Vertical migration as a mechanism to assimilate iron by the HAB dinoflagellate *Akashiwo sanguinea*

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Abstract

Prediction of harmful algal bloom (HAB) forming dinoflagellate events could be improved by better understanding vertical migration responses to nutrient limitation. Dinoflagellates have been documented vertically migrating to assimilate Nitrogen (N) and Phosphorus (P). During blooms surface waters are often depleted of both macro- and micronutrients. We hypothesize that the motile HAB forming dinoflagellate, *Akashiwo sanguinea*, from Monterey Bay, California will migrate for Iron (Fe) as well as N and P when nutrient limited. Experiments were conducted in a vertical migration chamber strongly stratified for both temperature and salinity. ^{57}Fe and ^{15}N were added below the pycnocline and measured in the particulate fraction by ICP-MS and MS. Chlorophyll measurements and observed depletion of inorganic nutrients at depth provides evidence for strong vertical migration. Particulate surface and bottom sampling indicates uptake of both ^{57}Fe and ^{15}N from depth. Dinoflagellate blooms are frequently preceded by Fe-limitation in surface water, and we propose that understanding the mechanism(s) for incorporating Fe will enhance our understanding of HAB dynamics.

Methods

- *Akashiwo sanguinea* isolated from Monterey Bay, California was grown in CCMP L1 media for 3 weeks and then reverse filtered into nutrient replete media with the addition of 50 nM desferoximine-B (an iron chelator) for 36 hours prior to experiment start.
- The pycnocline was established with temperature and salinity gradients, and kept stratified by the addition of an ice bath surrounding the bottom 5 liters.
- Nutrient additions were added as described (Table 1).
- Chlorophyll-a and inorganic nutrients (N, P) were sampled from 7 sample ports hourly (hours 0 -24) and at 48 and 72 hours. Samples were analyzed using typical methods.
- Cyclops-7 Turner Fluorometer was used to determine raw fluorescence units to establish vertical migration.
- Filtered Fe samples were sampled at hours 0, 6, 12, 18, 24, 48, and 72 hours of the experiment and digested for leachable particulate Fe (bio-available). They were processed by ICP-MS for ^{57}Fe and ^{56}Fe concentrations.
- Filtered ^{15}N samples were sampled at hours 0, 6, 12, 18, 24, 48, and 72 hours and analyzed on a CE Instruments NC25000 elemental analyzer using Dumas combustion coupled to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer.

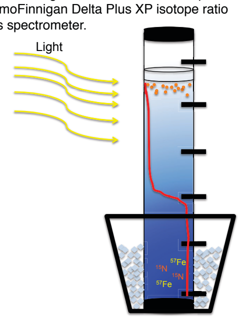

Fig. 1 Schematic of vertical migration chamber. The red line is a depiction of density, orange markers are dinoflagellates.

Objectives

- Determine vertical migration of *Akashiwo sanguinea* in a laboratory environment
- Establish if there is uptake of ^{15}N and ^{57}Fe at depth
- Determine enrichment of *Akashiwo sanguinea* for both ^{15}N and ^{57}Fe

Results

Parameters	Nutrient concentration					
	Start			End		
	Bottom (0 - 0.5m)	Middle (0.5 – 1.2 m)	Top (1.2-1.4m)	Bottom (0 - 0.5m)	Middle (0.5 - 1.2m)	Top (1.2-1.4m)
Temp (°C)	6	13	15	8	15	15
Salinity	32	29.5	29	31.5	29.5	29.5
P_i (uM)	2.8	3.2	4.3	3.7	3.9	4.3
N_i (uM)	22.0 (^{15}N)	1.3 (^{14}N)	58.0 (^{14}N)	20.4	40.4	45.4
Fe (nM)	7.75 (^{56}Fe)/ 10.0 (^{57}Fe)	-	-	-	-	-
Desferal (nM)	0.0	10.0	50.0	-	-	-

Table 1. The water column was kept vertically stratified by the continual replenishment of an ice bath surrounding the bottom 0.5m of the migration chamber. Desferal was introduced to the middle portion of the chamber prior to the addition of *Akashiwo sanguinea* to bind any bio-available Fe not introduced to the bottom 0.5m of the chamber. Drawdown of N_i and P_i was seen hours 12 – 24 of the experiment from the bottom 0.5m and from the surface from hours 4 – 72. From hours 24 – 72 there was a slight increase in N_i and P_i at depth.

