods to test hypotheses since we measure populations, communities, ecosystem properties, and organisms. There is also a strong bias against publishing replicated experiments (Vetter *et al.* 2013). Arguably this is strength since we can attack hypotheses from various angles, but the reader must cautiously interpret the conclusions of meta-analyses taking into consideration the diversity of the study set included. In meta-analyses, this is termed heterogeneity (Higgins and Thompson 2002). The combination of very different groups, species, processes, and places is integral to predictive and applied ecology (Stewart 2010). There are three sets of issues the reader should consider with respect to heterogeneity: model, low-quality studies, and sensitivity.

The choice of statistical model (fixed or random) should match the *a priori* purpose of the meta-analysis, i.e. is this treatment effective, are these groups different, has this hypothesis been successfully tested etc. The reader should inspect degree of fit, amount of heterogeneity explained, and appropriateness of the statistic generated in satisfying the purpose of the meta-analysis (Text box 2a). Furthermore, some ecologists have emphasized that different models and metrics may be appropriate depending on the type of variation associated with the set of studies (Osenberg *et al.* 1999), although some aspects of this recommendation are controversial (Hedges and Gurevitch 1999). As developed above, definition of the scope of review and evaluation of the respective evidence provides a robust means to assess whether the interpretations proposed by the authors are balanced and reasonable. In some instances, single summary estimates or models may not be adequate to encompass the range of natural variability associated with an ecological hypothesis and may warrant presentation of multiple estimates and models to

satisfactorily explain the context dependence (Text box 2a). A ‘great’ meta-analysis may focus more extensively on exploring the variation between studies and associated implications (Humphrey 2011) and less on rejection of a method or hypothesis.

In addition to “true” heterogeneity amongst studies or groups of studies, in some instances the studies may be of ‘low quality’ for various reasons, may be diverse in methodology, and vary in standards. Nevertheless it may still be valuable to do a systematic review or even preliminary meta-analysis with the intention of qualitatively describing the body of studies (Treadwell *et al.* 2007). In this situation, the primary purpose could be to determine net sign, sign differences between groups, or compare levels of variation (Text box 2a). In ecology, many sets of studies may fall into this qualitative category, and the purpose can be to assess sign, bias, consistency, and generality of outcomes. The ecological reader should expect these synthetic efforts to clearly differentiate between ‘no significant mean effect’ and ‘no differences’ depending on the purpose of the meta-analysis (Higgins and Green 2006). Interpretations rejecting support for an ecological process or hypothesis may not be correct if the evidence loaded into the meta-analysis has low capacity to test the ideas. As such, readers can also use magnitude, sign, sign changes, and comparison between groups as well as formal analysis of heterogeneity to assess meta-analyses.

Different categories of studies are also very common in ecology. In some instances, this can introduce heterogeneity, and the reader should be alert to effective exploration such as sensitivity analyses and contrasts of important categories (Text box 2a). There is a strong legacy of natural history and pattern analyses in ecology. Consequently, the reader of ecological meta-analyses

should also expect different categories of studies provided by authors. This not only provides the reader with the capacity to infer research gaps but also provides the substrate for the sensitivity analyses. One the most common groupings in ecological meta-analyses are observational versus mensurative experiments (Cote and Jennions 2013; Gates 2002). Differences between these coarse groups provide the reader with direct insights into how an ecological topic is explored to date and opportunities for novel research. Importantly, this same principle can be applied to contrast sets of methodologies, measures, or group of hypotheses depending on the extent the topic has been explored (Lamarque *et al.* 2011; Robinson *et al.* 2012). Heterogeneity is thus an instructive opportunity to explore scaling-up in ecology (Stewart 2010) and consistency of various methodologies applied.

Phylogenetics

Similar to heterogeneity, groups of species can introduce both significant patterns of variation and non-independence in meta-analyses. This topic is described succinctly in the meta-analysis handbook (Lajeunesse *et al.* 2013). Application of models to include phylogenetic dependence have also been developed in general (Hadfield and Nakagawa 2009; Nakagawa and Santos 2012), and a specific tool for phylogenetic meta-analyses is also available (Lajeunesse 2011). Conducting a meta-analysis and extracting the data needed to calculate effect sizes is not trivial. Adding evolutionary relationships of the taxa included is a also a challenge (Lajeunesse *et al.* 2013). Nonetheless, this is an important issue. A re-analysis of 30 published meta-analyses to include phylogenetic dependencies dramatically altered the overall pooled effect size of the syntheses in nearly 50% of the fixed-effect analyses and shifted effects from not significantly different from 0 to significance in up to 40% of the datasets (Chamberlain *et al.* 2012). If

