

## ***Interactive comment on “Spatial variability in iron nutritional status of large diatoms in the Sea of Okhotsk with special reference to the Amur River discharge” by K. Suzuki et al.***

**Anonymous Referee #2**

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In this paper the authors present the analysis of the ferredoxin to flavodoxin in relation to a size-fractionated isolation of phytoplankton for the waters in the Sea of Okhotsk. The authors present a though data set and attempt to relate the Fd/Fld ratio to the nutrient status of the phytoplankton - potentially suggesting it as a tool to monitor the nutrient status of diatoms in the field. Comments: The relationship between available Fe and dissolved Fe (measured in the paper) is complex to define - hence there are always issues in relating dFe to nutrient status. In addition a paper by Ryan-Keogh et al 2013 (L&O) demonstrates how the iron-stress is often only apparent when nitrate is also available in the system - i.e if both Nitrate and Fe are low the system may reduce biomass without showing significant iron-stress. This should be discussed in this paper.

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Much weight is given to the Fd/Fld ratio - not only to other phytoplankton species display this switch - but we should consider that there will be a mixed response of different diatoms to this ratio as well. A genetic analysis of Fd and Fld gene diversity in the communities would be of benefit in this study and may help explain situations where the community does not respond as predicted. Section 4.1 the relationship between dFe and Fd index is presented as being a possible marker for in situ Fe stress. I feel this comment is a little strong – there is a high range (from 0.2-0.6 in Fd index with little variation in dFe) i.e there are many different Fd index's at the same dFe concentration. This should be addressed. Figure 12 should perhaps lead to a discussion on if Fd index is better than Fv/Fm as a marker of Fe-stress. Potentially both require the artificial addition of Fe in bottle experiments to demonstrate they both increase with increasing available Fe to fully interpret these indices in complex systems? Specific comments section 2.3 - error in describing fractionation of phytoplankton Figure 2 should refer to Table 1 not Table 2 section 2.3 - What is the recovery of protein in your samples? This should be reported - are there stations where recovery is lower - does this effect the sensitivity of blotting. What evidence is there that the antibody reacts to all Fd/Fld in all diatoms species and does not cross react with other species (an example blot would be useful?)

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