

The effect of initial biomass in manipulative experiments on plants

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Mass is often used as a measure of plant performance in manipulative experiments (Eviner *et al.* 2000; Shen & Harte 2000). Experimental manipulations may be very diverse: from addition of nutrients, to changing abiotic growth conditions, to removing or adding biotic components such as neighbours, herbivores, etc. But, in general, researchers try to clarify the effect of one factor by comparing the biomass of naturally growing plants (or transplants, or planted phytometers) under control and manipulated conditions. Experiments usually start with individuals that are young enough to avoid previous environmental effects, but mature enough to ensure survival and correct identification if several species are used. As a consequence, target individuals in both treatment and control have an initial mass prior to experiments. Established individuals are grown in different conditions and then harvested. The performance of target individuals is assessed by their final dry mass, and the initial value is often ignored in these analyses (Goldberg *et al.* 1999). However, ignoring the initial mass may conceal the treatment effects. Here we explore this problem, and show how ignoring initial biomass can cause systematic bias, particularly when experimental manipulation reduces plant performance compared with the control.

Biomass accumulation during the experiment, or net increase in mass per unit time, can be described as:

$$G_{\text{Control}} = (M_{\text{Control}} - M_0)/t \quad (\text{eqn 1})$$

where G_{Control} is growth of control individuals; M_{Control} and M_0 are, respectively, their final and initial mass; and t is time. Likewise, the equation for treated individuals will be:

$$G_{\text{Treated}} = (M_{\text{Treated}} - M_0)/t \quad (\text{eqn 2})$$

where G_{Treated} is growth of treated individuals and M_{Treated} is their final biomass. Equations 1 and 2 can be used to derive an index measuring an effect of any kind of manipulation. Here we limit our analysis to measurements of plant–plant interactions ranging from competition to facilitation, which may be one of the most sensitive cases to the initial biomass problem. Several indices have been designed to measure these kinds of

interaction, and have been reviewed (Gurevitch *et al.* 1992; Freckleton & Watkinson 2000; Aarssen & Keogh 2002; Weigelt & Jolliffe 2003), and discussed regarding improvements (Armas *et al.* 2004; Brooker *et al.* 2005). One popular and widely used index is $\ln RR$ (Gurevitch *et al.* 1992). This is a natural logarithm of the ratio of observed growth responses, $\ln RR = \ln(T/C)$, where T and C are, respectively, biomass of treated and control individuals. For true biomass increments, initial biomass will be included in calculations:

$$\ln RR = \ln[(M_{\text{Treated}} - M_0)/(M_{\text{Control}} - M_0)] = \ln(\alpha) \quad (\text{eqn 3})$$

where we introduce $\alpha = (M_{\text{Treated}} - M_0)/(M_{\text{Control}} - M_0)$ to simplify algebraic conversions. It is easy to see that $G_{\text{Treated}} = \alpha G_{\text{Control}}$ (equations 1–3); values of $\alpha > 1$ correspond to situations where treated individuals grow better than controls ($\ln RR > 0$), and, *vice versa*, $\alpha < 1$ corresponds to situations where treated individuals grow more slowly than controls ($\ln RR < 0$). As we can see, calculation of true $\ln RR$ thus requires the known value of initial biomass, M_0 . But often initial biomass is ignored and calculations are based on final mass to produce an apparent value of $\ln RR$ (equations 1 and 2):

$$\ln RR' = \ln(M_{\text{Treated}}/M_{\text{Control}}) = \ln[(\alpha G_{\text{Control}}t + M_0)/(G_{\text{Control}}t + M_0)] = \ln[(\alpha + \beta)/(1 + \beta)] \quad (\text{eqn 4})$$

where $\ln RR'$ is the apparent value of the interaction index, and β is introduced to simplify the algebraic expression. $\beta = M_0/G_{\text{Control}}t = M_0/(M_{\text{Control}} - M_0)$ and, as a ratio, compares the initial biomass to the increment in biomass; β approaches 0 when $M_0 \ll (M_{\text{Control}} - M_0)$, that is, when growth is strong and the accumulated biomass is much larger than its initial value. It is evident from equation 4 that the apparent value $\ln RR'$ depends on α as well as on β . Using equations 3 and 4 it is possible to calculate the deviation of the apparent value of the index from its true value. Figure 1 shows a surface of the percentage deviation of apparent value of $\ln RR$ from its true value, where $\ln RR$ ranges from -2.3 to 2.3 (the range of α from 0.1 to 10), and β ranges from 0.05 to 1. An asymmetric bias in measuring plant–plant interactions is evident. For example, consider $\beta = 1$ (when the mass increment is comparable with the initial mass due to slow growth under severe conditions, or to the short term of the experiment). Then, at $\ln RR = 2.3$

