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Meta-research in aquatic ecology

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SARA LODI DE CARVALHO SPACEK

Meta-research in aquatic ecology

Tese apresentada ao Programa de Pós-graduação em Ecologia e Evolução do Instituto de Ciências Biológicas da Universidade federal de Goiás (UFG), como requisito para a obtenção do título de Doutor em Ecologia e Evolução.

Área de concentração: Ecologia e Evolução

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ATA DE DEFESA DE TESE

Ata Nº **106** da sessão de Defesa de Tese de **Sara Lodi de Carvalho Spacek** que confere o título de **Doutora em Ecologia e Evolução**, na área de concentração em **Ecologia e Evolução**.

Aos **trinta dias do mês de julho de dois mil e vinte e um (30/07/2021)**, a partir das **14h:00min**, por **videoconferência**, seguindo **portaria CAPES no. 36 de 16 de março de 2020** e recomendação da UFG, realizou-se a sessão pública de Defesa de Tese intitulada “**Meta-research in aquatic ecology**”. Os trabalhos foram instalados pelo Orientador, **Prof. Dr. Luis Mauricio Bini (Departamento de Ecologia/ICB/UFG)**, com a participação dos demais membros da Banca Examinadora: **Profa. Dra. Priscilla de Carvalho (Departamento de Ecologia/ICB/UFG)**, membro titular interno; **Profa. Dra. Jascieli Carla Bortolini (Departamento de Botânica/ICB/UFG)**, membro titular interno; **Profa. Dra. Thaísa Sala Michelin (ICB/UFPA)**, membro titular externo; **Prof. Dr. Ludgero Cardoso Galli Vieira (FUP/UnB)**, membro titular externo. Durante a arguição os membros da banca não fizeram sugestão de alteração do título do trabalho. A Banca Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Tese tendo sido a candidata **aprovada** pelos seus membros. Proclamados os resultados pelo **Prof. Dr. Luis Mauricio Bini**, Presidente da Banca Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Banca Examinadora, ao(s) **trinta dias do mês de julho de dois mil e vinte e um (30/07/2021)**.

TÍTULO SUGERIDO PELA BANCA



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Dedico este trabalho a todos vocês.

Abstract

Systematic reviews value transparency, systematic reporting and reproducibility and follow clear and direct guidelines. It is as simple as systematizing the qualitative review, as the name implies. A quantitative review (or meta-analysis) may also be conducted and goes a step further by providing empirical support for the conclusions. Using this technique, we can combine multiple, independent studies by calculating an average effect size weighted by study precision, which provides the analysis with an increased statistical power. This thesis addresses meta-analysis as its main theme, and is divided into three chapters, all concerning meta-research in freshwater environments. In Chapter 1, I evaluated the quality of meta-analyses in freshwater ecology and made recommendations on how we can increase quality of meta-analyses in our field. I evaluated another issue of quality in Chapter 2, specifically biases towards positive results. In Chapter 3 I conducted a meta-analysis using studies that evaluate the effects of land use on freshwater environments at regional and local scales. I compared the effect sizes obtained when using local and land scape variables. Interestingly, I also provide an example of a study with negative results, given that my hypotheses were not confirmed. I believe this thesis will expand the view of limnologists on the possibilities of the meta-analytical technique applied to our field of research.

Keywords: Meta-analysis, systematic review, land use, publication bias, negative results, quality of meta-analyses

Introduction

Meta-research is an option to organize and make generalizations from the plethora of articles published in a given field. It may be defined as ‘the study of research itself: its methods, reporting, reproducibility, evaluation, and incentives’ (Ioannidis, 2018). Meta-research has been well-developed in fields like psychology and medicine, where steps towards better research practices (Altman, 1994; Ioannidis, 2005, 2008, 2010; Ioannidis & Trikalinos, 2007; Fanelli, 2010b a, 2012; Fanelli, Costas & Larivière, 2015; Page & Moher, 2016; Munafò *et al.*, 2017; Brembs, 2018) and more well-systematized reviews have been taken (Moher *et al.*, 2009b; Liberati *et al.*, 2009; Willis & Quigley, 2011). For example, the Cochrane organization is a reference for producing quality systematic reviews and synthesis for decision makers to reach informed and reliable conclusions in the field of medicine (see <https://cochrane.org/> and the Cochrane Handbook of Systematic Reviews; Higgins & Green, 2011). Such advancements have also been reaching ecology (Hillebrand & Cardinale, 2010; Jennions & Lortie, 2013; Koricheva & Gurevitch, 2013a; Koricheva, Jennions & Lau, 2013; Gurevitch *et al.*, 2018; Lodi *et al.*, 2021), although more recently.

Systematic reviews value transparency, systematic reporting and reproducibility (Gurevitch *et al.*, 2018) and follow clear and direct guidelines (Moher *et al.*, 2009a and see Tools for Transparency in Ecology and Evolution as well; <https://osf.io/g65cb>, 2015). It is as simple as systematizing the qualitative review, as the name implies. A quantitative review (or meta-analysis) may also be conducted (Gurevitch *et al.*, 2018) and goes a step further by providing empirical support for the conclusions. A meta-analysis calculates an overall effect size, its significance, and provides a formal, transparent, and quantitative approach for exploring possible causes of variation in estimated effects (Koricheva & Gurevitch, 2013b; Senior *et al.*, 2016; Gurevitch *et al.*, 2018). Using this technique, we can combine multiple,

independent studies by calculating an average effect size weighted by study precision (defined as the inverse variance, see Borenstein *et al.*, 2009), which provides the analysis with an increased statistical power. This is especially important in Ecology as the effect size of individual ecological studies tends to be low (Jennions & Lortie, 2013).

This thesis addresses meta-analysis as its main theme, and is divided into three chapters, all concerning meta-research in freshwater environments. In Chapter 1, I evaluated the quality of meta-analyses in freshwater ecology and made recommendations on how we can increase quality of meta-analyses in our field. I conducted a standardized search and screening and evaluated meta-analyses in freshwater ecology one by one, seeking relevant quality traits in the studies. I observed that the term meta-analysis is frequently misused. However, despite the lower-than-expected quality of the papers, guidelines seem to be reaching researchers that use the term properly.

I evaluated another issue of quality in Chapter 2, specifically biases towards positive results. This is not a novel issue to be discussed in science but is being often revisited. I evaluated meta-analyses and observed the incidence of positive results; positive being the confirmation of the hypothesis in direction and significance. Positive results did prevail. I discuss the importance of bias-control practices in meta-analyses and possible influence of biased primary studies.

In Chapter 3 I conducted a meta-analysis using studies that evaluate the effects of land use on freshwater environments at regional and local scales. I compared the effect sizes obtained when using local and land landscape variables. Interestingly, I also provide an example of a study with negative results, given that my hypotheses were not confirmed. I believe this thesis will expand the view of limnologists on the possibilities of the meta-analytical technique applied to our field of research.

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Chapter I

Quality of meta-analyses in freshwater ecology: A systematic review

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Quality of meta-analyses in freshwater ecology: a systematic review

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Resumo

Revisões sistemáticas e meta-análises em ecologia tem sido cada vez mais usadas em ecologia. Logo, seus protocolos devem ser seguidos à risca para garantir a qualidade. Vários *checklists* (listas de verificação) estão disponíveis para orientar os pesquisadores rumo a uma meta-análise de alta qualidade. Os estudos de ecologia de água doce tradicionalmente realizam estudos experimentais, fornecendo dados ideais para testar hipóteses usando meta-análise. Aqui, avaliamos a qualidade de 114 meta-análises em ecologia de água doce e 86 meta-análises em ecologia e evolução para fins comparativos. Descobrimos que muitos estudos ainda usam o termo meta-análise incorretamente e que esse erro persistiu com o tempo. A qualidade dos estudos que conduziram uma meta-análise formal melhorou. Assim, especulamos que as diretrizes disponíveis estão sendo eficazes na melhoria da qualidade dos estudos meta-analíticos. A qualidade não foi associada ao fator de impacto do periódico onde as meta-análises foram publicadas ou ao número médio de citações. Além do uso incorreto do termo, descobrimos que muitos estudos falharam em: relatar estatísticas de heterogeneidade, avaliar mudanças temporais no tamanho do efeito, conduzir análises de viés de publicação, abordar a colinearidade entre moderadores e fornecer os dados. Em geral, meta-análises em

ecologia e evolução têm uma pontuação média ligeiramente melhor do que meta-análises em ecologia de água doce. Embora a qualidade das meta-análises em ecologia de água doce tenha melhorado ao longo do tempo, há muito espaço para melhorias. Os autores não devem nomear seus estudos como meta-análises se esses métodos não forem usados. A conformidade com as listas de verificação deve ser amplamente incentivada, pois as meta-análises estão cada vez mais sendo usadas para resumir resultados em diferentes áreas da ecologia. Autores, revisores e editores devem usar listas de verificação para melhorar a qualidade das meta-análises em ecologia de água doce.

Palavras-chave: sistematização, revisão quantitativa, limnologia, Prisma, checklist

Abstract

The protocols of systematic reviews and meta-analyses in ecology should be closely followed to ensure quality given the increasing use. Several checklists are available to guide researchers towards a high-quality meta-analytic study. Freshwater ecology studies have a tradition of using experimental studies, which provide the ideal data to test hypotheses using meta-analysis. Here, we evaluated the quality of 114 meta-analyses in freshwater ecology and 86 meta-analyses in ecology and evolution for comparative purposes. We found that many studies are still using the term meta-analysis incorrectly and that this error persisted over time. The quality of the studies that did conduct a formal meta-analysis has improved. Thus, we speculate that available guidelines are being effective in improving the quality of meta-analytic studies. Quality was not associated with the impact factor of the journal where the meta-analyses were published or with the average number of citations. In addition to the incorrect use of the term, we found that many studies failed to: report heterogeneity statistics, evaluate temporal changes in effect size, conduct publication bias analyses, address the collinearity among moderators and provide the data. In general, meta-analyses in ecology and evolution have only a slightly better average score than meta-analyses in freshwater ecology. Although the quality of meta-analyses in freshwater ecology has improved over time, there is much room for improvement. Authors should not label their studies as meta-analyses if these methods were not used. Compliance with checklists should be widely fostered as meta-analyses are increasingly being used to summarize findings in different areas of ecology. Authors, reviewers, and editors should use checklists to improve the quality of meta-analyses in freshwater ecology.

Keywords: systematization, quantitative review, limnology, Prisma statement, checklist

Introduction

Most review articles in ecology can be classified as narrative reviews, where a given research theme is qualitatively summarized in a narrative structure (Gates, 2002; Koricheva & Gurevitch, 2013). Narrative reviews are useful to explore perspectives, conceptual frameworks, historical advances, and to provide a broader interpretation of topics (Collins & Fauser, 2005; Gurevitch et al., 2018). However, narrative reviews tend to be plagued with subjectivity and biases (e.g., by not stating the methods used to compile the primary studies reviewed; Koricheva & Gurevitch, 2013; Lortie, 2014). In recent decades, a growing number of studies have shown that systematic reviews and meta-analyses, if properly conducted, can minimize these issues (Gates, 2002; Koricheva & Gurevitch, 2013; Lortie, 2014; Gurevitch et al., 2018). These methods are especially important considering the need for predictive power and generalization in the field of ecology (e.g., Houlahan et al., 2017).

Systematic reviews value transparency, systematic reporting, and reproducibility (Gurevitch et al., 2018). A meta-analysis may be conducted when enough data is available, going a step further by quantitatively summarizing empirical findings (Gurevitch et al., 2018). In a nutshell, a meta-analysis calculates a weighted mean effect size, its statistical significance and, importantly, explores possible causes of variation in effect sizes using moderators (Borenstein et al., 2009). Weighting effect sizes by their precision is a pivotal step (Borenstein et al., 2009), as it ensures that estimates will be more influenced by studies with high precision.

However, as emphasized by Olkin (1992), “doing a meta-analysis is easy, doing one well is hard” (see also Felson, 1992; Berman & Parker, 2002). The devil is in the details, and poorly conducted meta-analysis may do more harm than good. For example, results from meta-analyses, in the medical sciences, are generally regarded as providing stronger evidence than a single randomized control trial (but see Murad et al., 2016). However, if the meta-

analysis is conducted poorly, it may deliver misleading conclusions that may be believed as truthful and determine medical treatment regimens or health policies. Similarly, poorly conducted meta-analyses in ecology, in addition to delaying scientific progress, may lead to inappropriate conservation policies. For example, it is common that several primary studies in a meta-analysis contribute more than one effect size (e.g., when the same control is compared with two experimental groups or when the control group is compared with an experimental group at different time-points). Accordingly, a meta-analysis ignoring the fact that multiple effect sizes from the same primary studies are not independent would produce results with inflated type I error rates (see also Song et al., 2020; Mengersen, Jennions, & Schmid, 2013; Van den Noortgate et al., 2014). In general, issues associated with lack of transparency (e.g., in selecting studies and reporting), misinterpretation of results (Morrissey, 2016), incorrect methodology (López-López et al., 2018), premature use of meta-analyses where there is insufficient data (Borenstein et al., 2009; Ioannidis, 2010), and lack of systematization (Gurevitch et al., 2018), for example, have prompted critiques and challenged the credibility of meta-analytical results (Whittaker, 2010; Ioannidis, 2016; Morrissey, 2016). This shows a need to better disseminate good practices among different research fields and to improve the quality of meta-analysis studies (Hillebrand & Cardinale, 2010; Gurevitch et al., 2018).

Clear guidelines for conducting a systematic review have been published and updated as a strategy to improve the quality of meta-analysis (Moher et al., 2009; 2015; Fleming et al., 2014; Shamseer et al., 2015). Well-established guidelines for systematic reviews include, but are not limited to, the Cochrane Handbook of Systematic Reviews (Higgins & Green, 2011), PRISMA statement (Moher et al., 2009; 2015), Tools for Transparency in Ecology and Evolution (Parker et al., 2018) and other relevant checklists (Higgins et al., 2013; Nakagawa et al., 2017; see applications in Delaney et al., 2005; Willis & Quigley, 2011). In ecology, Koricheva & Gurevitch (2014) have proposed a checklist of quality criteria for

meta-analysis for research synthesists, peer reviewers and editors (see also Nakagawa et al., 2017). However, despite these calls for systematization, misuses and poor reporting are still recurrent in meta-analyses in many fields of research (Gates, 2002; Roberts, Stewart, & Pullin, 2006; Philibert, Loyce, & Makowski, 2012; Vetter, Rucker, & Storch, 2013; Koricheva & Gurevitch, 2014; Senior et al., 2016).

Here, we performed a systematic review of meta-analyses published within the field of freshwater ecology and evaluated the quality of these studies considering established criteria. We used the checklist proposed by Koricheva & Gurevitch (2014) as the main reference to evaluate the quality of meta-analyses in freshwater ecology. For comparative purposes, we also evaluated the quality of meta-analyses in the field of ecology and evolution (Senior et al., 2016). We expected that our measure of quality (see Methods section) would increase over time due to increased knowledge of best practices by authors, reviewers, and editors. We also asked whether quality was associated with citation metrics to test whether better developed articles were published in journals with higher visibility. We also expected that the quality score would be correlated with the average number of citations, assuming that readers would seek well-developed studies as references. We expected that the statistical issues related to phylogenetic relationship would be the least addressed criterion due to the lack of recognition that species cannot be regarded as independent points in statistical analyses (Felsenstein, 1985). Also, phylogenetic data are not readily available for many biological groups in the freshwater realm. We expected that the assessment of temporal changes in effect sizes using, for example, cumulative meta-analysis, would not often be conducted. Despite being a well-established tool (e.g., Leimu & Koricheva, 2004), ecologists have only recently considered its potential and importance (e.g., Ortega, Thomaz, & Bini, 2018; Koricheva & Kulinskaya, 2019). Methods to address the use of multiple outcomes or dependent effect sizes have only recently been published (e.g., Hedges et al., 2010; Van den

Noortgate et al., 2014) and used in ecological studies (e.g., Stein, Gerstner, & Kreft, 2014). Thus, we predict that this quality criterion would also often be neglected.

Methods

Search strategy and criteria

We searched for meta-analyses in freshwater ecology using the Web of Science (WoS) and Scopus databases (from 1990 to August 8th, 2017). Our search string (see details in Table 1) resulted in 443 and 382 hits for WoS and Scopus, respectively. We removed duplicates using the function “mergeDbSources” from the R package Bibliometrix (Aria & Cuccurullo, 2017) and screened the articles following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009; 2015). The PRISMA statement is a checklist, including a flow diagram, designed to help authors improve the reporting of systematic reviews (Moher et al., 2009; 2015). The first step consisted of reading the abstracts to exclude obviously irrelevant literature. Then, we read the papers for eligibility (full-text assessment). We included studies that contained formal meta-analyses and we excluded all reviews that were not ‘quantitative reviews using meta-analysis procedures’. Also, we only included studies with results from freshwater ecosystems and excluded studies that did not focus on freshwater organisms (Figure 1.1, Table S1. 1).

Table 1.1 Search string used to obtain meta-analytical studies on freshwater ecology published between 1991 to 2017 in the Web of Science (WoS) and Scopus databases. The Boolean codes were adjusted to each search database, but the same words and wildcard functions were used in both.