possible, inclusion of even coarse but accurate trees in meta-analyses can improve synthesis (Lajeunesse *et al.* 2013). General principles for the reader are to include as many species as possible in meta-analysis, to consider phylogenetic signal and size as potentially important factors, and to report size of dataset in general because this can generate nonindependence issues (Text box 2b). Important specific applications for the reader to be aware of also include fixed versus random effects models, number of species included in the meta-analyses, tree balance, distribution of nodes, reporting of phylogenetic correlations, phylogenetic signal as a form of bias, and alternative statistical approaches (Text box 2b). Another simple alternative for the reader to examine is classification of species into functional groups associated with the specific roles that species play in the ecosystem/context or process examined (Maestre and Cortina 2004). Phylogenetics added to all forms ecological syntheses provide an important and often necessary dimension of evolution to the discipline.

Summary

To summarize, we propose that there are both several general and specific heuristics that ecological readers should consider in using meta-analyses to discover broad patterns and reach general conclusions about a topic. These include general guidelines associated with meta-analyses such as literature and scope (transparency, replicability, and purpose) and critical appraisal of the results (focus on strength of evidence and not significance). Not necessarily specific to ecology but likely important, heterogeneity, sensitivity, and phylogenetics should be assessed by the reader in framing the ecological relevance of a synthesis endeavor. Readers should look for clear delineation of how the strength of evidence was used, reporting and exploration of variability in evidence and sign, and careful use of groups to determine the

importance of moderators, covariates, and subgroups. Ecology often takes place in less controlled environments (relative to some other scientific fields) and incorporates many aspects of natural variation in the field, sometimes using very diverse methods to test a hypothesis. Ecology is now in a position to practically apply many forms of synthesis to examine important issues. Interpretations derived from meta-analyses within this domain have the opportunity to not only enhance predictive ecology but also facilitate exploration at larger and novel scales.

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Text Box 1. General guidelines for ecological meta-analysis readers.

Overarching Principles

Transparency
Replicability
Statement of purpose

A. Literature & Scope

General Heuristic

Scope of search
Choice of relevant studies
Representativeness

Specifics

Defined inclusion/exclusion criteria for identification of relevant (evidence) studies
Reasons for inclusion/exclusion documented for each study
Inclusion/exclusion controlled & listed excluded studies in appendix
Assessment of study quality/validity: design, context, scale, and taxa
Data extraction methodology documented and repeatable
Reporting of aggregation methods across studies
Estimation of publication bias

B. Results & Interpretation

Heuristic

Larger context of evidence framed & interpreted
Variation effectively explored
Ecology of system included, i.e. generalizable results

Specifics

Reported number of studies (N) relative to number of effect size estimates (n)
Investigation of sources of variation including heterogeneity
Conducted sub-group analyses or meta-regression
Partial reporting of covariates in studies listed
Alternative response variables explored
Identification evidence gaps & proposed future designs and/or sample sizes
Common ecological drivers tested (latitude, climate, etc.)
Appropriate effect sizes calculated & statistical methods applied

Sources: Pullin, A. S., and G. B. Stewart. 2006. Guidelines for Systematic Review in Conservation and Environmental Management. Conservation biology 10.1111/j.1523-1739.2006.00485.x. Russo, M. W. 2007. How to review a meta-analysis. Gastroenterology & Hepatology 3: 637-642.

Text Box 2. Frequent considerations in ecological meta-analysis

A. Heterogeneity

Heuristic

Statistical model

Low-quality studies

Sensitivity

Specifics

Degree of fit of statistical model

Heterogeneity reported & statistically tested

Heterogeneity within & between groups interpreted & explanations proposed

Alternative models explored

Sign consistency & changes addressed

Observational versus mensurative methods contrasted

Studies coded whether directly tested question or reported associated data

B. Phylogenetics

Heuristic

Inclusion of many different species

Phylogenetic signal & size treated as factors

Size of dataset relates to nonindependence

Specifics

Fixed versus random effects models tested or justified

Number of species included in the meta-analyses provided

Tree balance, distribution of nodes, & reporting of phylogenetic correlations

Phylogenetic signal examined as a form of nonindependence bias

Alternative statistical approaches explored

Functional classifications considered

Sources: (1) Stewart, G. 2010. Meta-analysis in applied ecology. *Biology Letters* 6: 78-81. (2) Lajeunesse, M. et al. 2013. Phylogenetic nonindependence and meta-analysis. *Handbook of meta-analysis in ecology and evolution*: 284-299. (3) Chamberlain, S.A. et al. 2012. Does phylogeny matter? Assessing the impact of phylogenetic information in ecological meta-analysis. *Ecology Letters* 15: 627-636.