

On database biases and hypothesis testing with dilution experiments: Response to comment by Latasa

The comment by Latasa (2014) highlights an important point for the compilation and analysis of data on phytoplankton growth and microzooplankton grazing rates by the dilution technique—the desirability of reporting and utilizing all data. This is a useful and refreshing perspective given statements made elsewhere that dilution results with insignificant regression slopes (i.e., nonsignificant grazing) are uninterpretable or indicative of “failed” experiments (Dolan and McKeon 2005). Such thinking may have contributed to an emphasis on only the significantly negative slopes (i.e., significantly positive grazing rate estimates) in many papers, which underlies the underreporting issues discussed by Latasa (2014). As illustrated well for a small subset of published dilution studies, but hopefully obvious for data of any kind, the selective use or reporting of only higher rate estimates can substantially bias interpretations of natural trends and relationships. We agree on this key point, among others.

One issue that merits additional comment is the noted possibility for increased reporting bias for experiments run in low-temperature polar systems. While it is quite true that regression slopes are more difficult to distinguish statistically from zero when rates are inherently low, experimentalists can readily compensate by running their incubations proportionately longer, 2 or 3 d instead of 1 d, to amplify the differences in net biomass change that occur in the different dilution treatments (Landry et al. 2002; Sherr et al. 2009). This assumes that containment artifacts scale with temperature effects on metabolism or growth, such that they would be no worse after longer incubations of polar samples compared with 1 d experiments in warm-water systems. A practical suggestion would therefore be to encourage longer duration experiments in polar regions to achieve statistically comparable separation of dilution effects to those in other environments.

Besides low temperature, low grazing mortality estimates can arise in dilution results for various reasons. In oligotrophic subtropical regions, for example, low rates may reflect threshold feeding at low prey biomass (Lessard and Murrell 1998) or natural cyclical dynamics of predators and prey (Calbet et al. 2001). In richer coastal waters, grazing estimates (i.e., regression slopes) can be artificially low due to saturated feeding (Gallegos 1989). Additionally, low and even negative rate estimates can be expected to occur just by chance because they are calculated from imperfect measurements. Viewed in this way, negative grazing rate estimates are, in fact, statistically possible and might be considered a valid experimental outcome under some circumstances. For example, Landry et al. (2011b) included negative estimates in the mean grazing rate calculations assuming that they counterbalanced other experiments in which the rates may have been overestimated.

Nonetheless, there are circumstances that arise in which the practicalities of data handling may require some

manipulation. Latasa (2014) notes, for example, that analysis of the global dilution database by Calbet and Landry (2004) involved modifying negative grazing rate estimates to the lowest realistic values of zero. Although this comment was brought up mainly to illustrate that such a manipulation produces less of a data bias compared with leaving out the negative rate estimates entirely, a more thorough analysis of effect was lacking, leaving the impression of a still very significant bias. Two additional points need to be mentioned in this regard. The total number of data manipulated was relatively small. The seven studies listed in Latasa’s (2014) table 1 comprise, in fact, 40% of the data manipulated by Calbet and Landry (2004), but only 8% of the total experimental database. That is, they are among the more problematic of the studies and not representative of the general data quality; the total corrected grazing data is only ~ 2%. Calbet and Landry (2004) also did parallel corrections of growth rates, changing zero and negative values to a small positive number (0.01 d^{-1}). As explained in the original paper, these changes, again to a small subset of data, were done to avoid negative values and division by zero in the calculated rate ratios of grazing: growth ($g:\mu$), the proportion of phytoplankton production consumed by microzooplankton, which was the emphasis of the Calbet and Landry (2004) study. The net effect of these modest and largely compensatory manipulations was in the third decimal place—grazing as a fraction of phytoplankton growth was 0.1% lower in the corrected dataset relative to the raw values. This effect is much smaller and in the opposite direction of the bias that could be presumed from the overestimation in grazing rates noted by Latasa (2014). The conclusion that one should draw from this analysis is that bias discussions need to consider the full data set and the broader context of all manipulations made, not just to one parameter of interest, in order to quantify effect. As demonstrated by Latasa (2014), the alternative strategy of simply ignoring the questionable data would have led to more substantial overestimates of growth and grazing rates.

It is certainly the case that the null hypothesis $b > 0$ suggested by Latasa (2014) provides a more appropriate and rigorous framework for examining low grazing rate results in dilution databases than the presently used $H_0: b = 0$. This is an important advancement, but it begs the questions of whether it is necessary that each dilution experiment have its own hypothesis test or whether data quality can be adequately assessed from regression statistics alone. There are many subtle ways in which the results of dilution experiments may be biased or misleading. For example, most growth rate estimates based on measured changes in chlorophyll *a* are uncorrected for incubation effects on cellular pigment content due to photoacclimation or trace metal contamination (Selph et al. 2011). Such data, in isolation of other measured processes, might therefore

not shed useful light on natural rates and relationships, regardless how good the regression statistics. On a more promising note, however, some dilution results, done in large enough numbers to average over many experiments, have been shown to mesh well with complementary and independent measurements of primary production, net ambient phytoplankton growth, mesozooplankton grazing, and export flux in describing how natural systems work and in testing explicit hypotheses about process interactions and relationships (Landry et al. 2009, 2011a; Stukel et al. 2011). It seems to me that this is the level of hypothesis testing to which all process-estimating tools need to be applied in aquatic field studies. Thus, I would add to the excellent statistical point made by Latasa (2014) that advancing our understanding of natural system dynamics requires that we think beyond the performance of individual experiments viewed in isolation and evaluate the quality of results against independently measured variables that can serve as system-level constraints on growth and grazing estimates.

In summary, this is not a rebuttal of Latasa (2014), but rather a note of concord. We may depart on some minor issues of interpretation and approach; however, we agree on the central point that low grazing rate estimates (statistically indistinguishable from zero) contribute meaningful data to understanding process rate variability within and among regions of the oceans and need to be reported in original studies and integrated without bias in data analyses.

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