Terms	Search string
Meta-analysis terms	(meta-anal* OR metanal* OR "quantitative review")
Freshwater ecology terms	((freshwater OR aquatic* OR limnol* OR "inland water*" OR river* OR stream* OR creek* OR reservoir* OR lake* OR lagoon* OR pond* OR mere* OR loch* OR lakelet*) NOT (ocean* OR marine))

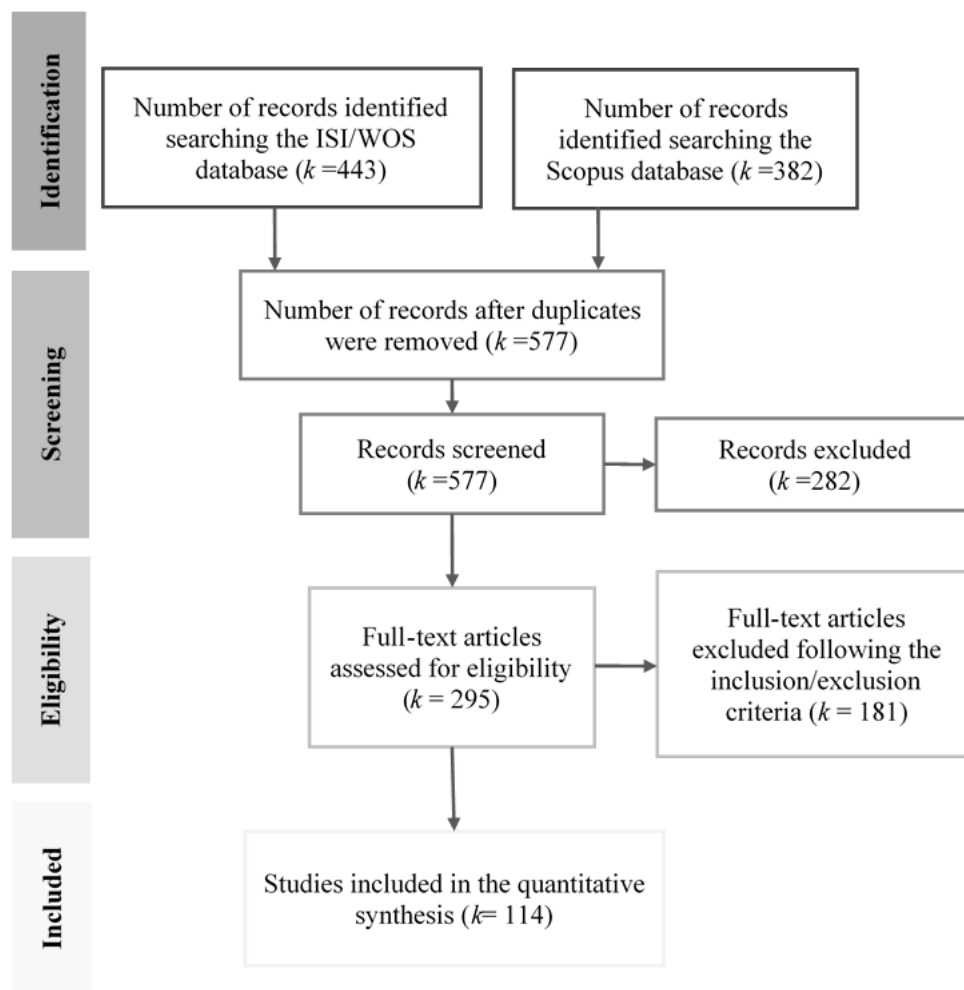


Figure 1.1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram summarizing study inclusion and exclusion phases (adapted from Moher et al., 2009)

A second reviewer re-evaluated 30% of the articles collected from the databases (174 out of 577 papers, selected using the function `sample()` in the software R; R Core Team, 2020). We then calculated the agreement rate (AR; Orwin & Vevea, 2009) and Cohen's Kappa (Cohen, 1960) to assess the reliability of the screening procedures conducted in this study.

Meta-analysis quality

We assigned scores to each study following the checklist of criteria proposed by Koricheva & Gurevitch (2014), which consisted of 16 criteria with references to support them (see their Table 1. 3). We altered the criterion “use of a Meta-Regression to appraise the issue of exploring existent heterogeneity” to “exploring the heterogeneity among studies” (Table 1. 2). In this case, the criterion was met whenever the authors explored causes of heterogeneity among studies using moderators irrespective of the approach (e.g., meta-regression or subset analyses). For each criterion met, we assigned one point to the study. Half points were assigned when the information was incomplete (e.g., lack of the specific search terms). To fulfil the third criterion, the studies had to have calculated weighted effect sizes before modelling. We also assigned one point to the third criterion when effect sizes were weighted by the number of observations (see Gurevitch et al., 2018). Some studies only sought to explain the variation among studies (without estimating a weighted mean effect size). Consequently, we assumed that studies reporting variance partitioning results met the requirements for criterion 3. Some criteria were not relevant for some studies depending on their design (Table 1. 2). For example, taking phylogenetic relatedness into account is not relevant for studies focused on a single species or ecosystem-level variables. Therefore, we calculated the ratio between the sum of points and the maximum possible number of points for the study. Accordingly, the maximum rating is no longer 16 as initially proposed by Koricheva & Gurevitch (2014) but ranged from 0 (lowest) to 1 (highest quality).

We tested for a temporal trend in the rating scores using the Spearman rank correlation between scores and publication year. Similarly, we tested for a correlation between scores and impact factor, and between scores and Normalized Citation Impact Index (NCII) using the Spearman rank correlation. The NCII is given by the ratio between the total

number of citations and the time elapsed (in years) since publication (see Coursaris & Van Osch, 2014 and references therein).

Table 1. 2 Quality criteria used to assign scores to the meta-analysis papers selected and observations explaining the criteria used and exceptions in punctuation. The quality criteria were obtained from Koricheva & Gurevitch (2014).

	Quality criteria	Observations
1	Are details of bibliographic search (electronic data bases used, keyword combinations, years) reported in sufficient detail to allow replication?	Half a point when the search terms are not provided; Not relevant for meta-analysis conducted with primary data.
2	Are criteria for study inclusion/exclusion explicitly listed?	Half a point when the criteria provided are not clear enough for reproducibility. Not relevant for meta-analysis conducted with primary data.
3	Have effect sizes been weighted by study precision or has the rationale for using unweighted approach been provided?	
4	Has the statistical model for meta-analysis been described?	
5	Has heterogeneity of effect sizes between studies been quantified?	
6	Have the causes of existent heterogeneity in effect sizes been explored?	
7	If effects of multiple moderators have been tested, have potential non-independence of and interactions between moderators been considered?	Not relevant if moderators were not considered
8	Have tests for publication bias been conducted?	
9	Have sensitivity analysis been performed to test the robustness of results?	
10	If meta-analysis combines studies published over considerable time span, have possible temporal changes in effect size been tested?	
11	If meta-analysis combined studies conducted on different species, has phylogenetic relatedness of species been considered?	Not relevant for studies considering single species or focused on community (<i>e.g.</i> , diversity or species richness) or ecosystem responses
12	Has the software used been described?	
13	Have full bibliographic details of primary studies included in a meta-analysis been provided?	Not relevant for meta-analysis conducted with primary data.
14	Has the data set used for meta-analysis, including effect sizes and variances/sample sizes from individual primary studies and moderator variables, been provided as electronic appendix?	
15	Have standard metrics of effect size been used or, if nonstandard metrics have been employed, is the distribution of these parameters known and have the authors explained how they calculated variances for such metrics?	
16	If more than one estimate of effect size per study was included in the analysis, has potential non-independence of these estimates been considered?	Not relevant for studies that used one estimate of effect size per study

We also rated the 86 meta-analyses in ecology and evolution compiled by Senior et al. (2016), to compare with our results. Senior et al. (2016) only included studies that quantified heterogeneity among effect sizes. Thus, for comparative purposes, we rated each of the 86 meta-analyses in ecology and evolution without considering the following criteria: “Has heterogeneity of effect sizes between studies been quantified?” (5th criterion), “Have the causes of existent heterogeneity in effect sizes been explored?” (6th criterion) and “Have standard metrics of effect size been used (...)” (15th criterion). We checked whether freshwater studies were included by Senior et al. (2016) and found that a total of 9 studies also evaluated the freshwater realm, none of which were included in our study, thus ensuring independence between datasets.

Results

Most of the 577 studies were excluded in abstract and full text screening because they did not conduct a formal meta-analysis (224 studies) or because they were not focused on freshwater systems (58 studies). The studies that were excluded during the analysis of the abstracts (282 papers) were clearly out of the objectives of our review. Specifically, 118 and 87 studies were not focused on freshwater systems and on ecological questions, respectively. Also, 76 studies did not conduct a formal meta-analysis and one was clearly methodological.

After reading the full texts, we excluded 147 and 34 studies for not being formal meta-analyses or focused on aquatic ecosystems, respectively. Most of these studies consisted of a re-analysis of primary data, without calculating an effect size per study. We found 114 meta-analytic studies that met our criteria (Figure 1.1) after following the different steps to select the studies. We found an agreement rate (AR) of 0.90 and a Cohen’s Kappa of 0.73 (95% CI = 0.62-0.84), indicating that most of the studies were consistently selected by both reviewers during the screening of the abstracts.

The number of meta-analytic studies focused on freshwater ecology increased over time, from the first study published by Wooster (1994), among the studies we selected, to 11 studies published in 2016 and 8 in 2017 (until the search date; Figure 1.2a). These papers were published mostly in *Ecology*, *Oikos*, *Freshwater Biology* and other specific journals within the field of freshwater ecology (Figure 1.2b).

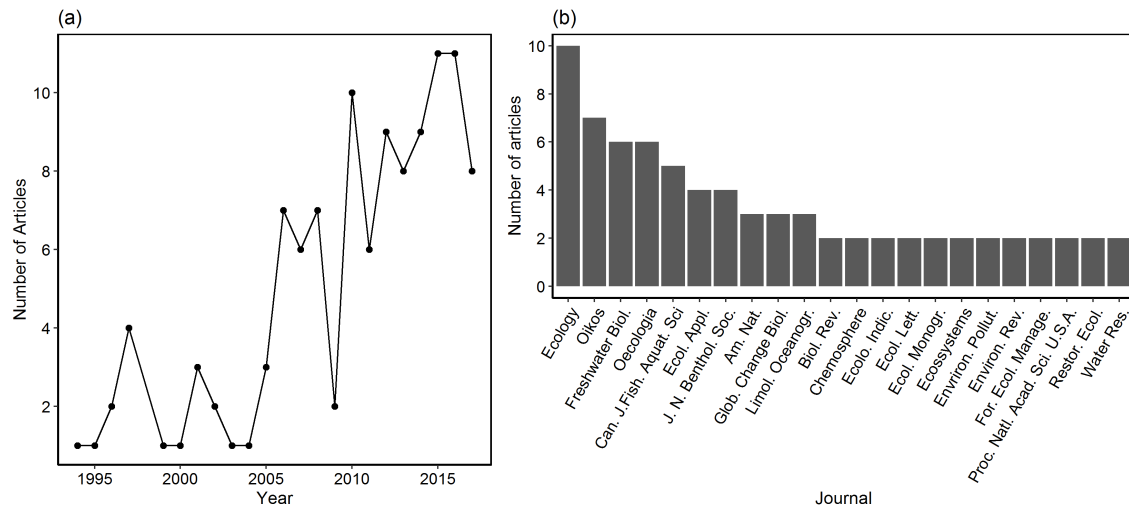


Figure 1.2 Annual number of studies on freshwater ecology using meta-analysis (a). Number of studies per journal (b). The dataset included a total of 114 studies published in 61 journals.

We found an average rating score of 0.56 ± 0.20 (mean \pm SD). Rating scores increased over time (Spearman correlation $r = 0.43$; $P < 0.001$) but were not related to journal impact factor ($r = 0.03$; $P = 0.720$) or to the Normalized Citation Impact Index ($r = 0.15$; $P = 0.121$). Most studies (86.8%, out of 114) did not test for temporal changes in effect sizes (10th criterion). Also, most studies did not perform sensitivity analyses (9th criterion), evaluate the dependence among moderators (7th criterion) and did not provide heterogeneity statistics (5th criterion; Table 1. 3). Nearly half of the studies did not check for publication bias (8th criterion) nor provided the data used for the meta-analysis (14th criterion). The issues of using multiple effect sizes per primary study (16th criterion) were also often ignored and approximately 36 % of the studies did not provide details of the full literature search (1st criterion; Table 1. 3). However, other important criteria were met more frequently; for

example, most studies used standard metrics of effect size (15th criterion), explored the heterogeneity in effect sizes (6th criterion) and used the inverse-variance method to weight effect sizes (3rd criterion; Table 1. 3).

Table 1. 3 Compliance with the criteria for reporting and following methodological standards assigned to freshwater ecology meta-analyses. This survey encompasses 114 studies published from 1991 to 2017. NR = Not Relevant for the study. A detailed description of the criteria is shown in Table 2.

	Quality Criteria	NR (%)	Yes (%)	No (%)	Partial (%)
1	Searching details	7.02	28.95	35.96	28.07
2	Inclusion/exclusion criteria	7.02	57.02	19.3	16.67
3	Weighted effect sizes	-	75.44	24.56	-
4	Meta-analytical model	-	71.93	28.07	-
5	Heterogeneity in effect sizes	-	41.23	58.77	-
6	Causes of heterogeneity	0.88	82.46	16.67	-
7	Collinearity analysis	15.79	22.81	61.4	-
8	Publication bias	6.14	39.47	54.39	-
9	Sensitivity analysis	-	37.72	62.28	-
10	Changes in effect size	5.26	7.89	86.84	-
11	Controlling for phylogeny	71.05	3.51	25.44	-
12	Software	-	69.3	30.7	-
13	Bibliographic details	6.14	75.44	18.42	-
14	Data	-	52.63	47.37	-
15	Standard effect size	-	93.86	6.14	-
16	Multiple effect sizes	27.19	26.32	39.47	7.02

Most of the criteria were met in ecological and evolutionary studies (average rating score of 0.61 ± 0.21 SD) with a higher frequency than in limnological meta-analyses (Table 1. 4). This was especially so for criteria 1, 3, 4, 8, 9, 11, 13 and 16 (Figure 1.3). In general, criteria 1, 7, 10, 11 and 16 were more frequently unmet (< 50% of the studies) in both fields.

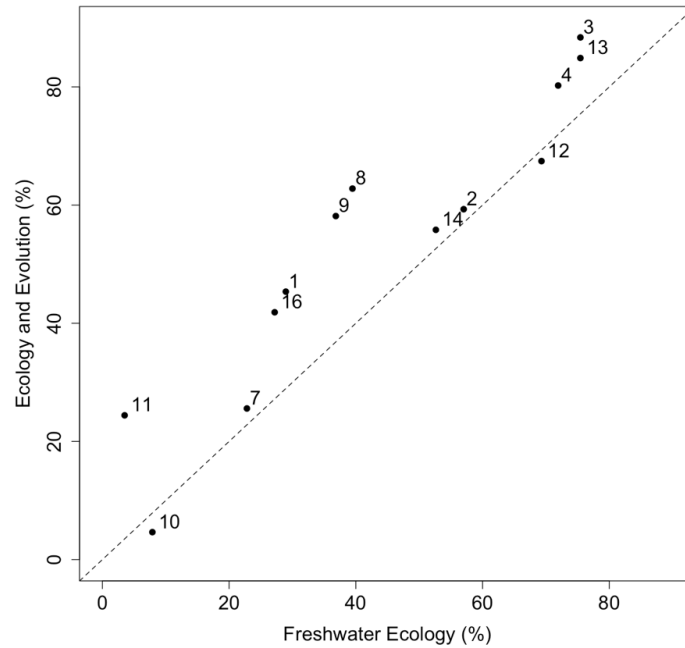


Figure 1.3 Relationship between percentages of meta-analytical studies complying with different criteria for both freshwater ecology (this study) and ecology and evolution (compiled by Senior et al., 2016). Numbers indicate the quality criteria (Table 1. 2). Values above the line represent criteria that were met more frequently by ecological and evolutionary meta-analyses, while values below the line were met more frequently by freshwater ecology meta-analyses.

Table 1. 4 Compliance with the criteria for reporting and following methodological standards assigned to 86 ecological and evolutionary meta-analyses reviewed by Senior et al. (2016). A detailed description of the criteria is shown in Table 2. * indicates criteria that were disregarded for ecological and evolutionary meta-analyses.

Quality Criteria	Yes (%)	No (%)	Partial (%)
1 Searching details	49.41	27.06	20.00
2 Inclusion/exclusion criteria	63.53	11.76	21.18
3 Weighted effect sizes	92.94	7.06	0.00
4 Meta-analytical model	84.71	15.29	0.00
5 Heterogeneity in effect sizes	*	*	*
6 Causes of heterogeneity	*	*	*
7 Collinearity analysis	27.06	63.53	0.00
8 Publication bias	65.88	30.59	0.00
9 Sensitivity analysis	61.18	38.82	0.00
10 Changes in effect size	5.88	90.59	0.00
11 Controlling for phylogeny	24.71	45.88	0.00
12 Software	71.76	28.24	0.00
13 Bibliographic details	89.41	7.06	0.00
14 Data	58.82	41.18	0.00
15 Standard effect size	*	*	*
16 Multiple effect sizes	42.35	35.29	4.71

Discussion

Meta-analysis is considered a powerful method for summarizing results of independent studies in different research areas (e.g., Lortie, 2014; Ellis, 2010; Gurevitch et al., 2019). However, the higher credibility of meta-analysis results may be a double-edged sword, especially if reliability is questionable (Ioannidis, 2010; 2016; 2017; Nakagawa et al., 2017). In this context, it was disturbing to find many studies stating that they conducted meta-analyses despite the use of different approaches (e.g., re-analysis of primary data, vote counting, scientometrics, qualitative reviews). Other studies failed to mention the criteria used to select studies, disregarded publication bias analyses (Koricheva & Gurevitch, 2014; Nakagawa et al., 2017) or focused mostly on statistical significance, often neglecting to model heterogeneity (Higgins & Thompson, 2002; Koricheva & Gurevitch, 2014; Senior et al., 2016). Many of the studies failed to address non-independence issues with the use of multiple effect sizes from the same studies (e.g., Hedges, Tipton, & Johnson, 2010), or at least to conduct a sensitivity analysis to demonstrate the robustness of their results (Gurevitch et al., 2018). The analysis of temporal stability in effect sizes was also disregarded in several meta-analyses, which is of concern as it may lead to misleading conclusions (e.g., Koricheva & Kulinskaya, 2019).

According to Koricheva & Gurevitch (2014), meta-analysis is a “set of statistical methods for combining outcomes (effect sizes) across different data sets addressing the same research question to examine patterns of response across these data sets and sources of heterogeneity in outcomes”. Here, 224 studies were excluded solely for not being a formal meta-analysis. Some of these exclusions occurred after reading the abstract (77) because the studies stated that their results were important for the development of future meta-analysis, while others cited the term meta-analysis in other contexts. The most striking result concerns the exclusion of 154 studies, after reading the full texts, for not using meta-analytic

approaches (e.g., vote-counting and re-analysis of primary data), despite being described as meta-analyses by the authors. This erroneous use of the term meta-analysis has also been observed in other areas of ecology (Vetter et al., 2013; Koricheva & Gurevitch, 2014; Senior et al., 2016), despite many studies urging authors to “apply the term ‘meta-analysis’ consistently and correctly and not to confuse it with other summary techniques” (e.g., Vetter et al., 2013). Indeed, we found that the number of studies erroneously self-classified as meta-analysis is still increasing (Spearman’s $r = 0.74$; $P = 0.0002$; $n = 20$ years, (1998-2017)).

