Simple study designs in ecology produce inaccurate estimates of biodiversity responses

Alec P. Christie¹, Tatsuya Amano¹,²,³, Philip A. Martin¹,⁴, Gorm E. Shackelford¹,⁴, Benno I. Simmons¹,⁵, William J. Sutherland¹,⁴

¹Conservation Science Group, Department of Zoology, University of Cambridge, The David Attenborough Building, Downing Street, Cambridge CB3 3QZ, UK.
²Centre for the Study of Existential Risk, University of Cambridge, 16 Mill Lane, Cambridge, CB2 1SB, UK.
³School of Biological Sciences, University of Queensland, Brisbane, 4072 Queensland, Australia
⁴BioRISC, St Catharine’s College, Cambridge CB2 1RL, UK.
⁵Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN UK.

*Corresponding author, apc58@cam.ac.uk

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2664.13499
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Abstract

1. Monitoring the impacts of anthropogenic threats and interventions to mitigate these threats is key to understanding how to best conserve biodiversity. Ecologists use many different study designs to monitor such impacts. Simpler designs lacking controls (e.g. Before-After (BA) and After) or pre-impact data (e.g. Control-Impact (CI)) are considered to be less robust than more complex designs (e.g. Before-After Control-Impact (BACI) or Randomised Controlled Trials (RCTs)). However, we lack quantitative estimates of how much less accurate simpler study designs are in ecology. Understanding this could help prioritise research and weight studies by their design's accuracy in meta-analysis and evidence assessment.

2. We compared how accurately five study designs estimated the true effect of a simulated environmental impact that caused a step-change response in a population's density. We derived empirical estimates of several simulation parameters from 47 ecological datasets to ensure our simulations were realistic. We measured design performance by determining the percentage of simulations where: (i) the true effect fell within the 95% Confidence Intervals of effect size estimates, and (ii) each design correctly estimated the true effect's direction and magnitude. We also considered how sample size affected their performance.

3. We demonstrated that BACI designs performed: 1.3-1.8 times better than RCTs; 2.9-4.2 times vs BA; 3.2-4.6 times vs CI; and 7.1-10.1 times vs After designs (depending on sample size), when correctly estimating true effect’s direction and magnitude to within ±30%. Although BACI designs suffered from low power at small sample sizes, they outperformed other designs for almost all performance measures. Increasing sample size improved BACI design accuracy but only increased the precision of simpler designs around biased estimates.

4. Synthesis and applications. We suggest that more investment in more robust designs is needed in ecology since inferences from simpler designs, even with large sample sizes
may be misleading. Facilitating this requires longer-term funding and stronger research-practice partnerships. We also propose ‘accuracy weights’ and demonstrate how they can weight studies in three recent meta-analyses by accounting for study design and sample size. We hope these help decision-makers and meta-analysts better account for study design when assessing evidence.

Foreign language abstract

1. 生物多様性の保全を効果的に行うためには、人為的脅威の影響や保全対策の効果を適切に評価することが重要となる。生態学ではこのような評価を行うために、様々な研究デザインが用いられている。対照区が存在しないBefore-After（BA）デザインやAfterデザイン、また処理以前のデータが存在しないControl-Impact（CI）デザインなど簡素な研究デザインは、Before-AfterControl-Impact（BACI）デザインやランダム化比較試験（RCTs: Randomised Controlled Trials）などの複雑なデザインよりも頑健さに劣ると考えられている。しかしながら、生態学においてこれら簡素な研究デザインがどれだけ正確度に劣るのか、定量的な評価はこれまで行われていない。研究デザインの正確度を定量的に評価することで、メタ解析やエビデンスの評価を行う際に、用いられた研究デザインの正確度に基づいて各研究の優先順位付けや重み付けを行うことが可能になるだろう。

2. 本研究では、環境変化が個体群密度に及ぼす影響を、5種類の研究デザインがどれだけ正確に推定することができるのか、シミュレーションを用いて検討した。より現実に即した状況を再現するため、シミュレーションで用いたパラメータは、47の生態學的データから抽出した。各研究デザインの正確度は、シミュレーションにおいて、（1）推定された効果サイズの95%信頼区間に真の効果が含まれる割合、（2）推定された効果が真の効果の方向・程度と一致した割合を算出することによって評価した。またサンプルサイズの違いが各研究デザインの正確度に及ぼす影響も検討した。

3. シミュレーションの結果、BACIデザインはランダム化比較試験に対して1.3-1.8倍、BAデザインに対して2.9-4.2倍、CIデザインに対して3.2-4.6倍、Afterデザインに比較すると7.1-10.1倍も正確に真の効果を推定できる（推定された効果が真の効果の方向と一致し、且つ真の効果の±30%に含まれる）ことが明らかになった（比較値のばらつきはサンプルサイズによる）。BACIデザインの正確度はサンプルサイズが小さい場合には低下したが、それでもほとんどの指標において他のデザインよりも高い正確度を示していた。サンプルサイズを増やすことでBACIデザインの正確度は向上したが、他の研究デザインでは偏った推定値の精度が向上するだけであった。

4. Synthesis and applications. 例えサンプルサイズが十分であったとしても、簡素なデザインに基づいた推論は正確でない可能性があるため、生態学においてもより頑健な研究デザインの利
Using strong research design is necessary to promote it. To promote it, long-term research funding and a stronger connection between research and practice are needed. This research further builds on these results by proposing a method for weighting each study based on research design and sample size in meta-analysis, and presents practical examples using three recent meta-analyses. These results are useful for decision-makers or researchers conducting meta-analysis to consider the evidence synthesized for decision-making.

**Keywords:** Before-After Control-Impact, causal inference, evidence synthesis, impact evaluation, meta-analysis, randomised controlled trial, study design, inverse-variance weighting

**Introduction**

Monitoring the impact of human activities on biodiversity is fundamental to understanding how to effectively conserve biodiversity. This includes monitoring the impacts of anthropogenic threats, as well as the effectiveness of management interventions to mitigate such threats. The main challenge for such monitoring is disentangling natural environmental change from anthropogenic change (Hewitt et al. 2001; Hipel et al. 1978), whilst considering the focal impact’s statistical (Osenberg and Schmitt 1996; Box & Tiao 1975) and ecological significance (Wolfe et al. 1987). The complexity of ecosystems, including various sources of spatiotemporal variation and confounding variables, has catalysed much research on understanding the best ways to design impact assessments (Lettenmaier et al. 1978; Stewart-Oaten et al. 1986; Osenberg et al. 2006). Whilst improvements in study design have helped ecologists to more accurately quantify human impacts on biodiversity, a range of designs with varying complexity and biases still persist (De Palma et al., 2018; Table 1).