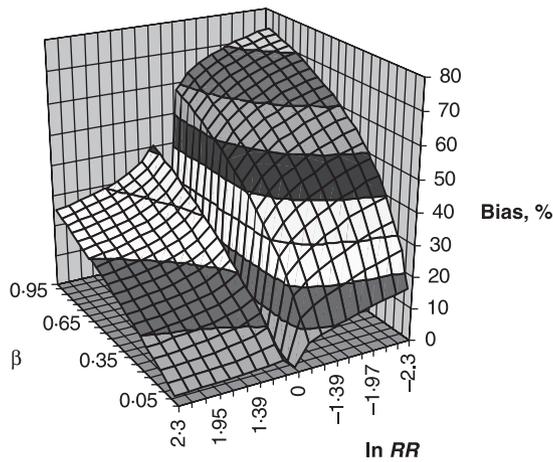


Fig. 1. Bias in measurements of $\ln RR$ depends on its true value, and on growth response as measured by β (= ratio of initial and accumulated biomasses). Bias is calculated as the percentage deviation of apparent value from true value of $\ln RR$.

(when $\alpha = 10$, corresponding to 10 \times increased growth of treated plants compared with controls), the apparent value of $\ln RR$ becomes 26% lower than its true value. However, at $\ln RR = -2.3$ (corresponding to $\alpha = 0.1$, or 10 \times reduced growth of treated plants compared with controls), the bias increases dramatically to 80%. The point where apparent and true values are equal occurs only when $\ln RR = 0$ (no interaction: $\alpha = 1$, equations 3 and 4). But, if initial biomass is used in calculations (as in equation 3), this bias disappears completely.

The above analyses show that ignoring initial biomass causes directional bias when treated plants grow more slowly than controls (when $\alpha < 1$). Such situations are expected in popular experimental designs for measuring plant–plant interactions. For example, in competition experiments conducted in greenhouses, researchers usually take isolated plants as controls to compare them with plants growing with competitors (Reynolds 1999). Because control plants will accumulate more biomass in the absence of competitors, we will always have $\alpha < 1$ (Fig. 1, negative values of $\ln RR$). If such experiments are conducted on a productivity gradient, ignoring initial biomass will corrupt the real trend because β will be greater under less productive conditions (equation 4).

Another example is the neighbourhood approach to multi-species interactions: comparing the performance of target individuals in the presence vs the absence of neighbouring vegetation. These experiments test both positive (facilitation) and negative (competition) neighbour effects on plants. In contrast to the previous example, researchers usually remove neighbouring vegetation around target individuals and consider them as treated to compare with control individuals with intact neighbours (Reynolds 1999). If plant–plant interactions are facilitative, as often happen in harsh and unproductive environments, removing neighbours will reduce growth in treated plants compared with

controls. Again, we will have $\alpha < 1$ and negative values of $\ln RR$ at higher values of β , in the most biased conditions (Fig. 1). This bias will corrupt the real trends of plant–plant interactions along environmental gradients: when initial biomass is ignored, strong facilitative effects will be considerably harder to see than similarly strong competitive interactions. $\ln RR$ is often recommended due to its convenience for meta-analysis (Gurevitch *et al.* 1992). However, when it is applied to unproductive conditions using only final biomass, the results of such analyses will be questionable. It is worth repeating that if initial biomass is used in calculations (equation 3) this bias disappears completely.

We also analysed other recommended indices of competition and facilitation such as *RNE* (Markham & Chanway 1996) and *RII* (Armas *et al.* 2004), which are mathematically related to $\ln RR$ (Goldberg *et al.* 1999; Armas *et al.* 2004). Not surprisingly, the analyses revealed a very similar bias related to the initial biomass (not shown).

We conclude that initial biomass should be determined and used to measure plant performance whenever possible. Sometimes direct determination of initial biomass may be difficult, for example when dry mass is used as a measure. Nonetheless, an acceptable precision can usually be achieved if, prior to the experiment, a sufficiently large set of young plants is selected and divided into three subsets: one for treatment; another as control; and the third harvested immediately to assess initial biomass. However, in some cases determination of initial mass may be impossible, for example in re-analyses of old data. In such cases the use of some mathematical approaches (always take plants that grow better as treated) can remove directional bias in a range of plant–plant interactions (see also Armas *et al.* 2004 for details). However, even if directional bias is avoided, ignoring initial biomass can still reduce the probability of discovering any effect at all, and this excessive conservatism should be taken into consideration.

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