We found that meta-analyses in freshwater ecology require improvements, in line with previous studies from different fields (Delaney et al., 2005; Koricheva & Gurevitch, 2014; Ioannidis, 2016). Meta-analysis quality was not correlated with journal impact factor, indicating that the most well-conducted meta-analyses are not necessarily published in journals with high impact factors (Brembs, 2018). The lack of correlation between the average number of citations an article received and its quality score, in turn, suggests that other factors (e.g., themes and specific results) are more important to “citability” than the quality of the meta-analysis itself (see also Padial et al., 2010 for a general analysis of citation frequency of ecological articles). However, we found that our rating scores were significantly correlated with publication year, suggesting that published guidelines to improve the use of meta-analyses (e.g., Moher et al., 2009; Koricheva & Gurevitch, 2014; Nakagawa et al., 2017) are being effective.

Reporting the search strings and databases (e.g., Web of Science and Scopus) used to find articles that provide data to a meta-analysis, is far from being a mere bureaucratic step (Nakagawa et al., 2007; Gusenbauer & Haddaway, 2020). For example, researchers may avoid wasting time through the repetition of an inclusive meta-analysis when search strings are reported or may detect the need for a new meta-analysis when/if the one published was too restrictive (see Babic et al., 2020). However, our results parallel those of Koricheva &

Gurevitch, (2014) by showing that only a few studies included full details of bibliographic searches. The problem becomes more pronounced when the inclusion/exclusion criteria are not reported (for a discussion on this theme, see Lortie & Callaway, 2006). Even the easy-to-meet quality criterion of providing the list of primary studies included in the meta-analysis was not met by 21 studies, despite being essential for reproducibility testing. Specifically, if the list of primary studies used in a published meta-analysis differs substantially from a list of primary studies retrieved by using a different search string (e.g., due to the use of synonyms or different technical terms), then, all else being equal, a new meta-analysis, considering the different set of primary studies, may produce different results and new insights (e.g. compare Westgate et al., 2014 to de Morais et al., 2018 and Stein, Gerstner, & Kreft, 2014 to Ortega, Thomaz, & Bini, 2018; see also Page & Moher, 2016 for a discussion about the problem of redundant meta-analyses). In addition, only nearly half of the meta-analyses in freshwater ecology provided the dataset used (60 studies). Proportionally, this figure was even lower in plant ecology (Koricheva & Gurevitch, 2014). Providing the dataset used is crucial for cumulative meta-analysis (Leimu & Koricheva, 2004) and to explore the same dataset using different moderators (e.g., Menegotto, Kurtz & Lana, 2019).

A critical step in a meta-analysis is to weight effect sizes by the inverse variance method (Borenstein et al., 2009; Koricheva & Gurevitch, 2013). Many of the studies we reviewed weighted the effect sizes (86 out of 114). Still, 28 of the studies included in our review did not use the inverse variance method. In some cases, the authors stated that the variance (or the data needed to estimate it) was not available in the primary studies. However, other weighting strategies are available, for example, estimating variance from partial data and weighting by n (Koricheva & Gurevitch, 2014). As the quality of reporting and transparency increases in primary studies, the frequency of these issues is expected to decrease (Gerstner et al., 2017).

Thirty-two studies (out of 114) did not report the meta-analytical model used to analyze the data. However, different models (fixed or random effects) have different assumptions, test different hypotheses, and may provide contrasting results (Borenstein et al., 2009; Koricheva & Gurevitch, 2013). In general, the use of a random-effects model is the best alternative because of the restrictive assumptions of a fixed-effect model (mainly that effect sizes are homogeneous across primary studies). Similarly, thirty-five studies did not report the software used, although this is important because it may influence results (Koricheva & Gurevitch, 2014).

Heterogeneity measures are crucial for a comprehensive interpretation of the weighted mean effect size (Senior et al., 2016; Nakagawa et al., 2017; Forstmeier, Wagenmakers & Parker, 2017). Interpreting the weighted mean effect size without the heterogeneity measures is comparable to drawing conclusions from the mean without showing the standard deviation. Few studies have quantified heterogeneity (47 out of 114; heterogeneity quantified as T^2 and Q). Still, many explored possible causes of variation (94 out of 114), because most meta-analyses are more interested in exploring variation in effect sizes among studies and in the causes of this variation (Koricheva & Gurevitch, 2014). However, not reporting the heterogeneity or variation of effect sizes is concerning for ecological meta-analyses, given the high complexity of natural systems (Vetter et al., 2013).

The basic strategy to explore causes of heterogeneity is to conduct a meta-regression with several moderators, which corresponds to a weighted multiple regression. Thus, the same assumptions of multiple regression also apply to meta-regression (e.g., checking for collinearity among moderators; Borenstein et al., 2009; Koricheva & Gurevitch, 2013; Nakagawa et al., 2017). While many studies explored the causes of heterogeneity in effect sizes, less than a quarter checked for collinearity. Issues such as instability of parameter estimates, inflated standard errors and biased inferences (Dormann et al., 2013) arise when

moderators are collinear. As in any other research approach, the issues with multicollinearity highlight the need for careful planning to conduct a meta-analysis (Berman & Parker, 2002). For example, many studies are screened to compile data on moderators hypothetically related to the effect size. However, without proper planning and theoretical reasoning, this hard work may be in vain if different pairs of moderators are highly correlated to each other or even worse, if redundant moderators are compiled at the expense of ignoring other relevant moderators.

Publication bias is said to exist when the results of a study influence the decision of authors, reviewers, and editors to publish a manuscript. This subject has been extensively debated (Møller & Jennions, 2001; Rothstein, Sutton & Borenstein, 2006; Dwan et al., 2013) due to its effects on systematic reviews and meta-analyses (and also on narrative reviews). Positive results are more likely to be published (Fanelli, 2012; Fanelli, Costas & Ioannidis, 2017; Forstmeier, Wagenmakers, & Parker, 2017). In this context, positive results may be over-represented in the pool of articles available for meta-analysis potentially leading to overestimation of the weighted mean effect size (Møller & Jennions, 2001). Although researchers highlight the need to study publication bias (e.g., Møller & Jennions, 2001; Tomkins & Kotiaho, 2004; Jennions, 2013), over half of the studies (62 out of 114) did not assess it. The lack of concern with publication bias is a recurrent issue in ecology (Roberts, Stewart, & Pullin, 2006; Nakagawa & Santos, 2012; Koricheva & Gurevitch, 2014), as are requests to analyze this issue (Nakagawa & Poulin, 2012).

Twenty-seven meta-analytical studies, out of the 45 that tested for publication bias, used only one method. However, the use of multiple approaches is advisable (Lin et al., 2018). The funnel plot, which may indicate publication bias through asymmetric distribution of effect sizes, was the most used approach (28 papers). The funnel-plot has been criticized on several grounds (Terrin, Schmid & Lau, 2005; Jennions, 2013; Lin, 2019). For instance,

asymmetry may be the consequence of other factors when the number of primary studies (k) is small (Egger et al., 1997). Consequently, many studies also used the funnel plot associated with other tests (19 studies – Fail-safe numbers, Quantile-plot, and correlation). The ‘trim and fill’ method is often used to “correct” for publication bias (Duval & Tweedie, 2000; Peters et al., 2007; and see Weinhandl & Duval, 2012 for an alternative method applied to meta-regression). However, this method tests how robust the results are to publication bias (Jennions & Møller, 2002a; Sutton, 2002) and, therefore, is more of a sensitivity analysis than a method to correct for publication bias (Rothstein, Sutton & Borenstein, 2006).

Conducting sensitivity analyses is also important to check the robustness of results. Basically, it consists of double-checking decisions made throughout the study (Noble et al., 2017). The use of multiple effect sizes, models, publication bias methods and the evaluation of temporal changes in effect sizes are all issues that can be evaluated. However, the best strategy needs to be determined on a case-by-case basis.

A useful strategy to analyze the results is to evaluate whether the effect sizes vary over time (e.g., using a cumulative meta-analysis; Leimu & Koricheva, 2004; Ortega, Thomaz, & Bini, 2018) or to use publication year as a moderator when exploring sources of heterogeneity (Wood & Eagly, 2009; Lehmann et al., 2012). Cumulative sum charts may also be used to detect possible outliers and temporal tendencies (Kulinskaya & Koricheva, 2010; Dogo, Clark & Kulinskaya, 2017). Causes of variation include the tendency of earlier studies to publish higher effect sizes (i.e., time-lag bias), publication bias, Proteus phenomenon (the attraction to contradictory results), true heterogeneity or even chance (Jennions & Møller, 2002b; Trikalinos & Ioannidis, 2006; Ioannidis, 2008). A decrease in effect size over time can be also attributed to the test of a “valid theory beyond its domain of application” (Wilson, Harris, & Wixted, 2020). Despite evidence that effect sizes may change over time (see Koricheva, Jennions, & Lau, 2013 for detailed examples) and the practical implications of

this change (Koricheva & Kulinskaya, 2019), only nine meta-analytical studies in freshwater ecology explored this topic.

Independent data points are essential for statistical testing (Forstmeier, Wagenmakers, & Parker, 2017). Non-independence of effect sizes occurs when the data exhibit a correlated or clustered structure, which may be driven by several factors (Lajeunesse, Rosenberg, & Jennions, 2013). For example, in a cross-species meta-analysis, one can find that the effect sizes are phylogenetically structured. Thus, by not accounting for the phylogenetic structure, we would violate the assumption of independence of effect sizes, which has been shown to change the results of meta-analyses (Chamberlain et al., 2012; Nakagawa & Santos, 2012). We found that 29 out of 33 studies for which this criterion was relevant did not control for phylogenetic structure. Nevertheless, methods to incorporate phylogeny have been extensively developed in evolutionary biology and are also available for different meta-analysis models (Adams, 2008; Lajeunesse, 2009; Lajeunesse et al., 2013).

Primary studies often report more than one effect sizes. For example, a primary study may test the relationship between a response variable and different explanatory variables (across the same sampling units). However, if multiple effect sizes per study are used, the total sample size of a meta-analysis will be inflated (Jennions & Schmid, 2013), resulting in increased type I error rates (Hedges et al., 2010; Noble et al., 2017; Forstmeier, Wagenmakers, & Parker, 2017). Only 30 freshwater ecology studies addressed this issue, primarily by comparing the weighted mean effect size obtained using multiple effect sizes to the weighted mean effect size obtained by using one (selected or averaged) effect size per study (e.g., following the approaches described in López-López et al., 2018). There are several statistical methods available to address effect size multiplicity (e.g., Jennions & Schmid, 2013; Tanner-Smith & Tipton, 2014; López-López et al., 2018). For example, Hedges et al. (2010) proposed a method called Robust Variance Estimate (RVE). Given the

availability of software (e.g., Fisher & Tipton, 2015), we think that the issue of multiple effect sizes per study will be addressed in most future studies.

Our overall results (mean quality) may be generalizable among different fields in ecology (i.e., considering the results for freshwater ecology, ecology and evolution (Senior et al., 2016) and plant ecology (Koricheva & Gurevitch, 2014)). However, ecological and evolutionary meta-analyses had a higher compliance with the quality criteria than freshwater ecology meta-analyses. It is noteworthy that few studies explored temporal dependence in effect sizes, checked for dependence among moderators, or controlled for phylogenetic dependence in either areas, suggesting this may be a recurrent issue. A similar pattern was observed for plant ecology (Koricheva & Gurevitch, 2014). Thus, we think that further studies should be especially aware of these issues, once lack of independence (temporal, phylogenetic or in explanatory variables), temporal trends in effect sizes and collinearity among moderators are issues of primary concern.

Our findings point to the need for a greater adherence to guidelines for systematic reviews. Thus, we reiterate the importance of applying the checklists, which are widely available, to improve reporting of meta-analyses in different areas of ecology (e.g., Parker et al., 2018; Roberts, Stewart, & Pullin, 2006; Moher et al., 2009; 2015; Koricheva & Gurevitch, 2014). We expect that the use of checklists will increase the quality of meta-analyses, as has been reported, for example, for biomedical research (Han et al., 2017). The need to enhance quality should not be taken lightly, given that their results are potentially used by decision makers as decisive evidence (Koricheva & Kulinskaya, 2019).

Conclusion

We found that the quality of meta-analyses in freshwater ecology has increased over time; however, there is much room for improvement in complying with important quality criteria. Firstly, authors should not mislabel their studies as meta-analyses when, in fact, the

method was not used. Secondly, compliance with checklists should be widely fostered as meta-analyses are increasingly being used to summarize findings in different areas of ecology. Thus, authors, reviewers and editors should comprehensively use checklists to improve the quality of meta-analyses in freshwater ecology.

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Supplementary Information

Table S1. 1. Complete reference of the studies included (n=114; see methods section for details on the selection procedure)

Number	Complete Reference
1	Albertson, L. K., D. C. Allen, & J. C. Trexler, 2015. Meta-analysis: Abundance, behavior, and hydraulic energy shape biotic effects on sediment transport in streams. <i>Ecology</i> .
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Chapter II

But the emperor is [still] not wearing anything at all! – excessive positive results in limnology

Resumo

O viés de publicação ocorre quando a magnitude ou a direção dos resultados diferem entre estudos publicados e não publicados. Os estudos de meta-análise tendem a incluir principalmente artigos com resultados positivos (ou seja, resultados significativos que apoiam a hipótese), uma vez que são mais propensos a serem aceitos para publicação. Essa tendência aumenta os possíveis problemas com viés de publicação. Avaliei a prevalência de viés de resultados positivos em meta-análises de ecologia de água doce, dividindo os resultados em três conjuntos de dados diferentes. Os resultados positivos prevaleceram sobre os resultados negativos para todos os conjuntos de dados considerados. Resultados possivelmente percebidos como mais relevantes tiveram maior incidência de resultados positivos. Além disso, a incidência de resultados positivos em tamanhos de efeito individuais e resultados de moderadores não foram relacionados ao país dos autores. A incidência de resultados positivos para efeitos globais foi positiva e não-linearmente relacionada ao ano de publicação. A alta prevalência de resultados positivos entre meta-análises pode refletir vieses em duas escalas diferentes, especificamente, em estudos primários e dentro das próprias meta-análises.

Abstract

Publication bias is said to occur when the strength or direction of the results differ between published and unpublished studies. Meta-analysis studies tend to include mostly articles with positive results (i.e., significant results supporting a hypothesis), which are more likely to be accepted by scientific journals. This tendency heightens possible issues with publication bias. I evaluated the prevalence of positive results bias in freshwater ecology meta-analyses, dividing the results into three different datasets covering different scales of results. Positive results prevailed over negative results for all the datasets considered. Results possibly perceived as more relevant had a higher incidence of positive results. Also, the incidence of positive results in individual effect sizes and moderator results was unrelated to the authors' country. The incidence of positive results for summary effects was positively and non-linearly related to publication year. A high prevalence of positive results among meta-analyses may reflect biases in two different scales, specifically, in primary studies and within the meta-analyses itself.

Introduction

The problem with the excessive importance given to statistical significance is by no means a novelty (Bakan, 1966; Cohen, 1994; Møller & Jennions, 2001; Jennions & Møller, 2002; Ioannidis & Trikalinos, 2007a; Fanelli, 2010a, 2012). Bakan (1966) reported that a “*great deal of mischief*” has been associated with the use of *P*-values and acknowledged that stating the existence of such mischief is “*to assume the role of the child who pointed out that the emperor was really outfitted only in his underwear*”. It may seem obvious, but the emperor is apparently still running around without his clothes, and the aforementioned ‘mischief’ may include bias against negative and non-significant results, selective reporting and different types of questionable research practices (Ioannidis, 2005; Ioannidis & Trikalinos, 2007a; Fanelli, 2012; Fanelli et al., 2017; Van Aert et al., 2019).

Publication bias is said to occur when the strength or direction of the results differ between published and unpublished studies (Møller & Jennions, 2001). It may be defined as “the tendency on the parts of investigators, reviewers, and editors to submit or accept manuscripts for publication based on the direction or the strength of the study findings” (Dickersin, 1990) or yet as “the selective publishing of research based on the nature and direction of findings, that occurs when studies with significant or favorable results are more likely to be published than those with nonsignificant or unfavorable findings”(Marks-Anglin & Chen, 2020). Studies with significant results that also support their main hypotheses, are more likely to be published than studies reporting non-significant and negative results (Dickersin & Min, 1993) due to several reasons (see below). In general, publication bias may be perceived as an umbrella under which other biases are encountered.

Statistical significance was a major determinant of the decision to submit and of the time it took for a paper in clinical trials to be submitted and published (Ioannidis, 1998),

showing that publication bias may occur in all stages involving the publication of an article (Møller & Jennions, 2001). Non-significant results are often considered uninteresting by investigators (Dickersin & Min, 1993), and may never be submitted or published (Knight, 2003), contributing to the file-drawer problem (Rosenthal, 1979; and see Knight, 2003; Fanelli, 2012; Graqvist, 2015). In addition, more prestigious journals tend to publish a lower proportion of non-significant results (Csada et al., 1996; Browman, 1999; Knight, 2003; Lawrence, 2003), directly or indirectly pressing for statistical significance, once funding agencies take into account journal prestige when assessing to whom funds will be directed (Csada et al., 1996). More troubling is when ‘significant’ results are the product of data fabrication or unreported post-hoc re-interpretation, re-analysis, and selection. Unfortunately, fabrication bias (Borenstein et al., 2009c; Ioannidis, 2010) has been documented in medicine (Ranstam et al., 2000; Al-Marzouki et al., 2005; Gardner et al., 2005) and may occur in other disciplines as well.

Finally, bias towards positive results entails our tendency to confirm expected or desired results (Rosenthal & Fode, 1963). To obtain the expected results authors may change hypotheses after knowing the results (Forstmeier et al., 2017; Fraser et al., 2018), select parts of the results to be reported (selective reporting) and/or try different analytical methods until reaching the desired result (Simmons et al., 2011). As reported by Csada et al. (1996), *“this raises the question as to how many studies reported to have significant results are nothing more than a researcher's expectations of such results”* and the question *“of how much this contributes to the publishing of fraudulent results”*.