Study design is composed of three major aspects: (i) pre-impact sampling, (ii) use of controls, and (iii) randomised allocation of independent sampling units (here we term these “sites”). Adding pre-impact sampling to an After design - where monitoring only occurs after...
the impact – produces the Before-After (BA) design (Table 1). This compares the system’s state before and after the impact, attempting to minimise bias from temporal variability and pre-impact conditions.

Addition of control sites to BA designs results in Before-After Control-Impact (BACI) designs, where the average difference between control and impact sites is compared before and after an impact (Table 1; Stewart-Oaten et al. 1986; Osenberg et al. 2006). BACI designs use the pre-impact differences between control and impact sites as a null hypothesis for post-impact differences that would exist if the focal impact was absent – avoiding bias from a lack of a control (Thiault et al. 2016). Problems with site-specific temporal variation in BACI designs can be addressed by sampling control and impact sites simultaneously, several times before and after the impact (Before-After Control-Impact Paired-Series (BACIPS) design; Stewart-Oaten & Bence 2001).

Random allocation of sites to control and impact groups represents the third major aspect of study design. Control-Impact (CI) designs, analogous with Space-For-Time Substitutions (França et al. 2016; De Palma et al. 2018) or Intervention Versus Reference Site designs (Stewart-Oaten & Bence 2001), compare non-randomly allocated control and impact sites after the impact (Table 1). However, this non-random allocation can violate the assumption that the only differences between control and impact sites are due to the focal impact, leading to biased results (De Palma et al. 2018; Damgaard 2019; Larsen et al. 2019; Table 1). Randomised Controlled Trials (RCTs) minimise this bias by truly randomising site allocation to impact and control groups (Table 1). This reduces the need to sample before and after the impact to account for any initial differences (i.e. BACI design) if sufficient numbers of sites and points in time are sampled (Larsen et al. 2019; De Palma et al., 2018).
Table 1 - Comparison of the key features of study designs. Graphs show how designs sample from impact (green points) and control (blue points) sites over time, before and after an impact (white versus grey areas, respectively). Solid horizontal lines show the average density of sites measured to calculate each design’s effect size estimate. Dashed horizontal lines for CI and RCTs represent the pre-impact differences between the mean densities of control and impact sites, which can cause bias – note less difference for RCTs (with high sample size) versus CI. Many design variants exist – e.g. MBACI for BACI with multiple sites, R for Reference in BARI (Webb et al. 2012).

| Design                          | Sampling regime | Relative cost | Relative difficulty in ecology | Suitability                                                                 | Ecological examples of use                          |
|--------------------------------|-----------------|---------------|--------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------|
| After                           |                 | Very low      | Very low                       | Most systems                                                               | Pond creation                                       |
| Before-After (BA)               |                 | Moderate       | Moderate                       | Predictable impacts                                                        | Wildlife tunnels under roads                         |
| Before-After Control-Impact (BACI) |                 | High           | High                           | Predictable impacts, Appropriate control, Availability of pre-impact data  | MPA effectiveness, renewable energy infrastructure   |
| Control-Impact (CI)             |                 | Low            | Moderate                       | Unpredictable impacts, Large-scale replicates that cannot be truly randomised | Oil spill or other pollution event                    |
| Randomised Controlled Trial (RCT) |                 | Low            | Very high                      | Unpredictable impacts, Small-scale replicates appropriate for randomisation | Peatland restoration, field margins                |
Despite the development of robust approaches to quantifying impacts, greater usage of less robust designs persists. Three systematic maps on the biodiversity impacts of different threats and interventions found that a low proportion of studies used BACI (6-29%) and BA designs (3-37%), but many more used CI designs (48-89%) (Bernes et al. 2015, Bernes et al. 2017, Papathanasopoulou et al. 2016).

The greater prevalence of CI designs in the ecological literature probably reflects that they can be easier to implement than more complex study designs. For example, RCTs are widely used in fields, such as medicine, where random allocation of small-scale experimental units to impact and control groups is possible (Tugwell & Haynes 2006; Downs & Black 1998). However, RCTs often cannot be used in ecology because true randomisation of experimental units is more difficult with large-scale sites (e.g. protected areas) compared to smaller, more readily-available plots (Larsen et al. 2019; Stewart-Oaten & Bence 2001). Therefore, ecologists tend to use pseudo-experimental designs lacking randomisation, such as BA, CI and BACI designs (Table 1; De Palma et al. 2018). Nevertheless, constraints due to cost, logistics and project duration often prevent the implementation of complex BACI and even simpler BA designs because of the need to revisit sites pre- and post-impact (França et al. 2016, Osenberg et al. 2011; Table 1).

The disparities between the robustness of study designs and their usage is concerning as many studies may be making misleading inferences about anthropogenic impacts. Some empirical comparisons of the consequences of using BACI, BA and CI designs have been undertaken (Osenberg et al. 2011; França et al. 2016; Mahlum 2018; Smokorowski & Randall 2017). However, we are yet to understand how inaccurate simpler designs are relative to complex ones, or the influence of sample size on these patterns (e.g. are simpler designs with large sample sizes equivalent to more complex designs with smaller sample sizes?). A quantitative comparison of the accuracy of different designs and their sample size would help us better understand these issues.
To address this knowledge gap, we simulate a hypothetical population’s response to an impact, and compare how accurately different study designs estimate that response. We use empirically-derived parameter estimates from 47 ecological datasets to generate realistic control and impact data, before and after an impact. BACI, RCT, BA, CI and After designs are then used to sample from the simulated data with various levels of spatial replication (control and impact sites). We compare the accuracy of each design by their ability first to predict the correct direction of the response, and second to estimate the response to within a given percentage. Our goal is to inform the development of a quantitative scale of the comparative accuracy of different designs. Such a scale would have utility for future monitoring of anthropogenic impacts, as well as assessing the quality of ecological studies used to inform policy and practice.

Materials and methods

We simulated a hypothetical population with true density $\lambda$ that varied over $T$ time steps before and after a chronic impact occurred (Fig.1). For example, if $T=10$, then time steps 1-10 were classified as the ‘before period’ (i.e. before the impact occurred) and time steps 11-20 were classified as the ‘after period’ (Fig.1). The true density was monitored in sites where the impact occurred (‘impact sites’) and where the impact was absent (‘control sites’).

