

Comment on “Nature of Phosphorus Limitation in the Ultraoligotrophic Eastern Mediterranean”

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Thingstad *et al.* (Reports, 12 August 2005, p. 1068) reported that in situ mesoscale phosphorus enrichment of the eastern Mediterranean Sea altered selected biological parameters and concluded that the added phosphorus was rapidly transferred from bacteria to mesozooplankton. However, because of a lack of replication and a misinterpretation of their statistical analyses, that conclusion is not supported by the data.

Thingstad *et al.* (1) reported that phosphate addition to surface waters of the phosphorus-starved eastern Mediterranean caused unexpected ecosystem responses and proposed that added P was rapidly transferred from bacteria to metazoan grazers, based on reported increases in copepod egg abundance and ciliate biomass. However, because very few data ($n = 1$ and $n = 3$, for copepod egg and ciliate abundances, respectively) were collected from unenriched waters, either before P addition or from outside the enriched patch, the authors are not able to determine whether differences between P-enriched and unenriched waters reflected natural or sampling variability, or were a response to P addition. The authors then proposed two non-mutually exclusive pathways of P transfer through the microbial food web to copepods: (i) “trophic bypass” of the phytoplankton compartment by heterotrophic bacterial uptake of P and (ii) “trophic tunneling,” through luxury consumption of P by phytoplankton and bacteria. Here, we critically examine the supporting evidence for the two pathways.

Thingstad *et al.* (1) used analysis of variance (ANOVA) to test for significant differences in ecosystem properties in P-enriched and unenriched waters. The authors concluded that chlorophyll *a* (chl *a*) and bacterial production (BP) inside the patch were significantly lower and higher, respectively, than in unenriched waters ($P < 0.05$). They attributed these differences to the direct or indirect effects of the P addition and proposed a trophic bypass. However, ANOVA tests for treatment effects by comparing the variability between treatment groups with the variability between experimen-

tal units within each group (2). The variability between experimental units can only be estimated if (i) the treatment is replicated and (ii) the replicates are independent, such that the outcome of a given replicate has no effect on the outcomes of any other replicates (3). By repeatedly sampling a single P-enriched patch at different depths, through time, and incorrectly treating the samples as if they were independent replicates in the ANOVA, Thingstad *et al.* (1) confounded the effects of the P addition with the inherent differences between the P-enriched and unenriched waters (4–6). Thus, significant differences cannot be unequivocally attributed to the effects of the P enrichment.

We reanalyzed the published data using analysis of covariance (ANCOVA) to assess whether temporal changes in chl *a* and BP were significantly different in P-enriched and unenriched waters. We have used this approach to examine the effects of mesoscale iron enrichment on bacterial properties. ANCOVA allowed the explicit incorporation of time into the statistical model by including “sampling day” as the covariate and “patch” (i.e., enriched or unenriched) as the fixed factor. This approach avoids the problems associated with initial differences in enriched and unenriched waters at this site.

To avoid potential confounding influences from samples that were collected at different depths on the same day, we used the means of all measurements in the upper 20 m for each day, as presented in figure 2 in (1). Where necessary, the data for each parameter were transformed (log or square root) so that the data showed a linear relationship with time and the residuals were normally distributed (2). A significant interaction between sampling day and patch indicates a significant difference in the temporal change of a parameter in P-enriched and unenriched waters. However, a significant difference cannot be attributed to the

effects of the P addition. Based on ANCOVA, there were no significant differences in the temporal trends of chl *a* ($F_{1,14} = 0.039$, $P = 0.847$) or BP ($F_{1,9} = 0.249$, $P = 0.630$) in the P-enriched patch compared with unenriched waters. Thus, the trophic bypass proposed by Thingstad *et al.* (1) is not supported by the data.

Thingstad *et al.* (1) also proposed a trophic tunneling pathway that was based on an increase in particulate P inside the patch, concurrent with a decrease in the maximum potential for orthophosphate uptake (V^{\max}). However, ANCOVA identified no significant differences in the temporal trends of particulate P or V^{\max} in P-enriched and unenriched waters ($F_{1,12} = 0.003$, $P = 0.960$; and $F_{1,9} = 0.010$, $P = 0.924$, respectively). In the absence of significant luxury P consumption by phytoplankton and bacteria, support for a trophic tunneling pathway is weak.

Replication of experimental units with randomly assigned treatments is not always feasible in large-scale experiments. However, it is crucial to recognize that in the absence of replication, significant differences between treatment groups cannot be unequivocally attributed to the effect of the treatment. Instead, the question of causality should be addressed by examining (i) whether the magnitude of changes in the parameter of interest is ecologically meaningful, (ii) whether the differences observed are in the direction predicted by the theory, and (iii) the likelihood that the treatment, as opposed to other factors, caused the response (6). Future mesoscale enrichment experiments should use experimental designs and statistical methods, such as the before-after-control-impact-pairs design (BACIP) (7, 8), which allow treatment effects to be tested.

References and Notes

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