These biases against non-significant results may bring serious consequences to science (Fanelli, 2012). It was regarded by Stephen Jay Gould as *“perhaps the most important effectively undiscussed subject in the entire methodology of science”* (Browman, 1999). The scientific progress relies heavily on unexpected results, without which Popper’s

falsifiability (Popper, 2005) would never occur, Khuns' paradigm (Kuhn, 1996) would never shift, and nor would Lakatos' auxiliary hypotheses (Lakatos, 2014) be altered or abandoned with the onset of new/different discoveries (see Alatalo et al., 1997 for an example of the importance of negative results for paradigm shifts in science). In medicine, for instance, an ineffective drug could cease to be used by the population by the onset of a published negative result. Of course, the undesirable and harmful consequences are more visible, or perceived by society at large.

Ecology and other fields of knowledge would equally be affected by a possible resistance to the negative results, which is comprehensible. For instance, take climate change, a serious matter that has been evidenced extensively. Even if the evidence was misinterpreted (type I error), we will have increased renewable energy sources, and reduced air pollution, oil exploration in sensitive ecosystems and anthropogenic fires. On the other hand, if we tolerate a high type II error rate and disregard the existence of climate change, the consequences may be catastrophic (Oreskes, 2019). Our resistance to accepting negative results is ultimately a debate between consequences of accepting a false positive result (type I error) or rejecting a true positive result (type II error). The issue with simply accepting results as they come lies in the fact that many published results are type I errors, and these results are too rarely discredited (Parker & Nakagawa, 2014); however, type II error may implicate in more serious consequences (Forstmeier et al., 2017).

Meta-analysis is a powerful tool that is being increasingly used in different areas and consists of weighting the effect sizes obtained from primary studies by the inverse of their variances and of searching for correlates of the heterogeneity among effect sizes (Borenstein et al., 2009a; Koricheva & Gurevitch, 2013; Gurevitch et al., 2018). Tough criticisms have been made to the use of meta-analysis (Berlin & Golub, 2014), among which the 'garbage-in, garbage-out' argument (Ioannidis, 2010; Whittaker, 2010; Berlin & Golub, 2014), once it's

reliability depends highly on the quality of primary studies, which in turn are severely biased towards positive results (Fanelli, 2012). Meta-analysis studies tend to include mostly articles with positive results (i.e., significant results supporting a hypothesis), which are more likely to be accepted by scientific journals. This tendency heightens possible issues with publication bias (Hopewell et al., 2006) and raises the question of whether the tools available for dealing with publication bias are being effective (Ioannidis, 2010). Indeed, according to Ioannidis (2010), “it would be naïve to think we are able to filter out or control all the effects of such biases”.

Freshwater ecology is a field that brings relevant contributions to the preservation and conservation of waterbodies, especially given the growing issues with water quality throughout the world. As such, evaluating the possibility of bias and consequently assessing reliability of results has important implications which varies among scientific fields and publication environments (Ioannidis, 2010), and therefore must be evaluated specifically for each discipline. In this paper I will evaluate the incidence of positive results in meta-analyses of freshwater ecology. I will focus on the proportions of positive results (the significant results supporting the hypothesis) and negative results (significant results against the hypothesis and non-significant results; following Fanelli, 2012). I expect to (i) find a higher proportion of positive results compared to negative and non-significant results in each meta-analysis evaluated. I also predict that the proportion of positive results per study would be (ii) negatively associated with time, (iii) more associated with countries where science is more competitive, and (iii) negatively so with the number of authors involved in the publication. I expect a negative association with time as an indication of increasing awareness of publication bias, given the increasing literature on the importance of publishing negative findings. In turn, I hypothesized that more competitive countries will tend towards having a higher incidence of bias towards positive results and of selective reporting due to a higher

pressure to publish (following Fanelli, 2010b; Fanelli et al., 2015). I also expect that the number of authors involved in a study is inversely related with the proportion of positive results once having more scientists involved could buffer the incidence of some biases (Ioannidis, 2010; Fanelli et al., 2017). Finally, I also expect that the number people holding a doctoral degree in the country of the first author will also be negatively associated with the prevalence of positive results, indicating an increased knowledge and responsibility with the papers published.

Methods

Data collection

I compiled studies from the Web of Science and Scopus databases published from 1991 to 2017. The detailed search string and initial hits are shown in Table 2.1. The Boolean operators were adjusted to each search database, but I used the same words and wildcard functions for both. Subsequently, I screened the studies following the Prisma protocol (Moher et al., 2009), including only formal meta-analyses in freshwater ecology and obtained a total of 113 meta-analyses (Lodi et al., 2021). I included studies that conducted formal meta-analyses about freshwater ecosystems; however, I also included studies that evaluated several systems at once (terrestrial, oceanic, freshwater, etc.) if freshwater environment was evaluated (provided an individual effect size). I excluded studies in any field of expertise other than freshwater ecology and studies carried out within the freshwater environment, but that did study freshwater dynamics or organisms (e.g., Yu and collaborators (2017) conducted soil analysis of a restored wetland). A total of 21 of these papers were also excluded for not including enough information to infer the direction of the hypothesis (see dataset in Table S2.1).

Table 2.1. The search string used in the Web of Science (WoS) platform and the number of hits in both WoS and Scopus databases.

	Search string	WoS hits	Scopus hits
Meta-analysis terms	(meta-anal* OR metanal* OR "quantitative review")	130.960	199.481
Freshwater ecology terms	((freshwater OR aquatic* OR limnol* OR "inland water*" OR river* OR stream* OR creek* OR reservoir* OR lake* OR lagoon* OR pond* OR mere* OR loch* OR lakelet*) NOT (ocean* OR marine))	1.001.200	1.481.920
(Meta-analysis terms) AND (Freshwater ecology terms)		443	382

(*) Designates a wildcard function that includes all possible word endings, and quoted words (“”) represent searches of exact phrases.

Classification of the studies

I used a fine-grained measure of positive results (finer than Fanelli, 2012, for example), as suggested by Fanelli (2017), following a more inclusive approach. For each paper, I extracted the hypothesis, and the significance and direction of each result. Results were either for summary effect sizes, effects of moderators, or individual effect sizes. I categorized the results as ‘positive’, ‘negative’ or ‘not significant’. Results were classified as ‘positive’ when they confirmed the hypotheses of the studies and ‘negative’ when the results did not support the hypotheses either because the tests were not significant or because they were significant but in the opposite direction specified by the hypotheses. Some studies did not explicitly present a directional hypothesis. In such cases I read the paper and observed the direction of the hypothesis following the conceptual construct presented by the authors. When this was not possible, I considered that the authors expected to confirm theoretical predictions most often confirmed in the literature. I excluded all papers for which neither of these approaches were feasible. I obtained data in different resolutions instead of including only one hypothesis per paper and considering only clear direct hypothesis (as done by Fanelli, 2012) to obtain data with a finer resolution and broader scope.

I created three datasets (ds), one with the results of summary effect sizes (ds1), a second one with effects of moderators (ds2), and a third with the results of individual effect sizes (ds3). This multi-level approach is more appropriate to evaluate ‘longer publications with multiple results’ (Fanelli, 2018). Multiple results were presented by most papers, for all datasets (summary effects included). Therefore, I calculated the ratio of positive to negative and non-significant results ($\text{positive}/(\text{positive}+\text{negative}+\text{non-significant})$) to produce a measure called “proportion of positive results” (PPR). My *sample unit* is the results of a meta-analysis within each ds category, and the *sample size* (n_{ds}) is the number of articles that provided summary effect sizes, moderators or individual effect size results, once multiple results were compiled into one measure per article. I obtained 33 results for summary effect sizes ($n_{ds1}=33$), 48 for moderators ($n_{ds2}=48$) and 81 for individual effect sizes ($n_{ds3}=81$) from a total of 114 selected papers.

To complement the information on the significance and direction of the results, I also obtained the year of publication, first author’s country, and the number of authors included in the paper using the function *biblioAnalysis()* available in the R package Bibliometrix (Aria & Cuccurullo, 2017). The first authors’ countries were grouped to reduce the number of levels (following Fanelli, 2012) as follows: NAm: United States of America and Canada; EU: Austria, Czech republic, Finland, France, Germany, Ireland, Italy, Netherlands, Portugal, Spain, Sweden, United Kingdom, Norway; AS: China, Hong Kong, India, Japan, Singapore, South Korea, Taiwan; and NZ-AM: New Zealand, Argentina, Chile. I also obtained the proportion of 25-64-year-olds people with a doctorate degree per country from the Organisation for Economic Cooperation and Development (OECD) library (available at https://www.oecd-ilibrary.org/education/education-at-a-glance-2019_f8d7880d-en).

Data analysis

I evaluated whether positive results for summary effect sizes (ds1) prevailed over negative ones, and if the incidence of positive results was associated with publication year, corresponding author's country, number of authors and proportion of doctorate degrees per country, using a nonlinear least squares and a linear model. The variable year exhibited a clear nonlinear pattern in ds1, such that I was not able to fit a reliable linear model including this variable directly. I started with simpler approaches using the arcsine and logarithmic transformations and fitting linear models with different distributions. However, the residues of simpler analyses did not meet the required assumptions, i.e., were not suitable. Finally, I fitted a nonlinear model (function *nls()* in R) with the PPR of summary effects as response variable and publication year as the explanatory variable and extracted the residuals to be used as response variable in the linear model.

The full model for ds1 consisted of the residuals of the nonlinear model as the response variable, and country, number of authors involved in the publication and proportion of doctorate degrees per country as the explanatory variables. The PPR obtained for ds2 and for ds3 were used as response variables in two linear models, one for each dataset, each using publication year, country, number of authors and proportion of doctorate degrees per country as the explanatory variables. All assumptions of heteroscedasticity and residual normality were met.

Results

The average number of positive results surpassed that of negative results for all the datasets considered (Figure 2.1a). In addition, the confidence interval of negative results overlapped zero in all cases (Figure 2.1a). Consequently, the average PPR were greater than 0.5 in the different datasets (Figure 2.1b).

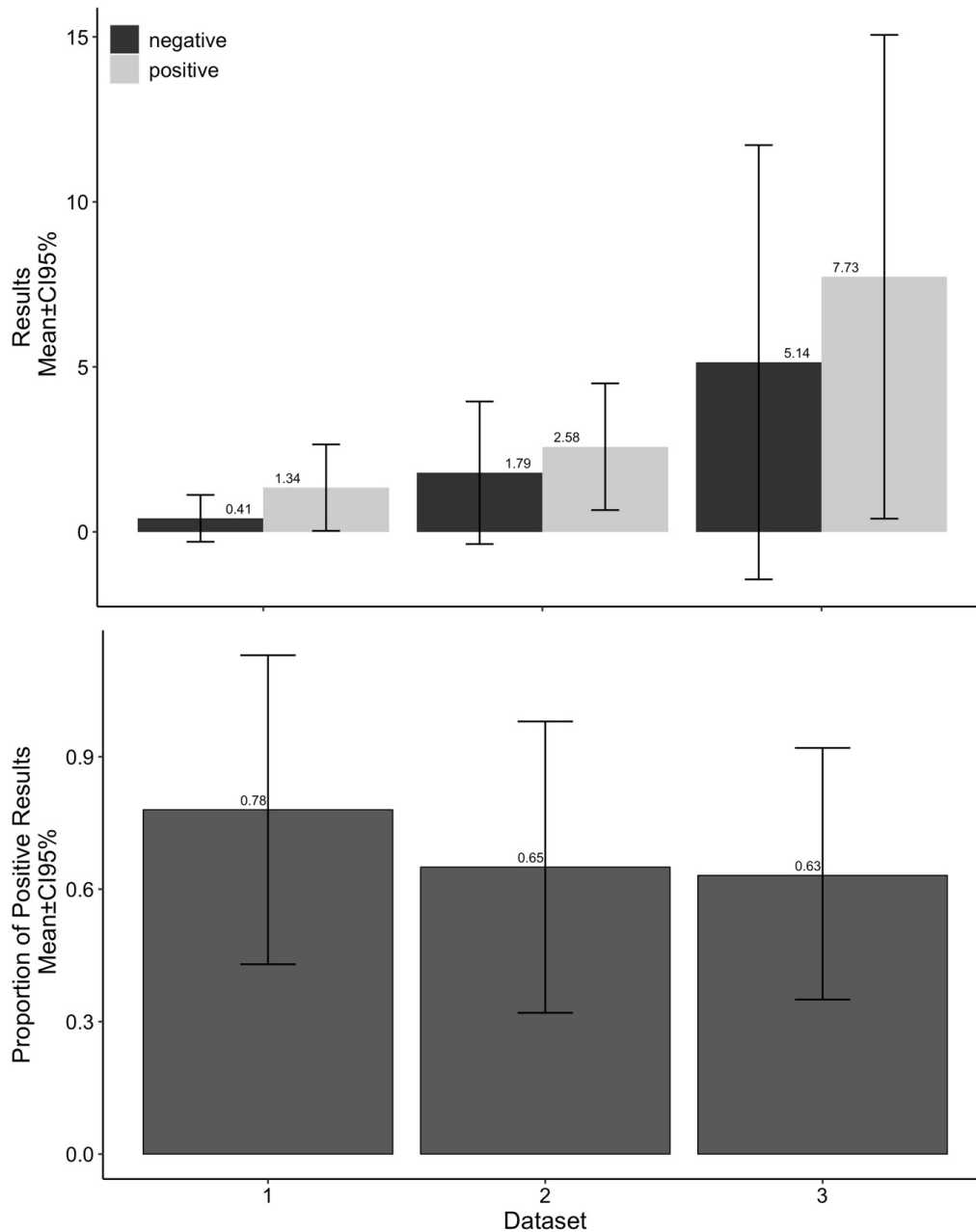


Figure 2.1(a) Mean positive and negative results obtained from the papers evaluated for each dataset and (b) ratio of positive to negative results, per dataset. The ratio varies from 0 to 1. Numbers shown above the bars are the mean PPR values and the error bars are 95% confidence intervals.

The incidence of positive results recorded for summary effects (ds1) was positively and non-linearly related to publication year (Table 2.2). Therefore, I fitted the linear model using the residues of the non-linear model as the response variable. The incidence of positive results varied among countries (Table 2.2), such that studies published in Europe had the highest mean positive results (as estimated in this study) and was followed by studies

developed in North America (Figure 2.2). However, North American studies exhibited a higher variation on the incidence of the positive results. The locations with the lowest incidence of positive results were New Zealand and South America.

Table 2.2. Proportion of positive results for summary effect sizes (ds1) modeled in response to publication year, location, and number of authors. A nonlinear regression (PPR ~ year) was first used due to the non-linear effect of the year (first part of the table) and the residuals were used as response variable in the linear model (second part of the table). The full model consisted of the residuals of the nonlinear model as the response variable, and country, number of authors involved in the publication and shares of doctorate degrees per country as the explanatory variables.

	Estimate	Std. Error	t-value	P	
Asymptote	0.83	0.06	13.76	>0.001	
Mean	2003	1.28	1562.40	>0.002	
Coefficient	0.63	1.45	0.44	0.67	
Residual standard error: 0.3247 on 29 degrees of freedom					
DS1	Number of iterations to convergence: 18				
	Achieved convergence tolerance: 9.962e-06				
	df	SQ	MSQ	F	P
Country	2	1.00	0.50	6.92	0.00
Number of authors	1	0.08	0.08	1.06	0.31
PhD graduates	1	0.04	0.04	0.50	0.49
Residuals	27	1.95	0.07		

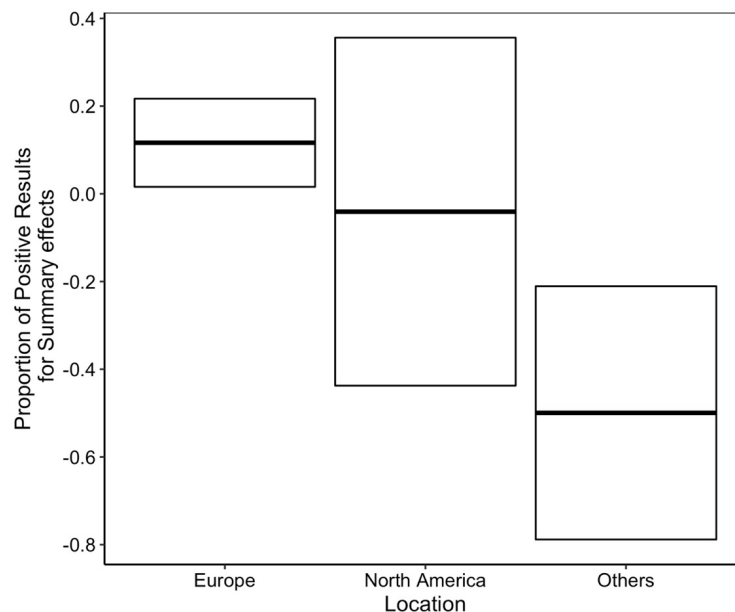


Figure 2.2. Proportion of positive results depending on the location where the article was published. Darker lines are the means and boxes represent the standard deviations. The PPR ds1 (proportion of positive results for summary effect size results) shown in the y axis are the residuals of the nonlinear least squares model.

The proportion of positive results recorded for the effects of moderators (ds2) was not associated with any of the response variables we proposed. The occurrence of positive results bias regarding individual effect size results (ds3), on the other hand, was predicted by the shares of doctorate degrees per country (Table 2. 3, Figure 2. 3). However, it explained only a small part of the variation.

