Biogeosciences Discussions, 2, S633–S635, 2005
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European Geosciences Union

Biogeosciences Discussions

Interactive comment on "Effect of incubation time and substrate concentration on N-uptake rates by phytoplankton in the Bay of Bengal" *by* S. Kumar et al.

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Received and published: 17 October 2005

This manuscript describes 3 experiments which were performed to investigate the uptake of nitrogen species nitrate, ammonium and urea using 15N tracers in the Bay of Bengal during September and October 2002. This study is reported by the authors to be the first of its kind in the Bay of Bengal, it therefore is of interest because of this. However, I have reviewed this manuscript previously and unfortunately it remains my opinion that the design and implementation of this work was not sufficiently rigorous to justify publication. The main overlying reasons for this, are the absence of measured ammonium and urea concentrations, and the lack of experimental replication. Because of this, the conclusions are drawn from rather tenuous evidence and are statistically questionable.

Ammonium & Urea concentration.

In order to perform these experiments there is a fundamental requirement to know accurately the concentration of dissolved nutrient, so that tracer additions can be made at ˜10% of ambient concentration and to determine the level of dissolved isotope enrichment for within the rate equation. Ammonium and urea concentrations were not determined during this study, but were derived from several speculative assumptions and associations based on seasonally averaged zooplankton biomass. I disagree with the authors statement that “regeneration of ammonium and urea by zooplankton is well known”, but even if it were true, the balance between regeneration rate and concentration is not straightforward. The authors recognise the uncertainty in their estimates by stating “Considering, the uncertainties involved in equations used for the calculation, the above values could well be near zero.” The rate of uptake of inorganic nitrogen is known to be concentration dependent and largely follows saturation kinetics, the recommendation to add tracer at 10% is therefore made so as not to stimulate uptake over and above natural rates. In experiment 1, all 3 tracers were added at 0.01 μmol l-1, this procedure is not ideal, but can be acceptable if full consideration is made during data interpretation, of the potential stimulation of uptake caused by over-addition of tracer - this was not done. In this case, estimates of NH4 and urea concentration were not provided, but, if they were similar to experiment 3 the additions were made at 12.5%, 73% and 277% for NO3, NH4 and urea respectively. In experiment 3, if the estimated concentrations of NH4 and urea were correct then additions were made at 73% and 833% respectively. It is little wonder that urea was the “preferred” nutrient. The time-series information from experiment 1 is interesting, but difficult to interpret, changes in NH4 and urea uptake may be due to substrate exhaustion but equally, the observations may not be statistically significant as no experimental replication was performed.

Lack of statistical evidence.

Conclusions are drawn in each case from a single experiment, and during each experiment no replication was performed - each data point is the result from a single

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incubated bottle. In Figures 1, 2 & 3 error bars of one SD are presented for each variable, however these appear to be derived from duplicate mass spectrometer analyses and as such provide information (which would not be statistically viable) on the analytical precision but not on sample variability.

Experiment 3.

Several problems are associated with experiment 3, as stated above, additions of NH4 and urea were likely to be far in excess of the recommended 10% of ambient; further to this are the conditions of incubation and the comparison with carbon fixation rates. The authors suggest that on-deck tanks return more realistic results relative to in-situ incubations, not only is this counter intuitive: in-situ incubations are at “real” conditions of light and temperature, whilst on-deck incubations are susceptible to anomalous conditions of light and temperature. These effects are exacerbated during this study as samples collected at 20, 40, and 60m were then incubated at light conditions equivalent to 41, 55 and 77m respectively. The conversion of nitrogen uptake rates to carbon through the Redfield ratio of 6.6 is not always appropriate as N-uptake and C-fixation mechanisms are de-coupled from each other, so that the C:N ratio for biomass and uptake rates are not always comparable, as recognised in the text. Because of the combined errors associated with the over addition of tracer and incubation under an unrealistic light regime, the agreement between on-deck nitrogen uptake and in-situ primary production is more than likely a circumstance of coincidence.

Interactive comment on Biogeosciences Discussions, 2, 1331, 2005.