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Rejoinder to: "Interpreting the results of oceanic mesoscale enrichment experiments: Caveats and lessons from limnology and coastal ecology"

Hale and Rivkin (2006; hereafter HR) provide a critique of the statistical treatment of our results (Oliver et al. 2004; hereafter O2004 as in HR) from the Southern Ocean Iron Experiment (SOFeX) study and question our conclusion that differences in bacterial properties between iron-enriched ("fertilized") and non-iron-enriched ("unfertilized") water masses were due to the iron addition. HR made the important point that iron enrichment studies have not been properly replicated. As a result, appropriate statistical analyses cannot be performed, and statistically rigorous inferences about responses and causative factors cannot be made. They also noted that few studies in the history of open-ocean iron fertilization experiments have addressed the issue of replication—or rather, the lack thereof. HR singled out our paper and that by Arrieta et al. (2004) for criticism because, unlike the vast majority of the iron enrichment literature, we actually performed statistical analyses of our results—just not the appropriate ones, according to HR. HR's critique of our analyses suggests some other analytical approaches and experimental and sampling designs that might be used in future studies.

HR point out that time-series measurements, as performed in a fertilized water mass ("patch"), may not be independent of each other, and should not be treated as replicates in a statistical analysis. This is especially important when considering oceanographic patches tagged with geochemical tracers and followed in the Lagrangian frame of reference. Such patches will exhibit some degree of biogeochemical and physical continuity or integrity over time. Whether the surrounding "control" patches exhibit similar coherence, and are subject to the same caveats about statistical replication, is an interesting question not explored by HR. It is likely, for example, that the tagged patch will flow past several distinct patches with different properties. Measurements within each of the distinct, nonexperimental patches would be independent. In the future, mesoscale enrichment studies should perhaps tag both the (replicated!) fertilized and unfertilized (control) patches, to provide better experimental controls on the treatment effects. But the logistics of following and sampling them would be daunting and at present are not practicable, given the overwhelming limitations in conducting open-ocean field work at this scale.

HR used ANCOVA to reanalyze our time-series results and concluded that "ANCOVA results for bacterial properties during SOFeX were generally similar to those reported by O2004 using two-sample *t*-tests." Next, they asked three questions about our results, "considering the following in turn: (1) whether the magnitude of changes in bacterial properties were ecologically meaningful; (2) whether the changes observed were in the direction predicted by the theory; and (3) whether it was likely that the addition of iron, as opposed to other factors, caused the response." They concluded that items (1) and (2) could be answered affirmatively. Item (3) was addressed by analyzing for possible differences in the physical environment of the "in" and "out" (fertilized and unfertilized) patches in order to determine whether factors other than iron addition could have caused the statistically significant differences between patches that we reported. They concluded that their analysis "showed no significant differences in the temporal changes in temperature and salinity inside and outside the patch.…Therefore, the increase in bacterial production inside the iron-enriched patch during SOFeX was probably due to factors other than differences in water column structure between the two water masses." Despite this affirmation, they argued that we could not definitively conclude that differences in bacterial production were linked to the availability of iron and that our conclusion was inferred and "not based on statistical analyses of the treatment effects."

HR's argument is carefully crafted in narrow statistical terms; however, we note here that they ignored what we considered to be our most important finding. We found that there was a significant positive regression between primary and bacterial production rates (PP and BP) within the fertilized patch (figs. 3 and 4 in O2004), but we found no relationship between these processes outside the patch. Significant regressions were obtained for several different size fractions of PP, as well as total PP. In fact, the tight correlation between BP and PP is one of the best we have seen reported from any study. Significant regressions between these two (presumably) coupled rates are often established at large scales, as in compilations of data from many different studies (e.g., Cole at al. 1988). But it is much rarer to establish them at the time and space scales pertinent to specifically mechanistic biological couplings among bacteria and phytoplankton (Ducklow 1984)—for example, the scale of an iron-enriched patch. It was this surprising finding that enabled us to suggest that bacterial production in the iron-enriched patch was organic carbon limited. The tacit implication is that phytoplankton responded to iron fertilization with increased photosynthetic rates and increased biomass, thereby making increased organic matter available to bacteria. The bacteria in turn responded to the organic matter. HR questioned this conclusion because of a lack of data of dissolved organic carbon or nitrogen. But the newly produced dissolved organic matter (DOM) would likely be removed quickly. That is why the BP and PP relationship is so important. We also provided arguments as to why we believed the bacteria were neither iron nor macronutrient limited.

We concede HR's point that direct or indirect bacterial (or phytoplankton) responses to the iron addition cannot be statistically established, and iron enrichment cannot be rigorously identified as the causative factor in any of the many studies that have appeared in the literature. But we stand by our conclusion that the bacteria did have enhanced production rates within the enriched SOFeX patch and concede that the response was either directly (i.e., if iron limited) or indirectly (i.e., if organic carbon limited) due to iron fertilization. Our findings are consistent with previous reports of bacterial responses to iron enrichment (Cochlan 2001; Hall and Safi 2001), including Arrieta et al. (2004). Although the lack of replication in open-ocean mesoscale iron fertilization may preclude making categorical statements on cause-and-effect responses to iron, reasonable inferences can still be made.

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