We set the mean true density to 50 and randomly sampled $T$ values from a Poisson distribution ($\lambda=50$) to vary the true density over $T$ time steps in the before period for control and impact sites. These $T$ values defined the true density in each time step before the impact occurred (e.g. $\mu_{t}$, for impact sites in the $t$th time step). To simulate a step-change response at both control and impact sites after the impact occurred (Fig.1), we sampled from a different Poisson distribution with $\lambda$ adjusted by an empirically-derived amount $I$ ($\lambda+I$;
Fig.1; Table 2) for impact sites and an empirically-derived amount \( C \) for control sites \((\lambda+ C;\) Fig.1; Table 2). \( I \) and \( C \) were varied using empirical estimates of the proportional change in control and impact sites in the before period versus the after period, \( p_I \) and \( p_C \), respectively, sampled from 47 ecological datasets \((I = \lambda \cdot (p_I - 1); C = \lambda \cdot (p_C - 1);\) Table 2). A list of published data sources for empirical estimates used in this study are provided in the Data sources section (see also Appendix S1 in Supporting Information).

While we focus on a step-change response in our simulation, temporal biodiversity dynamics following disturbances or interventions can follow different trajectories (Di Fonzo et al. 2013; Thiault et al. 2016). However, to simplify the simulation as much as possible, particularly in terms of computational demands, using a step-change response was most appropriate to test the relative accuracy of each design.

Using the simulated data for before and after periods we sampled various numbers of impact \((n_I)\) and control \((n_C)\) sites (these could also be plots or transects; Fig.1). For RCTs that use random allocation of sites to control and impact groups, we randomly sampled sites from two normal distributions for each time step: one with a mean, \( \mu_{I,t} \), for impact sites and one with a mean, \( \mu_{C,t} \), for control sites (Fig.1). The number of sites sampled was the same for all time steps. The standard deviation of each normal distribution represented the variation amongst sites and was calculated by multiplying the mean by the coefficient of variation (e.g. control sites: \( \sigma_{C,t} = \mu_{C,t} \cdot CV_s \); impact sites: \( \sigma_{I,t} = \mu_{I,t} \cdot CV_s \); Table 2). We varied \( CV_s \) by randomly drawing values from a truncated normal distribution: \( N(\mu = 0.1, \sigma = 0.05, \min = 0, \max = 0.2) \).
Table 2 - Definitions and summary statistics for all simulation parameters (termed ‘Sim.’) and empirically-derived parameters (termed ‘Emp.’; Appendix S1). Equations show how each parameter was calculated. For empirically-derived parameters, $\bar{x}$ refers to the average of sampled sites taken from 47 ecological datasets (e.g. $\bar{x_{AC}}$ refers to the average of all control sites in the after period; Appendix S1).

| Parameter | Definition | Source | Equation | Mean | SD  | Min | Max |
|-----------|------------|--------|----------|------|-----|-----|-----|
| $p_c$     | Change in control between before and after periods | Emp. | $p_c = \frac{\bar{x_{AC}}}{\bar{x_{BC}}}$ | 0.918 | 0.181 | 0.605 | 1.31 |
| $p_i$     | Change in impact between before and after periods | Emp. | $p_i = \frac{\bar{x_{AI}}}{\bar{x_{BI}}}$ | 0.967 | 0.230 | 0.579 | 1.46 |
| $p_{CIB}$ | Average value of control sites as a proportion of the average value of impact sites in the before period | Emp. | $p_{CIB} = \frac{\bar{x_{AC}}}{\bar{x_{BI}}}$ | 1.13 | 0.306 | 0.654 | 1.89 |
| $I$       | True change in impact sites from before to after impact | Emp. | $I = \lambda \cdot (p_i - 1)$ | -1.65 | 11.5 | -21.1 | 23.2 |
| $C$       | True change in control sites from before period to after period | Emp. | $C = \lambda \cdot (p_c - 1)$ | -4.10 | 9.05 | -19.8 | 15.4 |
| $d_{CIB}$ | Difference between true densities of control and impact sites in before period | Emp. | $d_{CIB} = \lambda \cdot (p_{CIB} - 1)$ | 6.60 | 15.3 | -17.3 | 44.5 |
| $\lambda$ | True density across all time steps | Sim. | $\lambda = 50$ | - | - | 50 | 50 |
| $T$       | Total number of time steps simulated | Sim. | $T = \{2,4,6,8,10\}$ | - | - | 2 | 10 |
| $n_T$     | Number of time steps sampled in each period | Sim. | $n_T = T$ | - | - | 2 | 10 |
| $\mu_{1,t}$ | True density in impact sites in time step $t$ | Sim. | Before: $\mu_{1,t} \sim \text{Poisson}(\lambda)$ After: $\mu_{1,t} \sim \text{Poisson}(\lambda + I)$ | - | - | - | - |
| $\sigma_{1,t}$ | Standard deviation of impact sites in time step $t$ | Sim. | $\sigma_{1,t} = CV_S \cdot \mu_{1,t}$ | - | - | - | - |
| $\mu_{c,t}$ | True density in control sites in time step $t$ | Sim. | Before: $\mu_{c,t} \sim \text{Poisson}(\lambda)$ After: $\mu_{c,t} \sim \text{Poisson}(\lambda + C)$ | - | - | - | - |
| $\sigma_{c,t}$ | Standard deviation of control sites in time step $t$ | Sim. | $\sigma_{c,t} = CV_S \cdot \mu_{c,t}$ | - | - | - | - |
To account for non-random allocation of sites to control and impact groups in BACI, BA, CI and After designs, we repeated the same approach but with one important modification. We adjusted the true density of control sites in every time step, $\mu_{C,t}$, by an empirically-derived amount, $d_{CIB}$ ($\mu_{C,t} + d_{CIB}$; Fig. 1; Table 2). To vary $d_{CIB}$, we used empirical estimates of the proportional difference between control and impact sites in the before period, $p_{CIB}$, sampled from 47 ecological datasets ($d_{CIB} = \lambda \cdot (p_{CIB} - 1)$; Table 2; Appendix S1). This simulated difference between control and impact sites accounted for different levels of site selection bias in non-randomised designs, including situations where little or no bias may be present (e.g. $d_{CIB} \approx 0$).

