Nitrogen Utilization by the Raphidophyte
*Heterosigma akashiwo*: Growth and Uptake kinetics in Unialgal Cultures and Natural Assemblages of San Francisco Bay

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Heterosigma akashiwo (Hada) Sournia

Phylum: Ochrophyta

Class: Raphidophyceae

- cells are 8-25 µm long
  6-15 µm wide
  8-10 µm thick
- variable number of chloroplasts (5-95)
- bi-flagellate
- ‘wall-less’, but are covered with a glycocalyx

25 µm
Presentation Outline

• Growth on the Various N substrates (nitrate, ammonium, and urea)

• Kinetics of N Uptake in unialgal cultures

• Nitrogen Uptake and Preference in San Francisco Bay blooms

• Nitrogen Substrate Availability in San Francisco Bay
Heterosigma akashiwo growth curves

Strain (CCMP 1912) isolated from Kalaloch, WA (R. Horner)

Semi-continuous batch cultures grown in 50 μmol·N·L⁻¹ nitrate, ammonium or urea ESAW at 110 μE·m⁻²·s⁻¹ in 50 cc PYREX® culture tubes (n=3) at 15°C.

lag phase

exponential

stationary phase

Used for Estimation of Growth Rates
*Heterosigma akashiwo* growth rates as a function of light and nitrogen source

No statistical* difference between N sources

Symbols denote means, error bars are the range of replicates (n=3), growth rates determined at 15°C.
**Heterosigma akashiwo** growth rates as a function of light and nitrogen source

- **NH₄⁺** statistically faster

Symbols denote means, error bars are the range of replicates (n=3), growth rates determined at 15°C.
Michaelis-Menten formulation for Nitrogen Uptake Kinetics
Dugdale (1967); Maclsaac and Dugdale (1969)

\[ V = V_{\text{max}} \cdot \frac{S}{(K_s + S)} \]

- \( V_{\text{max}} = \) maximum uptake rate
- \( K_s = \) half-saturation constant
- \( \alpha = \) initial slope, substrate affinity at low S (Healey, 1980; Cochlan and Harrison, 1991)
Two exponentially growing cultures of *H. akashiwo* - recently depleted of nitrate - replicate (n=18) sub-samples into 50-mL tubes.
For: Nitrate, Ammonium and Urea: duplicate incubations at all concentrations (0.1-12.0 μmol N·L⁻¹)

- Short (10 min incubations)
- Filtration of cells (PN) onto 5.0 μm Ag filters
- ¹⁵N/¹⁴N of PN (cells) determined by mass spectrometry
- PN Specific Uptake Rates re. Dugdale & Wilkerson (1986)

Nitrate and Ammonium uptake kinetics, (but not urea): were conducted on duplicate cultures separated by 4 days
$r^2 = 0.92$

**A: Nitrate**

$V_{\text{NH}_4} > V_{\text{NO}_3} > V_{\text{urea}}$

- $V_{\text{max}} = 18.0 \times 10^{-3} \text{ h}^{-1}$
  - (std. error = 2.28)
- $K_s = 1.47 \mu\text{mol N L}^{-1}$
  - (std. error = 0.25)
- $\alpha_N = 12.2$

$\checkmark$

$V_{\text{max}} = 28.0 \times 10^{-3} \text{ h}^{-1}$
  - (std. error = 2.17)
$K_s = 1.44 \mu\text{mol N L}^{-1}$
  - (std. error = 0.35)
$\alpha_N = 19.4$

$\checkmark$

$V_{\text{max}} = 2.89 \times 10^{-3} \text{ h}^{-1}$
  - (std. error = 0.24)
$K_s = 0.42 \mu\text{mol N L}^{-1}$
  - (std. error = 0.25)
$\alpha_N = 6.87$

$\checkmark$
4 very dense, widespread, episodic blooms in 2002
( > 300,000 cells/mL; > 500 μg/L of Chl a )

Light microscopy identification

Independent confirmation (Dr. R. Horner)

Molecular probe identification (Dr. C. Scholin)
Richardson Bay Nitrogen Utilization

\( ^{15}N\)-tracer methods

**IN BLOOM**
- NH\(_4\) : 0.39 \(\mu\)M
- Urea: 0.31 \(\mu\)M
- NO\(_3\) : 13.69 \(\mu\)M

**NON-BLOOM**
- NH\(_4\) : 4.14 \(\mu\)M
- Urea: 1.01 \(\mu\)M
- NO\(_3\) : 15.65 \(\mu\)M

Indicative of *in situ* utilization

Substantial Difference in Nitrogenous Nutrition
- 74% of bloom N uptake = Nitrate
- 29% of non-bloom N uptake = Nitrate
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$^{15}$N-tracer methods

6/27/02 Inside H. akashiwo patch

Indicative of N Preference

Bloom N preference for ammonium or nitrate $>>$ urea

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Indicative of N Preference

Bloom N preference for ammonium or nitrate $>>$ urea
Ammonium is found at equal or greater concentrations than nitrate, whereas [urea] is generally lower, averaging 25% of NH$_4$.
**H. akashiwo** Conclusions

1. Under saturating light conditions, *H. akashiwo* cultures grow faster on ammonium (statistically significant). At sub-saturating light, there is no difference (statistically*) in growth rate for nitrate, ammonium or urea.

2. Maximum uptake rates (preference) and substrate affinity ($\alpha$) values of N-sufficient *H. akashiwo* cultures were:

   ammonium $>$ nitrate $>$ urea.

3. Natural *H. akashiwo* blooms in San Francisco Bay during 2002 were fueled primarily by nitrate. Ammonium and urea were utilized first or simultaneously with nitrate (based on trace additions).

4. Nitrogen Preference in SF Bay was ammonium $>$ nitrate $>$ urea (based on saturating additions).

5. SF Bay is replete with both inorganic and organic N sources, with [ammonium] equal or greater than [nitrate], and substantial urea.
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