^{57}Fe Uptake

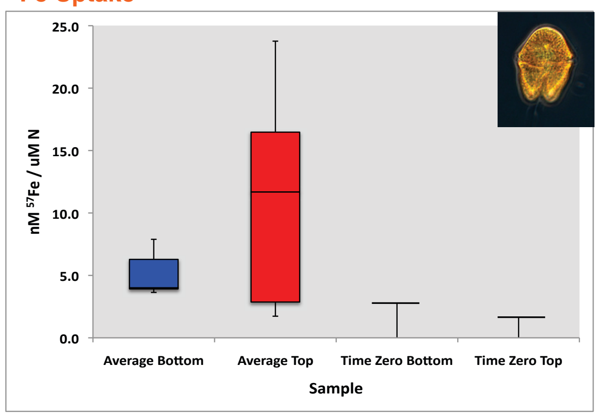

Fig. 2. Enrichment of ^{57}Fe shown in both the samples from the bottom (^{57}Fe replete) and top (^{57}Fe deplete) particulate data. Bottom samples are blue and top samples are red. All ^{57}Fe samples are normalized to μM of particulate N. Averaged samples include time points after the time zero (initial sample).

Nitrogen

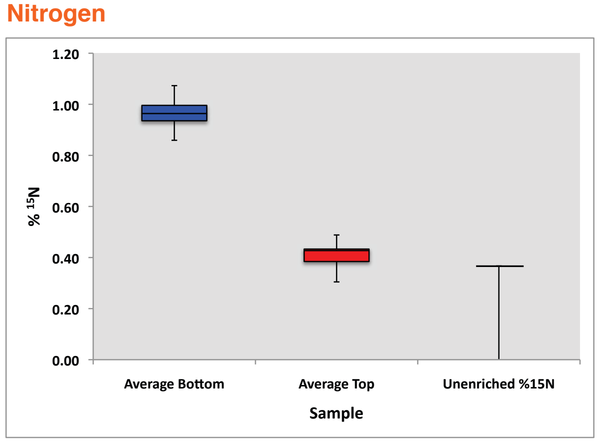

Fig. 3. Enrichment of ^{15}N shown in both the samples from the bottom (^{15}N replete) and the top (^{15}N deplete) particulate data. Bottom samples are blue, and top samples are red. ^{15}N samples are expressed in % ^{15}N. Averaged samples from the bottom and the top are compared to the known % ^{15}N value of 0.367.

Chlorophyll-a

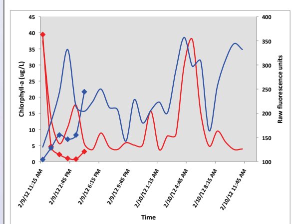

Fig. 4. Vertical migration of *A. sanguinea*. Chlorophyll-a (diamonds) at the surface (red) quickly migrates to the bottom (blue). Migration back to the surface at hour 16 is apparent from raw fluorescence provided by a Cyclops-7 fluorometer (red line) as is the migration to the bottom at hour 5 (blue line). After the initial migration chlorophyll-a was spread throughout the vertical migration chamber, with elevated concentrations in the bottom 0.5m.

Conclusions

- *Akashiwo sanguinea* migrated quickly to the ^{15}N and ^{57}Fe replete bottom water.
- Uptake of both ^{15}N and ^{57}Fe are apparent in the enrichment in the both the bottom and top samples.
- Enrichment is greater in the bottom samples compared to the top for ^{15}N, but there is obvious enrichment in the top samples, reinforcing the interpretation of migration of cells from the bottom back to the top.
- Enrichment for iron is much greater in the top compared to the bottom suggesting active uptake (rather than passive adsorption).
- Our results provide the first direct evidence for hypothesized micronutrient (Fe) uptake from depth in a vertically stratified water column. The greater assimilation of Fe compared to N suggests that *A. sanguinea* will vertically migrate for Fe in the presence of elevated surface N.

References

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Acknowledgements

We acknowledge Dyke Andreason and Chih-Ting Hsiegh for the analysis of ^{15}N samples, Dondra Biller for help with the ICP-MS and Rob Franks for the use of his analytical facilities. Funding was provided by NSF grant OCE-0726858.