We calculated effect size estimates for each design by first finding the mean density of sampled sites across all time steps for control and impact groups in the before period ($\text{Before}_{\text{Impact}}$, $\text{Before}_{\text{Control}}$) and the after period ($\text{After}_{\text{Impact}}$, $\text{After}_{\text{Control}}$). We assumed that sampling occurred in all time steps ($n_T = T$) in both periods. We did this as the investigator may wish to only estimate the effect over a certain timescale (which will be context-specific) and we lacked the computational capabilities to simulate all possible sampling permutations using fewer than the full number of time steps (e.g. sampling in certain intervals or continuous periods of time; Wauchope et al. 2019).
Fig. 1 - An overview of our simulation. Step 1 shows true densities of control and impact sites generated in the before period (white area). Step 2 shows true densities of control and impact sites generated in the after period (grey area) to reflect a step-change response (using I and C); the true density in each time step (t) is shown (μ_{i,t}, impact: green; and μ_{c,t}, control: blue). Step 3 shows how control and impact sites (SI and SC) are sampled (n_I and n_C = 10) for both randomised and non-randomised designs.

Effect size estimates were calculated using these mean densities, as appropriate for each study design (Table 3). For example, RCT effect sizes were found by subtracting
After_{Control} from After_{Impact}, whilst BA effect sizes were found by subtracting Before_{Impact} from After_{Impact} (Table 3). The exception was the After design, for which we found the mean of sampled sites in the first time step and subtracted this from the mean of sampled sites in the final time step of the after period (Table 3). We defined the true effect as the change in the mean of true densities of impact sites between the before and after periods minus the equivalent change in the mean of true densities of control sites (Table 3). As discussed previously, we did this because we wanted to compare each design’s relative accuracy at estimating the true effect over the number of time steps simulated.

We ran the simulation under 1000 different scenarios, varying: (i) the true change in control sites \((C)\); (ii) the true change in impact sites \((I)\); (iii) the mean difference between control and impact sites in the before period \((d_{CIB})\); and (iv) the variation between sites \((CV_S)\). For each simulation scenario, we varied the number of time steps simulated \((T = 2, 4, 6, 8 \text{ or } 10)\), as well as the number of impact sites \((n_I = 1, 5, 10, 25, 50)\) and control sites \((n_C = 2, 5, 10, 25, 50)\) sampled independently to use every possible pairwise combination - a total of 125 combinations. Overall, we simulated 1000 scenarios with 125 different sampling combinations in each, repeating each scenario 1000 times \((1000 \times (1000 \times 125) = 1.25 \times 10^8 \text{ runs})\).
Table 3 - Equations showing effect size estimate, variance and error calculation for each study design using mean densities of control or impact sites in each period (e.g. After\textsubscript{impact} refers to the mean of sampled impact sites across all time steps in the after period). For the After design, the effect size was calculated by finding the difference between the final time step (\( t = T \)) and the first

| Effect size estimate | Pooled variance \( (s_p^2) \) | Error |
|----------------------|-------------------------------|-------|
| RCT \( After\textsubscript{impact} - After\textsubscript{control} \) | \( \frac{(n_{AI} - 1)s_{AI}^2 + (n_{AC} - 1)s_{AC}^2}{n_{AI} + n_{AC} - 2} \) | \( \pm 1.96 \cdot \sqrt{\frac{s_p^2}{n_{AI}} + \frac{s_p^2}{n_{AC}}} \) |
| BACI \( \left( \frac{After\textsubscript{impact} - After\textsubscript{control}}{Before\textsubscript{impact} - Before\textsubscript{control}} \right) \) | \( \frac{(n_{BI} - 1)s_{BI}^2 + (n_{BC} - 1)s_{BC}^2 + (n_{AI} - 1)s_{AI}^2 + (n_{AC} - 1)s_{AC}^2}{n_{BI} + n_{BC} + n_{AI} + n_{AC} - 4} \) | \( \pm 1.96 \cdot \sqrt{\left( \frac{s_p^2}{n_{BI}} + \frac{s_p^2}{n_{BC}} + \frac{s_p^2}{n_{AI}} + \frac{s_p^2}{n_{AC}} \right)} \) |
| CI \( After\textsubscript{impact} - After\textsubscript{control} \) | \( \frac{(n_{AI} - 1)s_{AI}^2 + (n_{AC} - 1)s_{AC}^2}{n_{AI} + n_{AC} - 2} \) | \( \pm 1.96 \cdot \sqrt{\frac{s_p^2}{n_{AI}} + \frac{s_p^2}{n_{AC}}} \) |
| BA \( After\textsubscript{impact} - Before\textsubscript{impact} \) | \( \frac{(n_{BI} - 1)s_{BI}^2 + (n_{AI} - 1)s_{AI}^2}{n_{BI} + n_{AI} - 2} \) | \( \pm 1.96 \cdot \sqrt{\frac{s_p^2}{n_{BI}} + \frac{s_p^2}{n_{AI}}} \) |
| After \( After\textsubscript{impact,t=T} - After\textsubscript{impact,t=1} \) | \( \frac{(n_{I,t=T} - 1)s_{I,t=T}^2 + (n_{I,t=1} - 1)s_{I,t=1}^2}{n_{I,t=T} + n_{I,t=1} - 2} \) | \( \pm 1.96 \cdot \sqrt{\frac{s_p^2}{n_{I,t=T}} + \frac{s_p^2}{n_{I,t=1}}} \) |
| True effect \( \left( \frac{\mu_{IA,After,t=1,\ldots,\mu_{IA,After,t=T}}}{} - \frac{\mu_{IB,Before,t=1,\ldots,\mu_{IB,Before,t=T}}}{\mu_{IC,After,t=1,\ldots,\mu_{IC,After,t=T}}} - \frac{\mu_{IC,Before,t=1,\ldots,\mu_{IC,Before,t=T}}}{} \right) \) | | |

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2664.13499

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time step of the after period (1). n and s² refer to the number of sites and variance in that period (e.g. n_{A1} and s_{A1}² refer to the number of impact sites and variance in the After period).

Effect size estimates for each design were used to investigate their relative accuracy. We calculated 95% Confidence Intervals (CIs) using the pooled variance and associated error for each effect size estimate (Table 3). We used these 95% CIs to estimate the percentage of simulations repetitions where: (i) the true effect fell within the 95% CIs of effect size estimates (coverage probability); (ii) the correct direction was detected (95% CIs entirely above or below zero); (iii) the estimated effect size under- or overestimated the true effect (95% CIs entirely above or below true effect). We also investigated the percentage of simulation repetitions in which each design’s effect size estimate: (i) had the same direction as the true effect; and (ii) was both within a given percentage of the true effect and of the same direction. We believe these two measures capture the major aspects of accuracy and precision that are desirable in a study design.