Table 2. 3. The proportion of positive results of moderators (ds2) and of individual effect sizes (ds3) modelled separately in response to publication year, country, number of authors and shares of doctorate degrees per country as the explanatory variables.

		df	SQ	MSQ	F	p
DS2	Location	2	0.17	0.08	0.75	0.48
	Year	1	0.01	0.01	0.12	0.74
	Number of authors	1	0.02	0.01	0.13	0.72
	PhD graduates	1	0.15	0.15	1.37	0.25
	<i>Residuals</i>	42	4.67	0.11		
DS3	Location	3	0.41	0.14	1.89	0.14
	Year	1	0.00	0.00	0.05	0.82
	Number of authors	1	0.27	0.27	3.76	0.056
	PhD graduates	1	0.45	0.45	6.13	0.02
	<i>Residuals</i>	73	5.31	0.07		

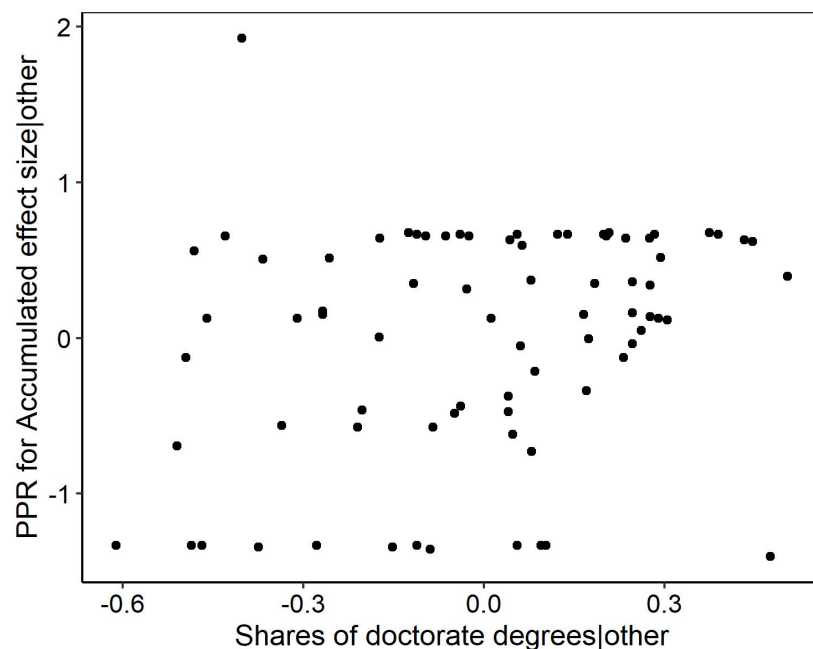


Figure 2. 3. Partial regression plot showing the influence of the shares of 25-64-year-olds with a doctorate degree per country on the proportion of positive results (PPR) for individual effect size results.

Discussion

Positive results were more frequent than negative results in the meta-analyses evaluated. In general, this result is in line with findings from studies focused on other disciplines (Csada et al., 1996; Jennions & Møller, 2002; Knight, 2003; Ioannidis, 2008; Fanelli, 2010b, 2012; Fanelli et al., 2017; Fraser et al., 2018) and may be a cause of concern (Ioannidis & Trikalinos, 2007a; Fanelli, 2012; Fanelli et al., 2017). A high incidence of positive results in meta-analyses illustrates how a high proportion of positive results in primary studies may be propagated to different research levels. Efforts are made in a meta-analysis to include data of different natures (e.g., grey literature) (Ioannidis, 2010). However, there is much room to increase the efforts to include grey literature data.

Excessive positive results in primary studies may indicate a difficulty in publishing negative results throughout the stages involved in publishing a paper (from the decision to submit to the acceptance for publication; Dickersin & Min, 1993; Ioannidis, 1998; Møller & Jennions, 2001; Knight, 2003), negatively affecting scientific progress (Alatalo et al., 1997; Browman, 1999; Fanelli, 2012). Concerns about the low occurrence of negative results have often been raised in the literature (Gould, 1993; Fanelli, 2010b, 2012; Graqvist, 2015). However, studies have indicated that negative results are in fact increasing (de Winter & Dodou, 2015) and only *seemingly* disappearing from papers (Fanelli, 2018). Negative results would be “increasingly embedded in longer publications that contain multiple results” and would “remain accessible to any researcher interested in finding them” (Fanelli, 2018). I included multiple results per study, in multiple levels, along with the negative results therein. Still, positive results prevailed in all three levels evaluated. In turn, the increased incidence of positive results may reflect more mature and well-developed theories (Fanelli, 2018).

A high prevalence of positive results in meta-analyses may reflect biases in two different levels, specifically, in primary studies and within the meta-analyses themselves. For

instance, the file drawer problem (Rosenthal, 1979), selective reporting, hypothesizing after the results are known (Fraser et al., 2018), P-hacking (e.g., by changing statistical methods and choosing how much data to include or remove based on significance; Head et al., 2015), and selective analysis (using analyses that provide positive results) are all biases related to ‘seeking positive results’ that can occur in primary studies. A file drawer problem may occur if a paper, in this case with negative results, is never submitted, or is submitted but rejected during the review process (Csada et al., 1996; Pautasso, 2010; Fraser et al., 2018). Prestigious journals tend to publish results that are significant (Csada et al., 1996; Fraser et al., 2018), with high effect sizes, and that support a new (usually counter-intuitive) hypothesis, based on a new theoretical framework. This tendency of prestigious journals may discourage authors from submitting their negative findings. However, the existence of journals or journal sections dedicated to publishing negative results shows that the scientific community is aware of the bias against negative results, and consequently may reduce the reluctance of authors to write up and/or submit a paper with negative findings (Graqvist, 2015; Ekmekci, 2017). Also, steps have been taken towards a more transparent (Parker et al., 2018) and less P-value oriented research (Nakagawa, 2004). Still, other issues associated with publishing negative findings, such as time and interest, go beyond the presence of journals dedicated to negative results (DeVito & Goldacre, 2019) and along with other factors (see Fraser et al., 2018) may still favor the file drawer problem.

Selective reporting was recorded by researchers in psychology who showed that 70% of the results in a study were not reported (Franco et al., 2016) based on statistical significance (Fraser et al., 2018; Van Aert et al., 2019). High rates (> 50%) of cherry picking and hypothesizing after the results have also been reported among ecologist and evolutionary biologists (Fraser et al., 2018). Some go as far as to suggest that conducting questionable research practices (QRP) is a norm among scientists (John et al., 2012).

Meta-analyses are also affected by QRP, however differently. Meta-analyses have been criticized based on the “garbage in garbage out” argument. The structure of meta-analyses itself calls for an objective methodological assessment that incorporates different results, regardless of the direction (Ioannidis & Lau, 1998). A prevailing incidence of positive results in meta-analyses of the same field, indicates an issue probably with biases in primary studies. For instance, no excess in positive findings is seen in a meta-analysis of individual-level data (prospective meta-analysis), where the study is designed in a more controlled manner (Ioannidis & Trikalinos, 2007a). Similarly, results of studies with an elevated contribution of non-published results and analyzed in homogenous subsets, yielded a low incidence of positive results (Van Aert et al., 2019).

Truly a meta-analysis composed of mostly published (and positive) results will probably yield a positive result itself, but other factors beyond primary studies also play a role in determining the results, for instance, P-hacking in more heterogeneous scenarios (van Aert et al., 2016). For instance, the frequent updating of a meta-analysis may “increase the chances of making a false positive conclusion” (AlBalawi et al., 2013). In addition, cherry picking and/or HARKing based on the analyses of moderator results can also increase the proportion of significant results. The prevalence of positive results in summary effects may also indicate that our theories are going in the right direction (Fanelli, 2018). On the other hand, excessive positive results in moderators may indicate cherry picking or a ‘fishing expedition’.

Meta-analyses do have several tools to manage publication bias (Ioannidis & Trikalinos, 2007a; Marks-Anglin & Chen, 2020). The meta-analytical method itself controls to some extent primary results derived from P-hacking (Head et al., 2015). However, we may be using such tools beyond their real capacity (Ioannidis & Trikalinos, 2005, 2007b; Lau et al., 2006; Ioannidis, 2008) because most publication bias tests are not intended to test

significance-chasing biases (Ioannidis, 2010), and may also mistake heterogeneity for bias when the adequate design is not used (Ioannidis & Trikalinos, 2007a). Still, conducting publication bias tests is important, and unfortunately often forgotten (Aytug et al., 2012; Koricheva & Gurevitch, 2014; Lodi et al., 2021). We previously reported that at least 50% of the meta-analysis did not conduct publication bias analysis (Lodi et al., 2021). A posterior exploratory analysis showed that those that did evaluate publication bias, conducted multiple tests, specifically funnel plot, followed by rank correlation and fail-safe numbers (Orwin's, Rosenthal's, Rosemberg's, and Egger's; see Figure S2.1), as recommended (Coburn & Vevea, 2015). The use of multiple publication bias evaluation methods is recommended because "some methods do not perform well in some conditions and none of the publication bias methods outperform all the other methods under each and every condition" (Van Aert et al., 2019).

Statistical methods have been continuously developed to address publication bias (Marks-Anglin & Chen, 2020). Thus, one would expect a reduction in the prevalence of positive results in time (de Winter & Dodou, 2015). However, only positive results of summary effect sizes increased time and non-linearly. I speculate that non-linearity may be a consequence the increasing number meta-analyses in freshwater ecology.

Researchers have reported a lack of cultural differences on the incidence of positive results along with a slow increase in negative results in primary studies (de Winter & Dodou, 2015). Similarly, my findings indicate that cultural differences (i.e., country) were irrelevant for the incidence of positive results in individual effect sizes and moderator results. Still, I have found a higher frequency of positive results (considering summary effect sizes) in Europe, followed by North America. Not only "location", but locations more prone to becoming involved in scientific endeavors, as represented by shares of doctorate degree holders, would have reduced incidence of positive results. However, I observed quite the

opposite for individual effect sizes with increased positive results in locations with higher shares of doctorate degree holders. However, the explanatory power was low, and these results should be observed with caution.

I had also expected that possible bias towards positive results would be reduced due to mutual control of team members (Fanelli et al., 2017), but the number of authors involved in the publication did not explain the proportion of positive results in any of the scales evaluated. The effect of collaboration in reducing bias has been reported as dependent on the geographical distance between collaborators such that the longer the distance the higher the probability of QRPs (Fanelli et al., 2017). However, we did not adjust for the distance factor. My results added to not having accounted for distance may have two implications. First, there is, in fact no relationship between the number of authors and bias towards positive results. Second, the effect of the distance between authors would buffer such relationship.

The ability to refute a very prestigious hypotheses may also be very attractive (for example, in molecular epidemiology high effects are published and quickly refuted; Ioannidis, 2010), and is known as the proteus phenomenon (Ioannidis & Trikalinos, 2005). A similar phenomenon has been reported for behavioral ecology (Chuard et al., 2019). However, this phenomenon is very discipline specific, and not likely to be occurring in freshwater ecology. Gould (1993) argues that the unwillingness of authors to publish negative findings is because positive results provide more interesting stories, while negative results “*often appear only in highly technical journals read by more restricted audiences and, as nonstories*”. Following his reasoning, a commitment to reporting negative findings under a more interesting narrative, as more of a “good story”, could correct publication bias to a large extent (Gould, 1993). Publication bias and the incidence of QRPs must continue to be studied empirically and using rigorous methods of investigation (Krosnick, 2019).

While reproducibility has been widely debated as a crisis (Baker & Penny, 2016) or a non-crisis (Fanelli, 2018; França & Monserrat, 2018), Fanelli (2018) argues that efforts to promote transparency and reproducibility would be better fostered under a narrative of transformation and empowerment. Following this reasoning, I add that more important than efforts to increase reproducibility would be to focus on encouraging researchers to turn their attention to writing up well-developed, well-founded manuscripts that answer *interesting/relevant* questions, regardless of the answer itself. Policies that encourage sharing data and data-analysis protocols are undoubtedly important for reasons that go beyond avoiding bias, for example, accelerating scientific advances by increasing exchange and efficiency (Woelfle et al., 2011; Fecher & Friesike, 2014). Still, I fail to see how science could truly advance without the continued increase in tolerance to unexpected/negative results. Especially as biological sciences are concerned, I believe ‘unexpected’ may be more common than not, and only by allowing the variability inherent to nature to surface, may ever more generalizable theories come forward.

Conclusion

Positive results were more frequent than negative results in the meta-analyses evaluated indicating that the high proportion of positive results in primary studies may be propagated to different research levels. I analyzed results in multiple levels per study; still, positive results prevailed in all levels evaluated. Such high prevalence of positive results in meta-analyses may reflect biases in primary studies as well as within the meta-analyses themselves. A meta-analysis composed of mostly published (and positive) results will probably yield a positive result, but other factors also play a role in determining the results. The prevalence of positive results in summary effects may also indicate that our theories are going in the right direction. Meta-analyses do have several tools to manage publication bias and the use of multiple publication bias evaluation methods is recommended. More important

than efforts to increase transparency and systematization would be to focus on encouraging researchers to return their attention to writing up manuscripts focused on interesting/relevant questions, and not on the answer itself.

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Supplementary Information

Table S2.1. Three datasets (ds1, ds2 and ds3) used in this paper. ds1 corresponds to the PPR of summary effect sizes, ds2 of effects of moderators, and ds3 of individual effect size results. The column PPR refers to ratio of positive to negative and non-significant results (positive/(negative + non-significant)). Neg = Negative; Pos = Positive, ns = Non-significant, Neg+ns = the total amount of negative results encountered. The column ‘Reference number’ indicates the reference evaluated, following (Lodi et al., 2021).

Reference Number	ds1					ds2					ds3				
	Neg	Pos	ns	Neg+ns	PPR	Neg	Pos	ns	Neg+ns	PPR	Neg	Pos	ns	Neg+ns	PPR
1	0	1	0	0	1	3	2	1	4	0.33	3	10	7	10	0.50
2	0	1	0	0	1	1	3	0	1	0.75	2	5	0	2	0.71
3	1	1	0	1	0.50	1	1	6	7	0.125	1	12	3	4	0.75
4	1	0	0	1	0	4	4	0	4	0.5	0	0	0	0	NA
5	0	1	0	0	1	0	1	0	0	1	0	7	0	0	1
6	1	1	0	1	0.50	0	0	0	0	NA	2	2	10	12	0.14
7	0	1	0	0	1	0	0	0	0	NA	0	0	0	0	NA
8	0	0	0	0	NA	0	3	0	0	1	1	5	1	2	0.71
9	0	1	2	2	0.33	0	0	0	0	NA	0	10	14	14	0.42
10	0	2	0	0	1	0	1	5	5	0.17	1	17	3	4	0.81
11	1	0	0	1	0	1	0	0	1	0	2	0	0	2	0
12	0	1	0	0	1	0	1	1	1	0.5	0	3	1	1	0.75
13	0	0	0	0	NA	0	1	1	1	0.5	0	3	5	5	0.38
14	0	0	0	0	NA	0	1	0	0	1	0	1	1	1	0.50
15	0	1	0	0	1	0	1	0	0	1	0	3	0	0	1
16	0	0	0	0	NA	2	4	0	2	0.67	1	7	10	11	0.39
17	0	0	0	0	NA	1	6	0	1	0.86	0	0	0	0	NA
18	0	0	0	0	NA	2	4	0	2	0.67	0	6	0	0	1
19	0	0	0	0	NA	0	1	0	0	1	0	5	0	0	1
20	0	1	0	0	1	1	3	0	1	0.75	5	11	1	6	0.65
21	0	3	0	0	1	0	0	0	0	NA	0	22	14	14	0.61
22	0	1	0	0	1	2	2	0	2	0.5	0	11	0	0	1
23	0	1	0	0	1	4	6	1	5	0.55	1	13	4	5	0.72
24	0	0	0	0	NA	0	8	1	1	0.89	0	22	0	0	1
25	0	0	0	0	NA	0	2	0	0	1	0	0	0	0	NA
26	0	0	0	0	NA	0	2	3	3	0.4	1	4	4	5	0.44
27	0	0	0	0	NA	0	0	0	0	NA	0	24	24	24	0.50
28	0	1	0	0	1	0	2	0	0	1	0	5	3	3	0.63
29	0	0	0	0	NA	0	3	2	2	0.6	0	11	4	4	0.73
30	0	0	2	2	0	4	1	0	4	0.2	4	6	2	6	0.5
31	0	0	0	0	NA	0	0	0	0	NA	0	6	0	0	1
32	0	0	0	0	NA	0	2	0	0	1	10	2	0	10	0.17
33	0	0	0	0	NA	0	0	0	0	NA	0	6	0	0	1
34	0	0	0	0	NA	0	0	0	0	NA	0	0	8	8	0
35	0	0	0	0	NA	0	4	1	1	0.8	3	18	1	4	0.82
36	0	0	0	0	NA	0	7	2	2	0.78	3	24	18	21	0.53
37	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA

38	0	0	0	0	NA	0	0	0	0	NA	0	2	1	1	0.67
39	0	0	0	0	NA	0	0	0	0	NA	0	2	3	3	0.40
40	0	0	0	0	NA	0	3	1	1	0.75	0	9	2	2	0.82
41	0	1	0	0	1	0	0	0	0	NA	0	0	0	0	NA
42	0	0	0	0	NA	0	0	0	0	NA	0	0	4	4	0
43	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
44	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
45	0	0	0	0	NA	0	0	0	0	NA	0	1	0	0	1
46	0	2	0	0	1	0	2	4	4	0.33	0	2	12	12	0.14
47	0	1	0	0	1	1	3	0	1	0.75	0	8	6	6	0.57
48	0	0	0	0	NA	0	0	0	0	NA	1	2	0	1	0.67
49	0	2	0	0	1	0	0	0	0	NA	0	0	0	0	NA
50	0	4	0	0	1	3	0	0	3	0	0	17	2	2	0.89
51	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
52	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
53	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
54	0	0	0	0	NA	0	0	0	0	NA	0	16	4	4	0.8
55	0	0	0	0	NA	0	7	1	1	0.88	0	0	0	0	NA
56	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
57	0	0	0	0	NA	0	0	0	0	NA	2	6	0	2	0.75
58	0	0	0	0	NA	1	2	0	1	0.67	0	0	0	0	NA
59	1	1	0	1	0.50	0	4	0	0	1	0	6	5	5	0.55
60	0	0	0	0	NA	0	0	0	0	NA	0	2	0	0	1
61	0	0	0	0	NA	0	3	11	11	0.21	0	1	5	5	0.17
62	0	0	0	0	NA	0	0	0	0	NA	0	1	1	1	0.50
63	0	0	0	0	NA	0	0	0	0	NA	1	1	2	3	0.25
64	0	0	0	0	NA	0	0	0	0	NA	1	2	0	1	0.67
65	0	0	0	0	NA	0	0	0	0	NA	1	4	7	8	0.33
66	0	0	0	0	NA	0	6	0	0	1	1	12	0	1	0.92
67	0	0	0	0	NA	0	0	0	0	NA	1	1	0	1	0.50
68	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
69	0	0	0	0	NA	1	1	0	1	0.5	9	37	29	38	0.49
70	0	0	0	0	NA	0	3	0	0	1	0	12	1	1	0.92
71	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
72	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
73	0	0	0	0	NA	0	0	0	0	NA	1	12	4	5	0.71
74	0	0	0	0	NA	0	0	0	0	NA	7	3	14	21	0.13
75	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
76	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
77	0	1	0	0	1	0	3	3	3	0.5	3	12	0	3	0.8
78	0	0	0	0	NA	0	0	0	0	NA	0	3	0	0	1
79	0	0	0	0	NA	0	0	0	0	NA	0	2	0	0	1
80	0	0	0	0	NA	1	1	3	4	0.2	0	23	14	14	0.62
81	0	0	0	0	NA	0	0	0	0	NA	0	9	13	13	0.41
82	0	0	0	0	NA	0	3	2	2	0.6	1	1	0	1	0.5
83	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
84	0	0	0	0	NA	0	0	0	0	NA	7	20	7	14	0.59
85	2	7	1	3	0.70	0	0	0	0	NA	0	0	0	0	NA