We calculated all these measures for all possible pairwise combinations of control and impact sites (e.g. two control and two impact sites, two control and five impact sites etc.). We set five thresholds to measure the percentage of times an effect size estimate was within a certain percentage of the true effect: 10, 20, 30, 40 and 50%. We also explored how varying the terms C and d_{CIB} (controlling bias in non-BACI designs) for three levels of magnitude (no bias: 1; low bias: 0.9 or 1.1; high bias: 0.7 or 1.3) affected this percentage (Figures S7 and S8).

We used Generalised Linear Models with a beta error distribution to determine the relationship between the performance of each design (the response variable; see below) and two explanatory variables (number of control sites and the number of impact sites). For the response variable we used the proportion of simulation repetitions where the effect size
estimate was within ±30% of the true effect and had the correct direction. We only considered results for an accuracy threshold of ±30% as this was deemed a reasonable level of accuracy and we wanted to simplify the interpretation of our results as much as possible. We also present results for other accuracy thresholds (±10% and ±50%) in Appendix S3.

Based on graphical observations of the relationship between the response and explanatory variables (Fig.4), we included impact and control sites as log transformed explanatory variables for models of BACI and RCT designs and tested models with and without an interaction term between these variables (Appendix S4). The BACI model with the interaction term had the lowest AIC by more than 2 units and was chosen as the best model. The RCT model without the interaction term was only lower by 1.8 units but was chosen as it was more parsimonious (Appendix S4). As the performance of BA, CI and After designs did not vary with the number of impact or control sites, we did not create any models for these designs (Fig.4).

We calculated quasi-\(R^2\) values (Appendix S4) to test model performance using the equation:

\[
\text{quasi}R^2 = 1 - \frac{\text{deviance}}{\text{null deviance}} \quad \text{equation 1.}
\]

Both models were only slightly over-dispersed (RCT model: \(\theta = 1.19\); BACI model: \(\theta = 1.25\)) and Pearson's \(\chi^2\) residuals were non-significant (p>0.05) suggesting no significant patterns remained in the residuals. There were also no observable patterns between residuals and explanatory variables or fitted values.

For BACI and RCT designs we converted estimated coefficients (\(\beta\)) from log odds (Appendix S4) to proportions to create an ‘accuracy weight’ equation for each design (eqn 2 and 3). We found the accuracy weights for BA, CI and After designs by simply taking the mean proportion of simulation repetitions where the effect size estimate was within ±30% of the true
effect and had the correct direction across all combinations of impact and control sites. These accuracy weights are on a continuous scale between a minimum of 0 (lowest accuracy) and a maximum of 1 (highest accuracy - see Results, Discussion and Appendix S2 for how to apply these weights):

$$\text{BACI accuracy weight} = \frac{1}{1+e^{-(\beta_{\text{Int.}} + \beta_{nI} \cdot \ln(n_I) + \beta_{nC} \cdot \ln(n_C))}} \quad \text{(equation 2)}$$

$$\text{RCT accuracy weight} = \frac{1}{1+e^{-(\beta_{\text{Int.}} + \beta_{nI} \cdot \ln(n_I) + \beta_{nC} \cdot \ln(n_C))}} \quad \text{(equation 3)}$$

where $\beta_{\text{Int.}}$ = Intercept coefficient, $\beta_{nI}$ = Impact sites coefficient, $\beta_{nC}$ = Control sites coefficient and $\beta_{nInC}$ = interaction coefficient between impact and control sites.

We applied our accuracy weights to three recent ecological meta-analyses: Bernes et al. (2018) on the effects of ungulate herbivory on vegetation and invertebrates; Eales et al. (2018) on the effects of prescribed burning on forest biodiversity; and Sandström et al. (2019) on the impacts of dead wood manipulation on forest biodiversity. We found these meta-analyses by searching the Environmental Evidence Journal and the Journal of Applied Ecology using the search terms: “meta analysis” OR “meta-analysis” and reviewing studies published since 2018. Only the three previously mentioned meta-analyses contained a sufficient range of study designs (Appendix S2) and readily available associated data on study design, replicates and effect sizes. We repeated analyses using random effects models following the authors’ methodology (e.g. including random factors such as Site IDs) using the metaphor package (Viechtbauer 2010; see Appendix S2). We were able to replicate 128 out of 130 summary effect sizes (comparisons) using the authors’ methodology and inverse-variance weighting, which we repeated with our accuracy weights. Two summary effect sizes could not be replicated from Bernes et al. (2018) due to lack of data labelling. We wanted to test how our weights altered the
conclusions of meta-analyses that used studies with a mixture of different study designs. Therefore, we only present results for 96 comparisons that used studies with at least one type of design. The mean number of studies of each design were: 9.0 BACI, 6.0 BA, 5.0 CI (see Appendix S2 for a breakdown of studies for each summary effect size).

We used R statistical software version 3.5.1 (R Core Team 2018) with the doParallel package (Microsoft Corporation & Weston 2017) to increase computational performance. We provide data to repeat all analyses on Zenodo (Christie et al. 2019).

Results

There was large variation in the performance of designs in accurately estimating the true effect. As overall patterns were similar across simulations with different time steps (Figures S1-S3), we present results when six time steps were simulated in both the before and after periods.

BACI designs performed best at correctly identifying the direction of the true effect (≥94.1% of simulation repetitions; Fig.2A), followed by RCTs (≥90.8% of the time). Both BACI and RCTs far outperformed CI, BA, and particularly After designs – BA designs slightly outperformed CI designs (approximately 76.3% versus 74.7%) and both strongly outperformed After designs (approximately 49.8%; Fig.2A). Unlike BACI designs, non-BACI designs showed negligible improvements in performance with increasing replication (increases from two control and two impact sites to 50 control and 50 impact sites: BACI = +4.6%; After = +0.0%; BA = +0.5%; CI= +0.2%; RCT= +1.0%; Fig.2A).
Taking account of the uncertainty around these effect size estimates (95% CIs) gave different results – where overlap with zero was classed as non-significant and non-overlap as either positive or negative (Fig.2B). With this measure, RCTs were most likely to correctly predict the direction of the true effect with two impact and control sites, followed by CI, BACI, BA and After designs in decreasing order of performance (Fig.2B). BACI designs showed the greatest proportional improvement in this measure, outperforming RCTs at sample sizes above 25 impact and control sites (Fig.2B). BA designs increased proportionally more than CI designs, reaching similar levels of performance above 25 impact and control sites (Fig.2B). BACI designs were also likely to produce non-significant effect sizes (overlap of 95% CIs with zero in ~45% of repetitions for 2 impact and control sites; Figure S3) at low sample sizes, but were extremely unlikely to produce significant effect sizes that had the wrong direction (~1% of repetitions for 2 impact and control sites; Figure S3).
Fig. 2 – Performance of designs in correctly predicting the direction of the true effect for multiple levels of spatial replication with equal numbers of control and impact sites (see Figures S1 and S2 for other combinations of sites). Fig. 2A measures this in terms of whether the effect size estimate was positive or negative, whilst Fig. 2B considers whether the 95% CIs of this estimate correctly fell entirely above or below zero. See Table 1 for the definition of each design.
If we consider the coverage probabilities of each design (i.e. proportion of times the true effect fell within the 95% CIs of effect size estimates), BACI designs substantially outperformed other designs (Fig.3A). The true effect fell within the 95% CIs of BACI effect size estimate approximately 99% of the time, with negligible change from increasing replication (Fig.3A). The coverage probabilities for other designs (RCT, BA, CI, After) declined asymptotically with increasing replication as 95% CIs narrowed (Fig.3A).

We also examined the tendency for designs to underestimate or overestimate the true effect (i.e. when 95% CIs did not overlap with true effect; Fig.3B). BACI designs rarely under- or overestimated the true effect (1% of repetitions), whilst both BA and CI designs were approximately twice as likely to underestimate than overestimate (Fig.3B). RCT designs and (to a lesser extent) After designs were equally as likely to underestimate as overestimate. With increasing replication, all non-BACI designs were increasingly likely to under- or overestimate the true effect, although this relationship was asymptotic – this probability increased at a higher rate for RCTs than other non-BACI designs (Fig.3B).
Fig. 3 – Percentage of simulation repetitions in which the 95% CIs of effect size estimates contained the true effect (coverage probability – Fig.3A) or were either greater than or less than the true effect (overestimate versus underestimate – Fig.3B). In Fig.3B, underestimates are shown by downward triangles, whilst overestimates are shown by upward triangles. This is shown for multiple levels of spatial replication with equal numbers of control and impact sites (see Figures S4 and S5 for other combinations of sites). See Table 1 for the definition of each design.

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Consistent patterns were also found when considering the percentage of repetitions for which the effect size estimate was both within a certain percentage of the true effect and had the correct direction (Fig.4). First, RCT and BACI designs still far outperformed CI, BA or After designs (for ±30% accuracy threshold: BACI ≥65.6%, RCT ≥51.3%, BA ≥22.4%, CI ≥20.4%, After ≥9.3%; Fig.4). Second, BA designs appeared to perform slightly better than CI designs, especially as the accuracy threshold rose from ±10%-50% (from ~2% higher to ~7% higher performance; Fig.4). Similarly, both BA and CI designs performed relatively better compared to After designs with an increasing accuracy threshold (Fig.4).

Third, BACI performance increased to a much greater extent with increasing replication than for other designs (Fig.4). For the ±30% accuracy threshold, increasing replication from two control and impact sites to 50 control and impact sites resulted in an increase of 26.7% for BACI compared to +3.8% for RCT, +0.2% for BA, +0.3% for CI and -0.4% for After (Fig.4). For BACI designs, increasing replication moderately in both control and impact sites resulted in greater performance than only increasing replication in just one type of site (±30% threshold: 76.6% at two impact and two control sites versus 72.8% at two impact and 50 control sites; Fig.3; Fig.S6).

We also considered how varying the simulation parameters $C$ and $d_{CIB}$ affected our results (Figures S7 and S8). Increasing the change in control ($C$) reduced the performance of BA designs substantially (Figure S7), whilst increasing the initial mean differences between impact and control groups in the before period ($d_{CIB}$) reduced the performance of CI designs substantially (Figure S8).
Fig. 4 – Performance of designs measured by percentage of simulation repetitions in which a design’s effect size estimate was both within ±10, 30 or 50% of the true effect and had the correct direction. This is shown for multiple levels of spatial replication with equal numbers of control and impact sites (see Figure S6 for other combinations of sites). See Table 1 for the definition of each design.
We used Generalised Linear Models to examine the factors that determined the performance of each design at estimating the direction and magnitude of the true effect to within ±30% (using data from Fig.4 and Fig.S6). For RCT designs, there was little difference in the importance of control versus impact sites in predicting performance, whilst control sites seemed to have greater importance in BACI designs. High Pseudo-$R^2$ values showed that our models explained far greater levels of variation in the data than null models (see Appendix S4).

Weights for studies in meta-analyses can be calculated from these relationships of performance with sample size, which we term ‘accuracy weights’. This requires information about a study’s design and the number of independent control and impact units used (see Appendix S4). For example, França et al. (2016) used a BACI design, 29 impact and five control units and thus receives an accuracy weight of:

$$\frac{1}{1+e^{-(0.661+0.0153\cdot \ln(29)+0.0647\cdot \ln(5))}} = 0.805$$

(see Appendix S4; eqn 2). More examples of calculating weights for studies are shown in Appendix S2.

We applied our accuracy weights to meta-analyses (Appendix S2) and found that they gave broadly similar results to conventional (inverse-variance) weighting (for 77% of comparisons). However, there was a tendency for our weights to alter the outcome to non-significant (12% from negative to non-significant and 8% from positive to non-significant; Table 4). A small proportion changed from non-significant to significantly positive (1%) or significantly negative (2%). No outcomes of summary effect sizes changed from positive to negative or vice versa (Table 4).
Table 4 – Comparison of outcomes for 96 summary effect sizes obtained using the accuracy weights proposed by this study versus conventional inverse-variance weighting. Summary effect sizes were extracted from 3 separate meta-analyses (see Methods). Cells show the proportion of effect sizes that were significantly positive, significantly negative or non-significant for both weighting systems.

| Weighting method          | Accuracy weight |
|---------------------------|-----------------|
|                           | Outcome         | Non-significant | -              |
| Inverse-variance weight   | +               | 9 (9%)          | 8 (8%)         |
|                           | Non-significant | 1 (1%)          | 53 (55%)       |
|                           | -               | 0               | 11 (12%)       |

Discussion

Using this simulation we have demonstrated that BACI and RCT designs are far more accurate than BA, CI and After designs. When estimating the true effect to within ±30% and correctly identifying its direction, BACI designs performed: 1.3-1.8 times better than RCTs; 2.9-4.2 times better than BA; 3.2-4.6 times better than CI; and 7.1-10.1 times better than After designs (depending on sample size). This is because increasing sample size tends to only increase precision in non-BACI designs around a biased estimate of the true effect.