86	0	1	1	1	0.50	0	1	0	0	1	2	19	0	2	0.90
87	0	0	0	0	NA	0	0	0	0	NA	0	11	2	2	0.85
88	0	0	0	0	NA	0	1	3	3	0.25	0	12	2	2	0.86
89	0	0	0	0	NA	0	0	0	0	NA	0	3	0	0	1
90	0	0	0	0	NA	0	0	1	1	0	0	4	9	9	0.31
91	0	0	0	0	NA	0	0	0	0	NA	0	1	2	2	0.33
92	0	0	1	1	0	0	0	0	0	NA	0	3	8	8	0.27
93	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
94	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
95	0	0	0	0	NA	0	0	0	0	NA	1	3	2	3	0.5
96	0	0	0	0	NA	0	0	0	0	NA	0	1	0	0	1
97	0	0	0	0	NA	0	0	0	0	NA	0	2	1	1	0.67
98	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
99	0	0	0	0	NA	0	2	0	0	1	0	5	1	1	0.83
100	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
101	0	1	0	0	1	0	1	0	0	1	0	12	11	11	0.52
102	0	1	0	0	1	0	2	0	0	1	0	3	0	0	1
103	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
104	0	1	0	0	1	0	0	0	0	NA	0	3	0	0	1
105	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
106	0	1	0	0	1	0	0	0	0	NA	0	0	0	0	NA
107	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
108	0	0	0	0	NA	0	0	0	0	NA	1	14	5	6	0.7
109	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
110	0	2	1	1	0.67	0	0	0	0	NA	0	16	18	18	0.47
111	0	0	0	0	NA	0	0	0	0	NA	0	2	7	7	0.22
112	0	0	0	0	NA	0	0	0	0	NA	0	0	0	0	NA
113	0	0	0	0	NA	0	0	0	0	NA	0	4	3	3	0.57

Table S2.2. Ratios obtained considering the direction of the hypothesis partitioned among three explanatory variables, namely number of authors, year of publication and location. Effects of each category of location are shown.

Source of variation	Estimate	Std.Error	t	P
Intercept	-49.165	26.162	-1.879	0.066
Number of authors	-0.011	0.031	-0.355	0.724
Year	0.025	0.013	1.917	0.061
Location - CN	0.221	0.485	0.456	0.650
Location - EU-15	0.368	0.480	0.768	0.446
Location - NZ-AM	0.250	0.575	0.435	0.666
Location - US	0.436	0.481	0.906	0.369

Table S2.3. Ratios obtained when I considered significant values as positive results (inclusive ratio) partitioned among three explanatory variables, namely number of authors, year of publication and location. Effects of each category of location are shown

Source of variation	Estimate	Std.Error	t	P
Intercept	-52.551	19.004	-2.765	0.007
Number of authors	-0.017	0.013	-1.259	0.211
Year	0.027	0.009	2.816	0.006
Location - CN	0.235	0.267	0.882	0.380
Location - EU-15	0.276	0.256	1.079	0.284
Location - NZ-AM	0.025	0.327	0.075	0.940
Location - US	0.432	0.260	1.662	0.100

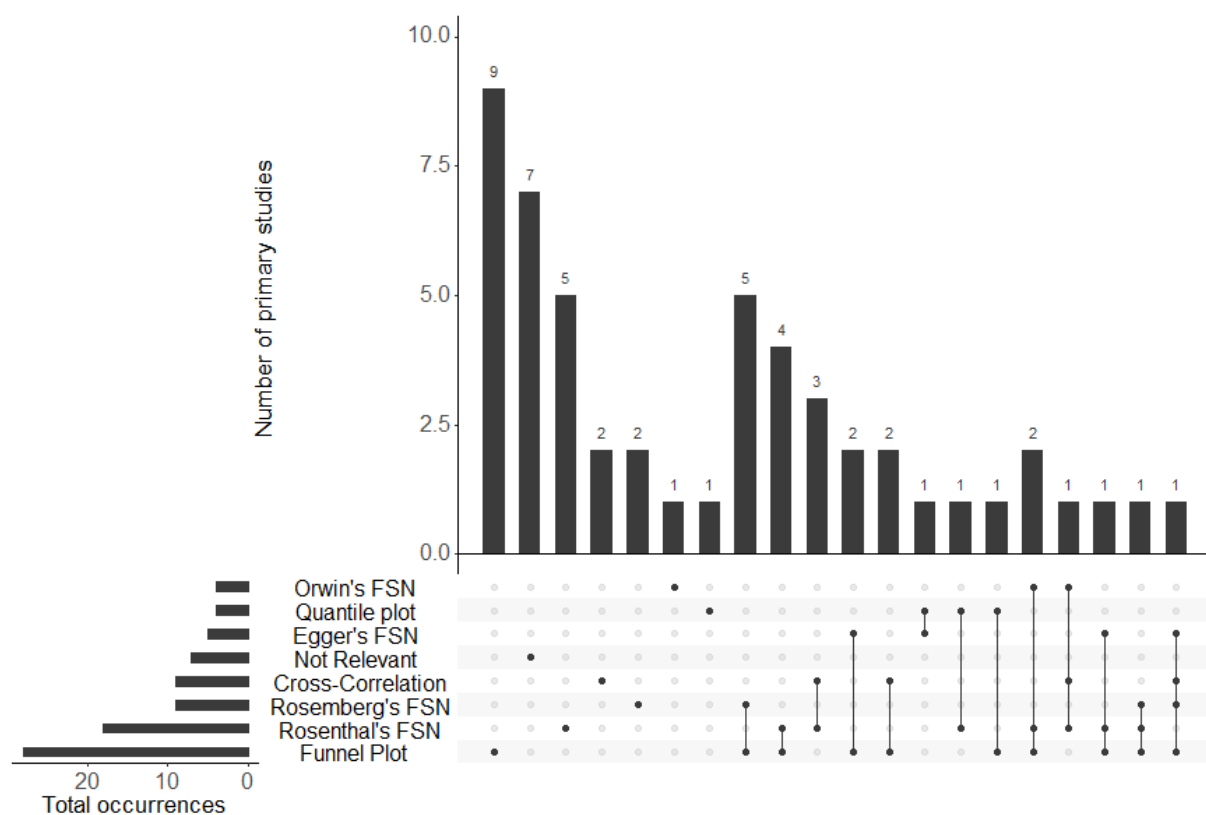


Figure S2.1 Strategies used to check for de occurrence of publication bias. The histogram with vertical bars shows the number of studies that used each combination of assessment strategy shown below the graph. The dots indicate the usage of a strategy, and connected dots indicate the multiple strategies used. The histogram with horizontal bars (to the left) shows the occurrence of each combination used in the studies evaluated. Note that 62 papers didn't use any strategy to check for publication bias, despite its relevance, and were therefore not included in this graph. FSN = Fail Safe Number

Chapter III

Relative importance of land use and local scale predictors of freshwater communities

Resumo

Ecólogos têm utilizado variáveis ambientais obtidas em diferentes escalas para tentar prever a estrutura das comunidades aquáticas. Para alcançar este objetivo, as variáveis de uso do solo podem complementar as variáveis obtidas em escala local. Neste estudo, utilizei resultados de análises canônicas para avaliar a importância relativa das variáveis locais e de uso do solo na estruturação de comunidades aquáticas, utilizando uma abordagem meta-analítica. Os resultados não apoiaram a noção de que as variáveis de uso do solo são melhores para explicar a estrutura das comunidades do que as variáveis locais. Verifiquei que os dois tipos de variáveis são importantes no estudo de correlatos da estrutura de comunidades aquáticas. Para um tipo de tamanho de efeito que analisei (correlação máxima com o primeiro eixo de ordenação), a importância relativa dos tipos de variáveis dependeu da diferença entre o número de variáveis representando cada grupo. Aproximadamente metade das variáveis incluídas nos estudos foram utilizadas apenas uma vez, indicando uma falta de consenso sobre variáveis devem ser utilizadas. Verifiquei a necessidade de melhorar a apresentação dos resultados em estudos que utilizam métodos multivariados para facilitar futuros estudos de meta-análise.

Abstract

Ecologists have become interested in integrating terrestrial and limnological knowledge to better understand the structure of aquatic communities. Land use variables may complement local scale variables in explaining the effects of the environment on aquatic communities. In this study, I used results from canonical analyses to evaluate the relative importance of local and land use variables in determining the composition of freshwater communities using a meta-analytical approach. The results did not support the notion that land use variables are better at explaining community structure than local variables. I found that both variable types are important when studying correlates of aquatic community structure. For one type of effect size (maximum correlation with the first ordination axis), the relative importance of the types of variables was dependent on the difference between the number of variables used to represent each group. Approximately half of the variables included in the studies were used only once, indicating a lack of consensus on which variables to use among studies. I found the need to improve the reporting of multivariate studies to facilitate future meta-analysis studies.

Introduction

Limnology has traditionally considered numerous environmental variables to describe aquatic ecosystems in different spatial and temporal scales (see Jenkins & Buikema, 1998; Beisner *et al.*, 2006). However, despite the large number of variables that are often used, multivariate models aiming to unveil the processes that shape the communities of both lotic and lentic environments have, in general, very low explanatory powers (e.g., Soininen, 2014; 2016). An explanation for this imbalance between describing and understanding community patterns may occur due to the historical assumption that aquatic environments are closed systems, with little influence from the surrounding environment (Forbes, 1925; Hansson *et al.*, 2013).

More recently ecologists became interested in integrating terrestrial and limnological knowledge to better understand the structure of aquatic communities (Wiley, Kohler & Seelbach, 1997; Allan & Castillo, 2007; Nessimian *et al.*, 2008) following the principle that aquatic and terrestrial ecosystems would be linked through the movement of animals, energy and matter (Soininen *et al.*, 2015). There would be four dimensions regulating stream dynamics: lateral (exchange between terrestrial and aquatic environments), longitudinal (movement of aquatic organisms and material downstream), vertical (movement within the water column and the hyporheic zone) and temporal dimensions (Ward, 1989; Mello *et al.*, 2020). Lake dynamics is also affected by anthropogenic and catchment scale factors (Liu *et al.*, 2012). Land use variables would add temporal information that the “in-water body” variables would disregard (Carneiro *et al.*, 2014). Local variables would be a snapshot representation of the environmental conditions whilst land use variables would show the events that the water body was, across time, subject to. Considering this framework, land use variables would complement (Mikulyuk *et al.*, 2011; Carneiro *et al.*, 2014; Soininen *et al.*, 2015; Mello *et al.*, 2020) or even be a better predictor of community structure than local

variables (Miserendino *et al.*, 2011). In addition to the theoretical considerations, variables obtained from the environment surrounding water bodies (e.g., land use), often obtained by geoprocessing, enable large-scale analyses (see Wiley *et al.*, 1997 for an example). Ecologists do agree that large-scale processes are important (Ricklefs, 1987; Levin, 1992; Root & Schneider, 1995; Wiley *et al.*, 1997). Effects of biotic interactions and more local factors driving species distributions are averaged out at large scales becoming Eltonian noise (Eltonian factors usually associated with interactions may not affect distributions at large extents and low resolutions; Soberon & Nakamura, 2009). In addition, at least one checklist with standardized variables associated with land-use is available and widely used (e.g., Nessimian *et al.*, 2008).

Despite many calls for the inclusion of land use variables as potential predictors of aquatic community structure, evidences show contrasting results on how these variables affect different biological groups (Vannote *et al.*, 1980; Allan, Erickson & Fay, 1997; Sponseller, Benfield & Valett, 2001; Hoffmann & Dodson, 2005; Carneiro *et al.*, 2014; Rocha *et al.*, 2020). For instance, land use has been shown to structure macroinvertebrate communities (Sponseller *et al.*, 2001; Fierro *et al.*, 2017). On the other hand, Omoto *et al.* (2000) are of the opinion that these communities are mainly structured by local factors. Zooplankton community has been reported to be more affected by lake development (indicating land-use intensity; Hoffmann & Dodson, 2005), by land-use (Sługocki *et al.*, 2018) and by spatially structured environmental factors (Rocha *et al.*, 2020). Studies report that the fish community diversity is affected by land use (Stanfield & Kilgour, 2013; Dala-Corte *et al.*, 2016) depending on the scale (Allan *et al.*, 1997). Also, the influence of land use may depend on the dispersal ability of the taxa being studied (Firmiano *et al.*, 2021).

There is also considerable redundancy in the variables usually considered, leaving room to improve parsimony in data gathering and, consequently, increase sample sizes with

the resources saved. The number/redundancy of the variables used to study a relationship between community structure and environmental factors directly relates to the reliability of the results obtained. When the number of explanatory variables increases, so does the odds of multicollinearity (especially if variables are describing similar/linked phenomena). Multicollinearity inflates the fit of models and, according to Graham (2003), “multicollinear explanatory variables are difficult to analyze because their effects on the response can be due to either true synergistic relationships among the variables or spurious correlations”, yielding possibly confusing results.

The degree of relationship between the environment and biological communities is typically examined using direct gradient analyses or canonical analyses (Canonical Correspondence Analysis or Redundancy Analysis) (Cottenie, 2005; Soininen, 2014, 2016). In this study I use results from canonical analyses to evaluate the relative importance of local and land use variables in determining the composition of plankton, macroinvertebrate, and fish communities using a meta-analytical approach. I aim to evaluate the hypotheses that land use variables will be important determinants of aquatic communities. Different components of the benthic macroinvertebrates and fish communities have been reported sensitive to land-use changes (see Harding *et al.*, 1998; Allen *et al.*, 1999; Cortes *et al.*, 2011; Cunha & Juen, 2017), while the effects of land use on planktonic communities tend to be smaller (Rocha *et al.*, 2020). The relative importance of land use variables will vary depending on the scale studied, reducing at a local scale, and increasing as we focus on larger scales.

Methods

Search strategy and criteria

I selected studies from the Web of Science and Scopus platforms. The terms “(assemblage* OR communit*) AND (fish* OR macroinvertebrate* OR *plankton) AND

("land us*" OR disturbance* OR "soil us*" OR "land cov*" OR regional)" (Moher *et al.*, 2009b, 2015) were searched in the title of the articles and filtered by Web of Science categories excluding irrelevant categories (e.g., psychology experimental, hematology, psychology applied, food science technology, immunology, or infectious diseases). I included articles conducted in freshwater environments, that studied the simultaneous effects of land-use and of local scale variables on aquatic community structure (specifically for zooplankton, phytoplankton, fish, and macroinvertebrate data; see results). Also, I only included studies that used canonical analyses and reported the correlation between the environmental variables (local or land use) and the ordination axes (following Siefert *et al.*, 2012). These results were retrieved from the ordination diagrams (see below). Studies that did not meet the inclusion criteria were excluded. Specifically, our exclusion criteria were not being i) ecology, ii) freshwater, iii) land use or vi) fish, zooplankton, phytoplankton, or macroinvertebrates, v) a primary study. I also excluded studies that vi) studied phytotelmata, vii) did not use canonical methods, viii) studied functional or beta diversity, ix) conducted a partial canonical analysis (because partial correlations depend also on correlations among predictor variables and not only on the relationship between the predictor and the response variable following Siefert *et al.*, 2012), x) used only land use variables, xi) was in Chinese or French, xii) was unavailable for download and/or not sent by authors, and that xiii) reported only local environmental variables. I collected and screened the articles following the PRISMA protocol. Study selection is summarized in the PRISMA flow diagram (Figure 3.1).