This bias is generated by violating the assumptions underpinning these non-BACI designs (De Palma et al. 2018). BA designs assume there is no average change in control group mean before versus after the intervention, whilst CI designs assume the only differences that exist between control and impact sites are due to the focal impact (C and $d_{CIB}$ in Methods; Figures S7 and S8). RCTs also suffer from bias because they only minimise initial differences between impact and control group means (in the before period), which can be increased by spatiotemporal variation (De Palma et al. 2018). Blocking, pairing or matching sites in CI designs or including a proxy variable in statistical analysis could theoretically account for some
of these biases, but they cannot guarantee lower levels of bias. BACI designs better account for these initial differences, effectively removing this bias, and therefore have higher accuracy – they can also deal with spatiotemporal variation through repeated sampling through time (Thiault et al. 2016).

The fact that increasing the sample size (precision) of non-BACI designs reduced the coverage probability (probability that the true effect fell within the 95% CIs of an effect size estimate; Fig.3A) supports this conclusion as 95% CIs converged on biased estimates. The coverage probability for BACI designs remained almost constant because the effect size estimates converged on the true effect – greater precision translated into greater accuracy for BACI designs. Greater accuracy is therefore best achieved using more robust designs that remove biases, such as BACI, with greater sample sizes.

We nevertheless note that BACI designs are known to suffer from noise (Osenberg et al. 2006), which was demonstrated by their tendency to classify the direction of the true effect as non-significant at low samples sizes (Figure S3). The low statistical power of BACI designs at small sample sizes is an issue and reinforces the need to ensure sufficient numbers of replicates are used in BACI designs (Osenberg et al. 2006), as well as to consider using Bayesian approaches to interpret effect sizes (Conner et al. 2016). When only considering the direction of effect size estimates, however, BACI and RCT designs gave the correct direction ~20-30% more often than CI and BA designs, and ~40-45% more than After designs (Fig.2A).

Our results provide strong evidence that simpler designs (e.g. After, BA and CI) often yield different inferences to BACI designs, as observed empirically by previous studies (Osenberg et al. 2011; França et al. 2016; Mahlum 2018; Smokorowski & Randall 2017). We also found that BA and CI designs were more prone to underestimation than overestimation.
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for their usage is important. For example, BACI design usage should be encouraged whenever prior knowledge exists of the timing of an impact or where suitable pre-impact data is available retrospectively (e.g. infrastructure projects, Protected Area designation). We also recognise the expensive nature of BACI designs, due to the need to revisit study sites before and after the impact, often hampers their implementation (De Palma et al. 2018). This means BACI designs can be impossible to use during short term projects limited by grant or studentship duration. Therefore, we suggest that longer-term funding and stronger research-practice partnerships are required to facilitate the use of BACI designs (Osenberg et al. 2011; De Palma et al. 2018).

Some investigators may also avoid using BACI designs due to concerns over the difficulty in interpreting their results for lay audiences and stakeholders. We suggest that this can be overcome using Bayesian approaches that present easily interpretable probabilities to managers and practitioners (Conner et al. 2016) or new measures for BACI designs that aid ecological interpretation (Chevalier et al. 2019).

Given the use of simpler designs will probably persist in the near future, we further argue that our results have major implications for decision-making and meta-analysis in ecology. We have proposed a novel weighting system that could help when meta-analyses are faced with studies that vary markedly in their design. Conventional meta-analysis typically uses inverse-variance of studies as weights to attempt to account for study quality (Marín-Martínez & Sánchez-Meca 2010; Koricheva & Gurevitch 2014). However, this can greatly reduce the number of suitable primary studies since not all studies report variance (Koricheva & Gurevitch 2014). Alternative approaches of meta-analysis to tackle poor data reporting, such as non-parametric weighting by sample size, have been proposed (Mayerhofer et al. 2013; Adams et al. 1997), but fail to consider wider aspects of study quality such as study design (Spake & Doncaster 2017). Whilst recent efforts to assessing evidence quantitatively by study design are
welcomed (Webb et al. 2012; Mupepele et al. 2016; Mupepele & Dormann 2017), their weights are relatively simplistic (e.g. simple integer scores or categories) and lack a quantitative or objective grounding.

Our weighting system is informed by the relationships we have found between accuracy, precision, study design and sample size, arguably accounting for more aspects of study quality than weighting by sample size or inverse-variance. We have shown how our accuracy weights can be easily used in meta-analyses to give greater influence to studies with more accurate designs (Appendix S2). We have also demonstrated that our weights tend to reduce the number of positive and negative significant results in meta-analyses compared to using inverse-variance weighting (Table 4). We argue that our simulation results imply that inverse-variance weighting may erroneously reward studies with non-BACI designs when they have higher precision (lower variance), possibly leading to more significantly positive or negative results. This is problematic because we have shown that increasing precision of non-BACI designs often leads to biased estimates and not greater accuracy. Weighting by a combination of accuracy and precision using our weights seems more sensible given these results.

Although we acknowledge that our weights only consider some aspects of study quality, we believe that they could be modulated using the percentage of criteria met in subject-specific quality checklists to incorporate more context-specific factors (e.g. size of sampling unit, temporal replication and internal validity; Mupepele et al. 2016; Bilotta et al. 2014). Adding extra components to the evidence assessment process, however, must be balanced against the effort expended in doing so. Our weights could also assign studies to different accuracy categories (Appendix S2), giving a rapid, easily interpretable way to communicate the robustness of evidence to decision-makers - e.g. in evidence toolkits such as Conservation Evidence
(Sutherland et al. 2019). We welcome future research to explore how best to apply our accuracy weights within evidence assessment and decision-making processes.

Overall, we have shown for the first time how much less accurate simpler study designs are compared to more complex ones, generating a new quantitative understanding of the relative accuracy of different designs. Our accuracy weights could also offer a powerful, yet versatile new approach to weighting evidence where studies use a range of different designs, with major implications for the future of meta-analysis and decision-making. We hope our work encourages greater discussion of study design by scientists, managers and policy-makers across ecology and demonstrates the need to tackle the serious consequences of using different designs to make inferences in ecology.

Acknowledgements

We thank the following for providing empirical data used to parameterise simulation: Anna Sher, Ricardo Rocha, Annelies de Backer, Aurora Torres, Carlos Palacín, Juan Carlos Alonso, Barry Baldigo, Brendan Kelaher, Daniel Mateos, Doriane Stagnol, Dominique Davoult, Filipe França, Heather Major, Ian Jones, Jake Bicknell, Jenyffer Vieira, Maria Ruiz-Delgado, Ruben Heleno, Joachim Claudet, Kade Mills, Kevin Stokesbury, Bradley Harris, Mehdi Adjeroud, Michael Craig, Michele Meroni, Norbertas Noreika, Janne Kotiaho, Patrick Edwards, Rafael Barrientos, Carlos Ponce, Carlos Martín, Beatriz Martín, Ricardo Ceia, Roland Pitcher, Sarah Clarke, Oliver Tully, Shailesh Sharma, Just Cebrian, Thomas Stanley, Tyler Eddy, Jonathan Gardner, Anjali Pande, Adrià López-Baucells, Christoph Meyer, Alvaro Antón, Bob McConnaughey, Corrine Watts, David Abecasis, Luciana Cibils, Monica Montefalcone, Teppo Vehanen, Aki Mäki-Petäys, Ari Huusko, Juan Schmitter-Soto, Matt Rinella, Garth Hodgson, Hartwell Welsh, Mikael van Deurs, Mary Donovan, Axel Schwerk, Jill Shaffer, Deborah Buhl, Alberto Velando, Dolores River
Restoration Partnership, Javier Pinilla, Andrew Page, Matt Dasey, David Maguire, Jos Barlow, Júlio Louzada, Rachel Buxton, Carley Schacter, Melinda Conners, Koniambo Nickel, Ginger Soproner, CSIRO, Arturo Elosegi, Loreto García-Arberas, Joserra Díez and Ana Rallo. Thanks also to Grania Smith for proofreading drafts. Author funding sources: TA was supported by the Grantham Foundation for the Protection of the Environment, Kenneth Miller Trust and Australian Research Council Future Fellowship (FT180100354); WJS, PAM and GES were supported by Arcadia and The David and Claudia Harding Foundation; BIS and APC were supported by the Natural Environment Research Council via Cambridge Earth System Science NERC DTP (NE/L002507/1).

Authors’ contributions

APC, WJS and TA conceived the ideas and designed simulation; APC analysed the data and led the writing of the manuscript. All authors contributed critically to drafts, as well as giving final approval for publication. TA provided Japanese language abstract.

Data Availability Statement

Simulation R script and empirical data used in simulations are available via Zenodo http://doi.org/10.5281/zenodo.3373462 (Christie et al. 2019). Published datasets from which some of this empirical data was derived are cited in the Data Sources section.

Supporting Information

Supporting information contains a PDF containing supporting figures, tables and explanations of the empirical derivation of parameter estimates and demonstrations of how our accuracy weights can be applied to meta-analyses and evidence assessment.

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**Figure and Table legends**

Table 1 - Comparison of the key features of study designs. Graphs show how designs sample from impact (green points) and control (blue points) sites over time, before and after an impact (white versus grey areas, respectively). Solid horizontal lines show the average density of sites measured to calculate each design’s effect size estimate. Dashed horizontal lines for CI and RCTs represent the pre-impact differences between the mean densities of control and impact sites, which can cause bias – note less difference for RCTs (with high sample size) versus CI. Many design variants exist – e.g. MBACI for BACI with multiple sites, R for Reference in BARI (Webb et al. 2012).
Table 2 - Definitions and summary statistics for all simulation parameters (termed ‘Sim.’) and empirically-derived parameters (termed ‘Emp.’; Appendix S1). Equations show how each parameter was calculated. For empirically-derived parameters, $\bar{x}$ refers to the average of sampled sites taken from 47 ecological datasets (e.g. $\bar{x}_{AC}$ refers to the average of all control sites in the after period; Appendix S1).

Fig. 1 - An overview of our simulation. Step 1 shows true densities of control and impact sites generated in the before period (white area). Step 2 shows true densities of control and impact sites generated in the after period (grey area) to reflect a step-change response (using $l$ and $c$); the true density in each time step (t) is shown ($\mu_{l,t}$, impact: green; and $\mu_{c,t}$, control: blue). Step 3 shows how control and impact sites ($SI$ and $SC$) are sampled ($n_I$ and $n_C = 10$) for both randomised and non-randomised designs.

Table 3 - Equations showing effect size estimate, variance and error calculation for each study design using mean densities of control or impact sites in each period (e.g. $After_{impact}$ refers to the mean of sampled impact sites across all time steps in the after period). For the After design, the effect size was calculated by finding the difference between the final time step ($t=T$) and the first time step of the after period (1). $n$ and $s^2$ refer to the number of sites and variance in that period (e.g. $n_{AI}$ and $s_{AI}^2$ refer to the number of impact sites and variance in the After period).

Fig. 2 – Performance of designs in correctly predicting the direction of the true effect for multiple levels of spatial replication with equal numbers of control and impact sites (see Figures S1 and S2 for other combinations of sites). Fig.2A measures this in terms of whether the effect size estimate was positive or negative, whilst Fig.2B considers whether the 95% CIs of this estimate correctly fell entirely above or below zero. See Table 1 for the definition of each design.

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Fig. 3 – Percentage of simulation repetitions in which the 95% CIs of effect size estimates contained the true effect (coverage probability – Fig.3A) or were either greater than or less than the true effect (overestimate versus underestimate – Fig.3B). In Fig.3B, underestimates are shown by downward triangles, whilst overestimates are shown by upward triangles. This is shown for multiple levels of spatial replication with equal numbers of control and impact sites (see Figures S4 and S5 for other combinations of sites). See Table 1 for the definition of each design.

Fig.4 – Performance of designs measured by percentage of simulation repetitions in which a design’s effect size estimate was both within ±10, 30 or 50% of the true effect and had the correct direction. This is shown for multiple levels of spatial replication with equal numbers of control and impact sites (see Figure S6 for other combinations of sites). See Table 1 for the definition of each design.

Table 4 – Comparison of outcomes for 96 summary effect sizes obtained using the accuracy weights proposed by this study versus conventional inverse-variance weighting. Summary effect sizes were extracted from 3 separate meta-analyses (see Methods). Cells show the proportion of effect sizes that were significantly positive, significantly negative or non-significant for both weighting systems.