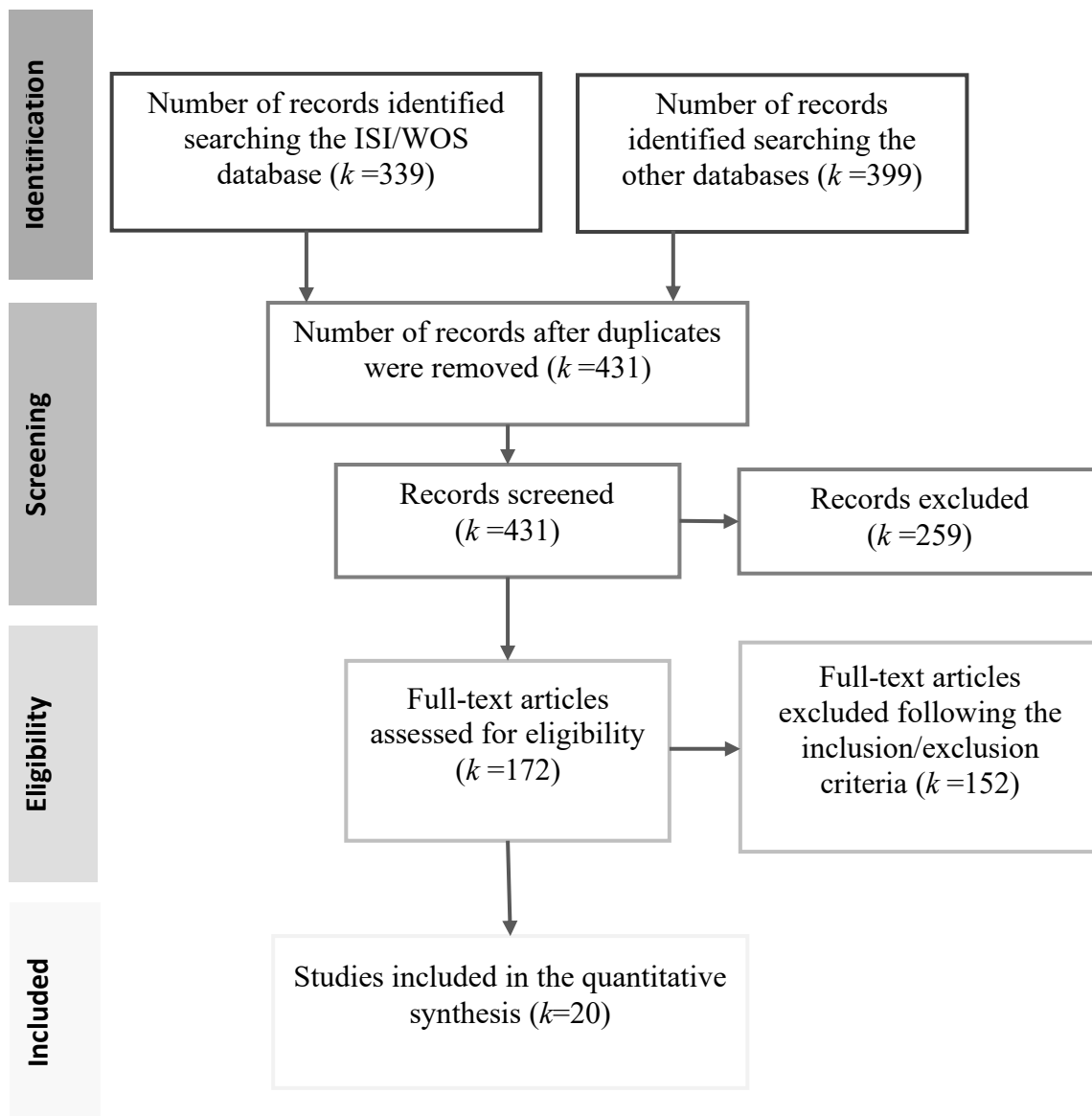


Figure 3.1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram summarizing study inclusion and exclusion phases (adapted from Moher *et al.*, 2009a)

Data and Statistical analysis

Considering an ordination plot from a study, we first retrieved the correlations (i.e., loadings) between the first axis and a given type of environmental variable (local and land use). Then, we averaged the absolute values of the correlations for each type of variable. After repeating this procedure with our set of 20 studies (totaling 24 ordination plots), we

created two vectors: one with the average correlations between the local variables and the first axis ($r_{Ax1,local}$) and other with the average correlations between the land use variables and the first axis ($r_{Ax1,land\ use}$). These procedures were repeated for the second axis ($r_{Ax2,local}$ and $r_{Ax2,land\ use}$) and for the maximum correlation values for each ordination axis (instead of averages; generating $r_{maxAx1,local}$, $r_{maxAx2,local}$, $r_{maxAx1,land\ use}$ and $r_{maxAx2,land\ use}$). Figure 3.2 summarizes these procedures.

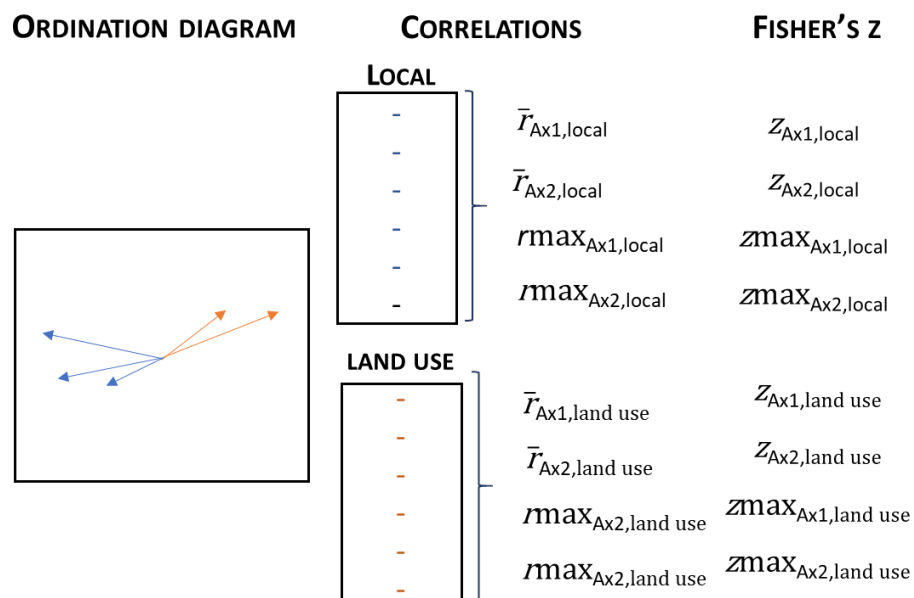


Figure 3.2. Schematic representation of the analyses conducted in this study. max=maximum value, Ax= Axis of an ordination diagram.

Each type of correlation (mean of the correlations between an axis and the local and land use variables and their respective maximum values) was transformed into Fisher's z (see equation 6.2 from Borenstein *et al.*, 2009). We then estimated a random-effects weighted effect size (following procedures described in Borenstein *et al.*, 2009) for each type of environmental variable (local and land use; $z_{-local++}$ and $z_{-land\ use++}$, respectively) and considering the different types of correlation. We used the overlap between the confidence intervals to ascertain whether the weighted effect sizes differed between local and land use

variables. All estimates were made using the metafor package (Viechtbauer, 2010) for R (R Development Core Team, 2020).

The comparison between the two correlation coefficients (e.g., between $r_{Ax1,local}$ and $r_{Ax1,land\ use}$), in our study, should consider that the correlations are overlapping. In other words, the two variables being compared (i.e., local and land use) have at least one variable in common, an aquatic community (Diedenhofen & Musch, 2015). Thus, we used the cocor package (Diedenhofen & Musch, 2015) to compare the strengths of the relationships between each type of environmental variable (local and land use) and an aquatic community for each study (for the different statistical tests available in this package, see Table 2 in Diedenhofen & Musch, 2015). We assumed that the correlation between local and land use variables was zero (a conservative criterion); however, other values (e.g., 0.25, 0.50 and 0.75) produced similar results (not shown).

Moderators

For each study, I also collected the following moderators (along with the effect sizes described above): biological group (zooplankton, phytoplankton, fish, or benthic macroinvertebrates), type of environment studied (lotic or lentic), number of variables included for each type of environmental variable (local and land use) and study spatial extent (defined in this study as the largest distance between two sites in a straight line). The effects of moderators were evaluated using the Z-tests.

Results

Most of the articles were excluded for not studying land use variables (125 out of 411 excluded), for not having analyzed the data using a canonical multivariate analysis (119 out of 411), and for not being conducted in freshwater ecosystems (116 out of 411; Figure 3.3). After applying these criteria, I included 24 analyses from 20 articles.

The number of local and of land use variables used in the canonical analyses varied greatly among studies (from 2 to 18 and from 1 to 14, respectively). However, the number of variables considered in the primary studies were larger than those used in the canonical analyses (from 6 to 33 and 1 to 20, respectively).

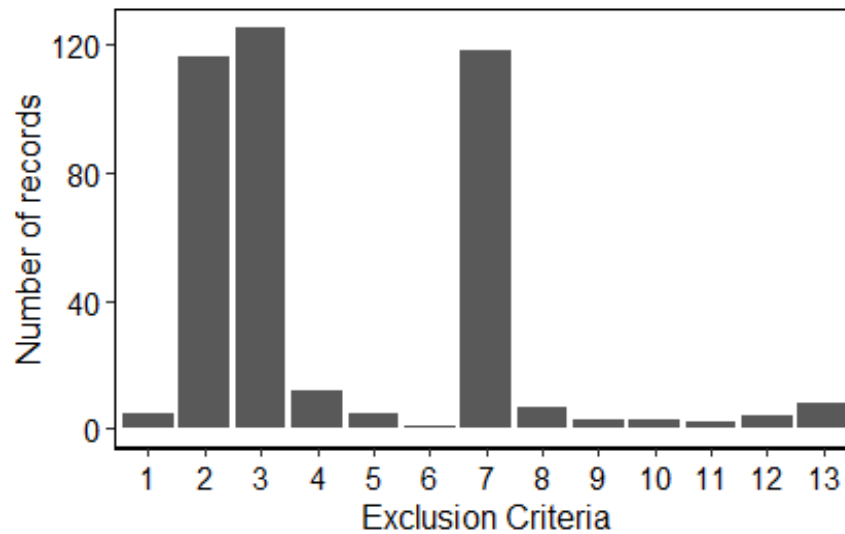


Figure 3.3. Number of records excluded per exclusion criterion. 1- Not ecology; 2- Not freshwater; 3- Not land use 4 Not fish, zooplankton, phytoplankton, or macroinvertebrates; 5- not a primary study; 6- Phytotelmas; 7- Did not use canonical methods; 8- used functional or beta diversity; 9- conducted a partial canonical analysis, 10- reported only land use variables; 11 – was in Chinese or French; 12 – data not available; 13 – reported only local environmental variables.

A total of 64 local scale variables and 65 land use variables were reported by the primary studies. Many of the variables were reported in only one or two studies, especially concerning land use variables (see Figure S3.1). The most used local-scale variables were dissolved oxygen (9 studies), alkalinity (8 studies), width of the water body (7 studies), water flow (7 studies), depth (6 studies), transparency (5 studies), salinity (4 studies), total suspended solids (4 studies), total dissolved solids (4 studies), Nitrogen/Phosphorous ratio (4 studies), and total organic carbon (4 studies). The variables permanganate index, total

phosphorous, conductivity, total nitrogen, NO₃, NH₃, NH₄, and pH were used by 3 studies. The most used land use-scale variables were forest (6 studies), crop (4 studies), grass (4 studies), urban (4 studies), grazing (3 studies), tree removal (3 studies) and catchment area (3 studies).

For the first ordination axis, the weighted effect size of the relationship between the local environmental variables and the aquatic communities ($z\text{-local}_{++} = 0.316$; 95% CI = 0.304-0.418) did not differ substantially from that estimated for the land use environmental variables ($z\text{-land use}_{++} = 0.445$; 95% CI = 0.302-0.587). However, the heterogeneity among the effect sizes estimated with the local environmental variables ($Q = 20.74$, $k = 23$, $P = 0.5971$) was clearly lower than the heterogeneity among the effect sizes estimated with the land use variables ($Q = 121.57$, $k = 23$, $P < 0.0001$). In general, the results of the comparisons between the local and land use variables, using the tests summarized by Diedenhofen & Musch (2015), also indicated that these groups of variables were similarly correlated with the aquatic communities. Indeed, we found that in only three studies the land use variables were more strongly correlated with the aquatic communities than the local variables (Figure 3.4a). We also did not find evidence that the differences between the types of variables (local and land use) were dependent on the group of organisms under study (Figure S3.2a), type of environment (lotic x lotic, not shown), on the difference in the number of variables of each type ($r = 0.177$; $P = 0.4083$) and on the spatial extent of the study ($r = -0.168$; $P = 0.468$).

The results for the second axis also indicated that the weighted effect sizes of the relationships between the environmental variables and the aquatic communities were similar ($z\text{-local}_{++} = 0.305$; 95% CI = 0.237-0.372; $z\text{-land use}_{++} = 0.241$; 95% CI = 0.177-0.587). However, the heterogeneity among the effect sizes was much lower ($Q = 21.20$, $P = 0.3852$ and $Q = 11.32$, $P = 0.9375$, for local and land use variables, respectively). Similar to the first axis, the differences between local and land use variables, in terms of their effect sizes on

aquatic communities (Figure 3.4b), did not vary consistently with the group of organisms (Figure S3.2b), type of environment (not shown), the difference in the number of variables in each type ($r = -0.086$; $P = 0.712$) and the spatial extent of the studies ($r = 0.043$; $P = 0.854$).

The maximum correlations between the two types of environmental variables (local and land use) and the aquatic communities (along the first ordination axis) were also similar ($z\text{-local}_{++} = 0.562$; 95% CI = 0.408-0.715; $z\text{-land use}_{++} = 0.584$; 95% CI = 0.431-0.737). The amount of heterogeneity was high and similar for the two types of environmental variables ($Q = 142.52$, $P < 0.0001$ and $Q = 141.46$, $P < 0.0001$, for local and land use variables, respectively). The number of significant differences between the maximum correlations was more frequent as compared to mean correlations (Figure 3.4c). Specifically, the correlations between local environmental variables and aquatic communities were stronger than the correlations between land use environmental variables and aquatic communities in four datasets. In other four datasets, we found the opposite ($r_{max_{Ax1,local}} < r_{max_{Ax1,land\ use}}$). The effects of types of environments (not shown), spatial extent ($r = -0.287$, $P = 0.207$), or biological groups (Figure S3.2c) were also negligible; however, the difference in the number of variables used in the primary studies to represent local and land use variables was positively and significantly correlated with the difference in effect sizes ($r = 0.483$, $P = 0.0168$). Thus, local variables tended to have a variable with higher maximum correlation, as compared to land use variables, when the former type contained a larger number of variables, and vice-versa.

Considering the maximum correlations with the second axis, the difference between the weighted effect sizes of local and land use variables was the highest among the metrics we used ($z\text{-local}_{++} = 0.632$; 95% CI = 0.504-0.761; $z\text{-land use}_{++} = 0.422$; 95% CI = 0.310-0.534). Heterogeneity values among the effect sizes were significant for both types of environmental variables ($Q = 65.05$, $P < 0.0001$; $Q = 48.53$, $P = 0.0004$; for local and land

use variables, respectively). All five datasets with significant differences between the correlation coefficients indicated that local variables were more strongly correlated with aquatic communities than land use variables (Figure 3.4d). None of the moderators [group of organisms (see Figure S3.2d), types of ecosystems, differences between the number of variables ($r = 0.108$, $P = 0.640$) and spatial extent ($r = -0.053$, $P = 0.821$)] were associated with the difference between $r_{max_{Ax2,local}}$ and $r_{max_{Ax2,land\ use}}$.

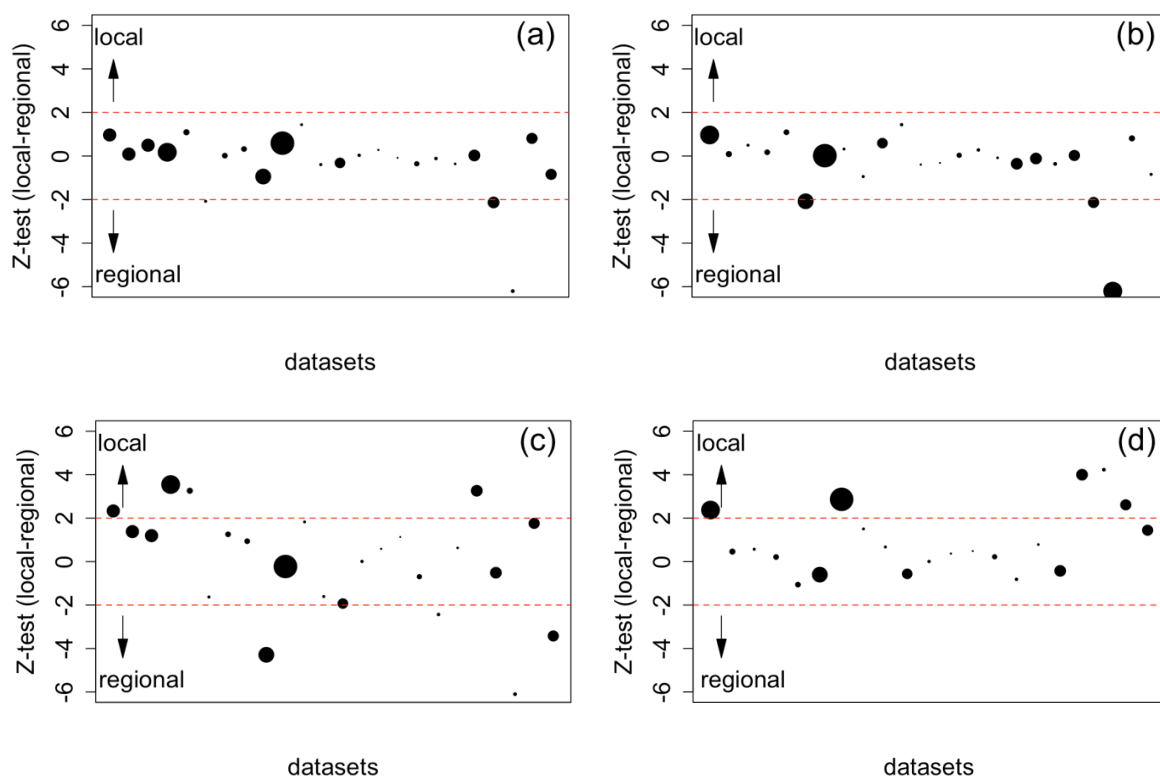


Figure 3.4. Z-tests evaluating the difference in the strengths of the correlations between environmental variables and (local - land use variables) and aquatic communities. Positive values indicate that the relationships between local variables and aquatic communities were stronger than the relationships between land use variables and aquatic communities. Negative values indicate the opposite. Z-values outside the dashed lines are statistically significant. Sizes of the circles are proportional to sample sizes. The analyses were based on the mean correlation for axis 1 (a), mean correlation for axis 2 (b), maximum correlation value for axis 1 (c), and maximum correlation value for axis 2 (d). The results were similar for the different tests discussed by Diefendorf & Musch (2015).

Discussion

Several studies have emphasized the need to consider variables measured in different scales to better understand the factors underlying community structure (Ward, 1989; Allan & Castillo, 2007; Nessimian *et al.*, 2008; Chessman & Townsend, 2010; Soininen *et al.*, 2011, 2015; Mello *et al.*, 2020). Land use and other regional scale variables have been used to describe processes that structure aquatic communities. This idea relies on the assumption that land use variables can be a better proxy of, for example, nutrient loading (Allan *et al.*, 1997) than local scale variables. Also, land use variables may better indicate the long-term impacts to which a water body is subjected (e.g., Miserendino *et al.*, 2011; Soininen *et al.*, 2015). If so, one would expect that land use variables would be more important predictors of aquatic community structure than local environmental variables (e.g., pH, conductivity, dissolved oxygen, etc.). However, our results did not support this view.

The maximum correlation values along the second axis suggested a greater importance of local rather than land use environmental variables. These results seem to be consistent for different biological groups and ecosystem types. The heterogeneity among the effect sizes estimated with the local environmental variables was lower than the heterogeneity among the effect sizes estimated with the land use variables. Therefore, local scale variables seem to be less variable in predicting the structure of biological communities. On the other hand, the small difference between local and land-use correlations does not allow us to argue that only local variables should be used. Therefore, we support the idea that using both variable types is important (Root & Schneider, 1995) when studying putative correlates of aquatic community structure (e.g., Lenat & Crawford, 1994; Brown & Swan, 2010; Cañedo-Argüelles *et al.*, 2015; Firmiano *et al.*, 2021). For example, the explanatory power of the analyses (e.g., RDA) would be reduced upon the use of only one group of variables.

The relative importance of different environmental factors may be dependent on the spatial scale (Sponseller *et al.*, 2001; Soininen, 2007; Siefert *et al.*, 2012; Zhang *et al.*, 2019). Spatial dynamics can affect the identification of important relationships since environment-community relationships have a strong spatial component (mainly due to spatial autocorrelation). A group of variables structured in space, for example, could emerge as more important (Rocha *et al.*, 2020), resulting in biased results if we seek to disentangle local and land use effects disregarding spatial structure. Spatially structured environmental data have been reported to have higher effect on diversity than pure environmental effects (Soininen, 2007; Zhang *et al.*, 2019), which shows the importance of also considering the spatial structure. Land use variables are to some extent spatially structured. As scale increases the importance of larger scaled variables should increase as well (Soberon & Nakamura, 2009), and the opposite is also expected. Still, we did not find a relationship between the difference between local and land use variables, in terms of their effect sizes on aquatic communities, and spatial extent.

Space must be regarded in variance partitioning to avoid that either the effects of local or regional environmental variables be overestimated due to autocorrelation (Lennon, 2000). If local variables are autocorrelated, then the difference in the correlation that I estimated would be underestimated (i.e., regional effects could be more important after spatial control, implying in more negative Z values in Figure 3.4). On the other hand, if regional variables included were more autocorrelated, then I would be overestimating the difference in correlations (i.e., local effects could be more important after spatial control, implying in more positive Z values in Figure 3.4). However, we were not able to assess the effect of taking the spatial component into account because of our exclusion criterion and also because few studies conducted variation partitioning analyses. Despite the problems of using the results of

variation partitioning analyses (as discussed by Siefert *et al.*, 2012), I believe that analyzing this effect, within the context of this work, would be an interesting avenue for future studies.

Our results based on average correlations did not evidence that the strength of the association between environmental variables and aquatic communities are dependent on the type of variable (whether local or land use). The relative importance of the variables was dependent on the difference between the number of variables used to represent each group when the maximum correlations values along the first axis were considered. The higher the number of explanatory variables included (local or land-use) the higher the probability of a variable with larger maximum correlation being encountered. This is a double-edged sword once limnologists could firstly be inclined to increase (even more) the number of variables included in a study. However, as the number of explanatory variables increases, so does the incidence of spurious correlations (Garsd, 1984) and of possible issues with multicollinearity. In addition, the explanatory power increases while our ability to explain our findings is greatly reduced with increasing number of explanatory variables (Weiner, 1995). It has been shown that correlated variables tend to be artificially more important in a regression (Lennon, 2000). Therefore, we reinforce recommendations that we must be cautious when choosing which variables to include in a study.

There seems to be a context dependency concerning the association between communities and the environment (Soininen, 2014). This dependency would be due to differences in environmental variation among regions or differences in the variables chosen as predictors (number and/or specific variables). Notably, over half of the variables included in the studies were used only once (see Figure S3.1). Few land use variables were used frequently, indicating a lack of consensus on which variables to use among studies. Moving our focus to a set of relevant environmental variables is paramount if we want to advance our knowledge on how communities are affected by land use. I observed in this study that

dissolved oxygen, alkalinity, width of the water body, flow of the water body, depth and transparency were the most used local variables, and forest, crop, grass, urbanization, grazing, tree removal and catchment were the most used land use/large scale variables. Note that these were the most used variables in the ordination plots. This implies that they were more selected by significance and other procedures, for example, the forward selection was used to select variables in some of the studies.

A gradient analysis (canonical correspondence analysis, CCA; or redundancy analysis, RDA) is expected in studies of the effects of environmental variables on communities (Soininen, 2014, 2016). A good reporting of the results is necessary for a thorough understanding of the study. Regardless of the method used, reporting a single effect size statistics for each response variable, depicting the amount of variation explained by it, is essential in future studies (Siefert *et al.*, 2012). Reporting the loadings of each environmental variable included and the biplot, in addition to (and not only) the adjusted R^2 of the model is crucial. Knowing how much of the variance (adjusted R^2) was explained shows the strength of the model, while the relationship between predictor variables and response variables (loadings) shows how the variance was partitioned (Legendre & Legendre, 2012). However, methods used varied greatly among studies sampled (114 studies excluded for not conducting or accurately reporting a canonical analysis); this variation was the biggest challenge in this study.

When designing a meta-analysis, one must determine which effect sizes will be used. A highly heterogenous array of methods (as I encountered) hinders the inclusion of many papers. This heterogeneity in the methodological approach in studies that evaluate the effects of variables of different natures/scales on biological communities is not a trivial matter, and has hindered data obtention in this and other meta-analyses (Siefert *et al.*, 2012). We highlight the need to standardize basic reporting items of studies aiming to evaluate the

effects of local scale and/or land use variables on biological communities. As such, I recommend that well-reported gradient analyses be called for in papers studying local/land use effects. Specifically, I recommend that studies clearly include (i) a table with the correlations between the environmental variables and sampling scores derived from the biological data (and not only the biplot), (ii) sample size, (iii) spatial extent and (iv) eigenvalues of the axes included. I believe more specific hypotheses and increased parsimony (using relevant variables) in choosing the explanatory variables will produce more reliable results. In addition, I recommend that future studies also partition variance into environmental and spatial components.

Conclusion

In general, the strengths of association between environmental variables and aquatic communities do not depend on the type of variable (local or land use). Thus, using both types of variables is important when studying aquatic communities to increase the explanatory power of the analyses. However, the relative importance of the variables depended on the number of variables used to represent each group for maximum correlation values. We recommend caution and parsimony when choosing to include many variables in a study, in addition to choosing variables that have been reported as relevant for biological communities. A more detailed reporting of multivariate results is also recommended.

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Supplementary information

association between communities and the environment

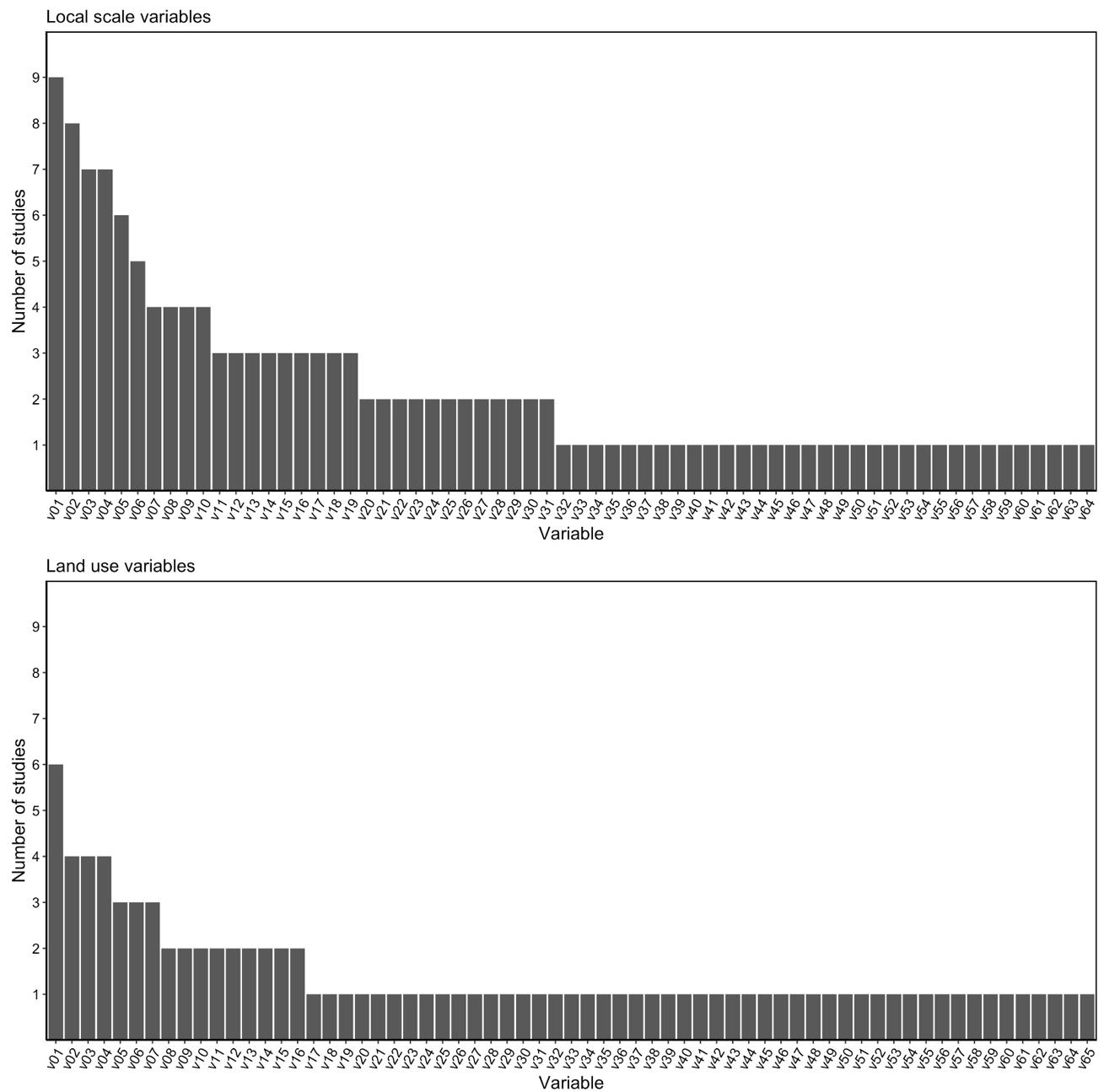


Figure S3.1. Number of local scale and land use variables included per study. Name of the variables are listed in Table S3.1 following the respective numbers.

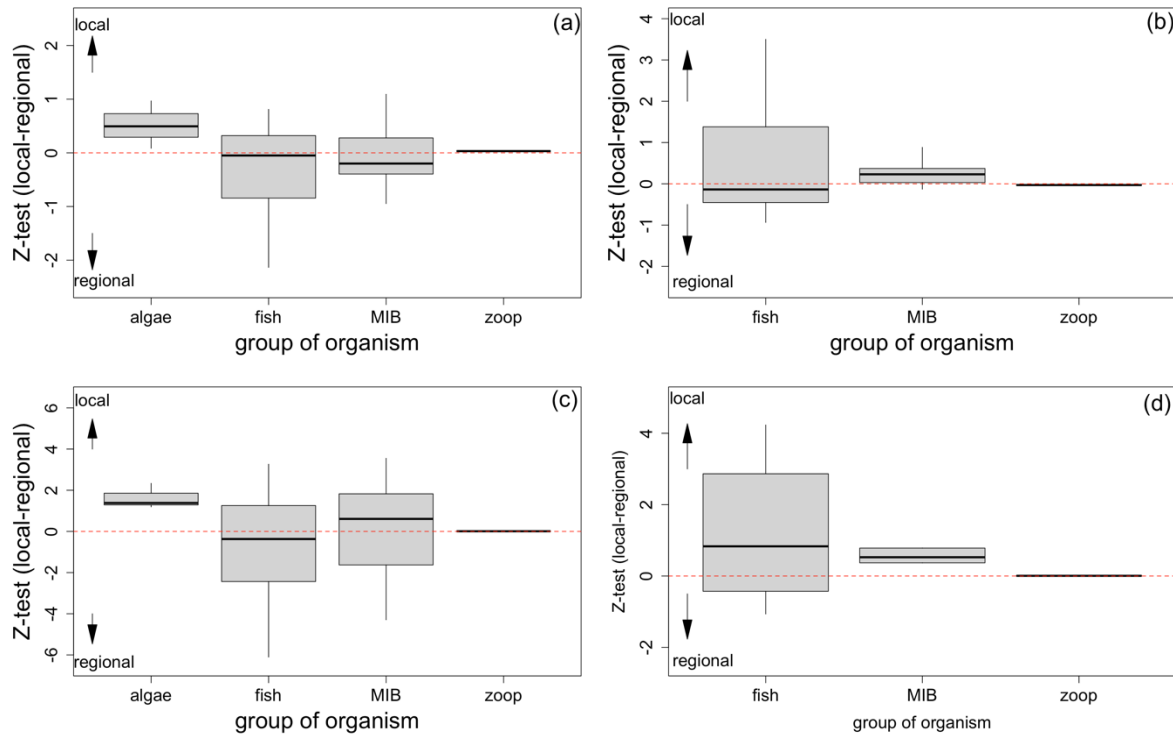


Figure S3.2. Z-tests evaluating the difference in the strengths of the correlations between environmental variables and (local - land use variables) and aquatic communities. Results were aggregated by type of organisms (individual results are shown in Figure 3.4). The analyses were based on the (a) mean correlation for axis 1 (a), mean correlation for axis 2 (b), maximum correlation value for axis 1 (c), and maximum correlation value for axis 2 (d)

Table S3.1. Local and land use scale variables included per study and the number of studies that used the respective variables

	Land use		Local	
v1	Forest	6	Dissolved oxygen	9
v2	Crop	4	Alkalinity	8
v3	Grass	4	Width	7
v4	Urban	4	Flow	7
v5	Grazing	3	Depth	6
v6	Tree removal	3	Transparency	5
v7	Catchment	3	Salinity	4
v8	Wetland	2	Total suspended solids	4
v9	Direct human activity	2	Total dissolved solids	4
v10	Preserved riparian	2	N/p	4
v11	Pca1	2	Total organic carbon	3

v12	Pca2	2	Permanganate index	3
v13	Agriculture	2	Total phosphorus	3
v14	Woody debris	2	Conductivity	3
v15	Impervious surface	2	Total nitrogen	3
v16	Altitude	2	Nitrate	3
v17	P fertilization	1	Ammonia	3
v18	K fertilization	1	Ammonium	3
v19	N fertilization	1	Ph	3
v20	C fertilization	1	Biological oxygen demand	2
v21	Pesticides	1	Temperature	2
v22	Total fertilizer	1	Chlorophyll	2
v23	Gross product	1	Phosphate	2
v24	Gross product agriculture	1	Discharge	2
v25	Gross product industry	1	Turbidity	2
v26	Gross other industry	1	Velocity	2
v27	Landfill	1	Altitude	2
v28	Irrigation	1	Wood in stream	2
v29	Tillage	1	Canopy	2
v30	Land slide	1	Coarse substrate	2
v31	Siltation	1	Boulder	2
v32	Area frag	1	Drainage to lake area	1
v33	Northing	1	Rifles	1
v34	Distance from source	1	Sand	1
v35	Regional anthropization index	1	Mud	1
v36	Order	1	Clay	1
v37	Shrub	1	Pca depth	1
v38	3rd order	1	Pca morphometry	1
v39	4th order	1	Pca chemical	1
v40	January temperature	1	Submerged vegetation	1
v41	July temperature	1	Macrophytes	1
v42	Precipitation	1	Litter	1
v43	Turf	1	Shrub	1
v44	Field	1	Debris	1
v45	Debris	1	Tree in stream	1
v46	Litter	1	Manganese	1
v47	Ph	1	Dissolved solids	1
v48	Soil OM	1	Sas5	1

v49	Open water	1	Aspt	1
v50	Other	1	Artificial substrate	1
v51	Sem	1	Hanging vegetation	1
v52	Nd2k (local index)	1	Hag	1
v53	Nd (local index)	1	Mmd	1
v54	Thalweg	1	Silt	1
v55	Riparian width	1	Coarse particulate organic material	1
v56	Bank stability	1	Wet channel width	1
v57	IHAS (local index)	1	Concrete	1
v58	Rain 15 days	1	No plant	1
v59	Rain 30 days	1	PC	1
v60	Rain 7 days	1	Width rapids	1
v61	Latitude	1	Under bank	1
v62	Longitude	1	Sd depth	1
v63	Exotic forest	1	Sd inc	1
v64	Qbr (local index)	1	Sd bank	1
v65	Habitat	1		-

Conclusion

Meta-analysis is a method increasingly used in freshwater ecology. There are issues that need to be addressed as quality is regarded. My primary concern is the use of the term 'meta-analysis'. Authors should be more careful when labeling their study a meta-analysis when, in fact, different methods were used. I find that compliance with checklists should be widely fostered especially due to the implications of meta-analysis results as a robust summary evidence. I reiterate that authors, reviewers and editors should comprehensively use checklists to improve the quality of meta-analyses in freshwater ecology. The checklists and guidelines available provide the means for a good quality meta-analysis to be conducted.

Bias towards positive results is still a matter for concern, despite being historically discussed. I found a prevalence of positive results in freshwater ecology meta-analyses. This prevalence indicates that the high proportion of positive results in primary studies may be propagated to different research levels once a meta-analysis composed of mostly published positive results will probably yield a positive result as well. Meta-analyses have several tools to manage publication bias and using multiple publication bias evaluation methods is recommended. However, a thorough search of the grey literature also helps the inclusion of negative results, reducing possible biases towards positive results. A long-term approach is also recommended. It's important that we, as researchers, encourage colleagues and students to focus always more on the questions and proper development of a paper than on the results obtained.

Although some of the items of the quality checklist were not met, interesting results were obtained in our meta-analysis. I found no evidence of differences between land use and local scale variables, although local scale variables seemed to be more precise. In addition, I

detected a need to narrow down which variables are included in land use studies as a step towards generalization and stronger analyses. I also recommended more detailed reporting of multivariate results to facilitate future meta-analyses.