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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PICES HAB Section
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Heterosigma akashiwo (Hada) Sournia

Phylum: Ochrophyta

Class: Raphidophyceae

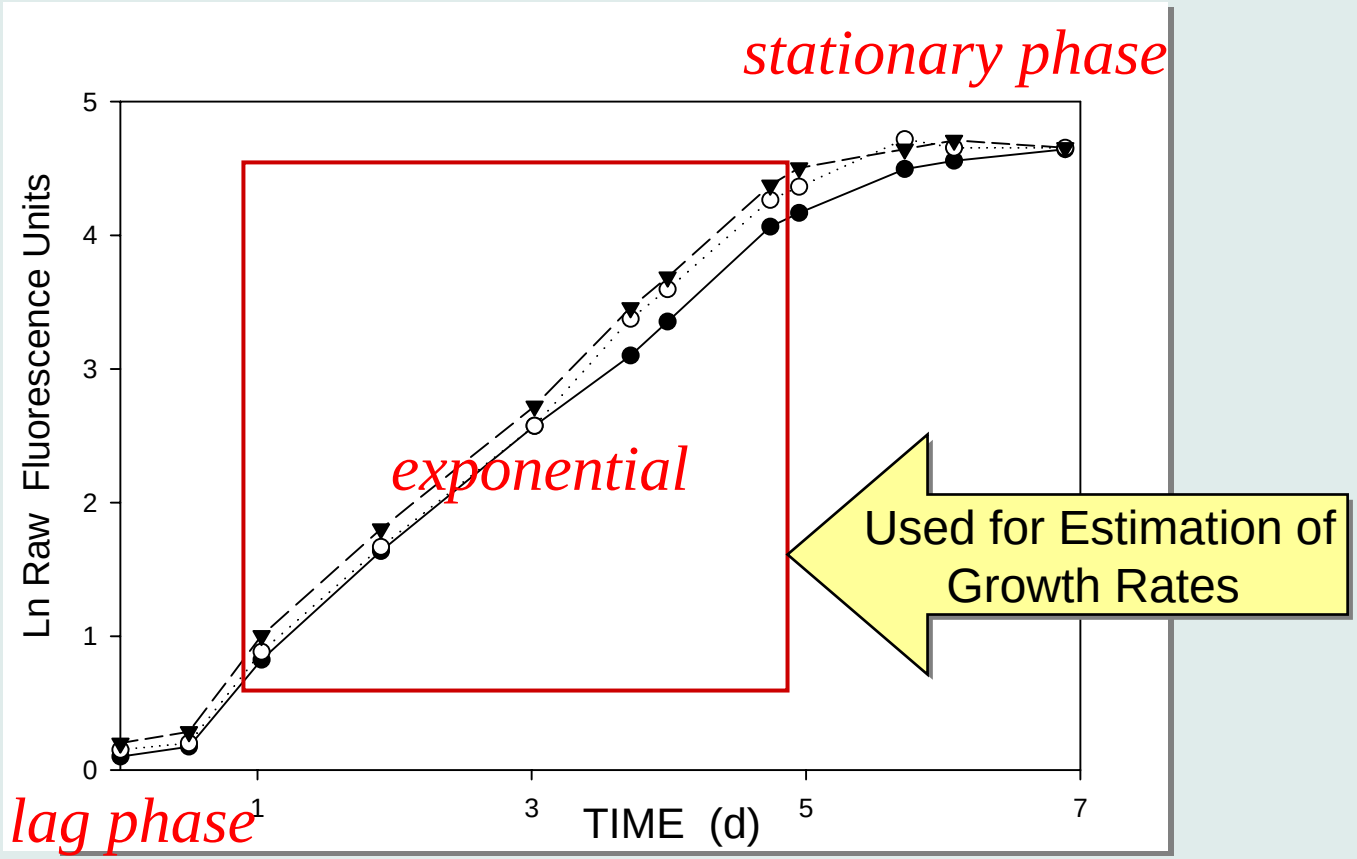
25 µm

- **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**

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

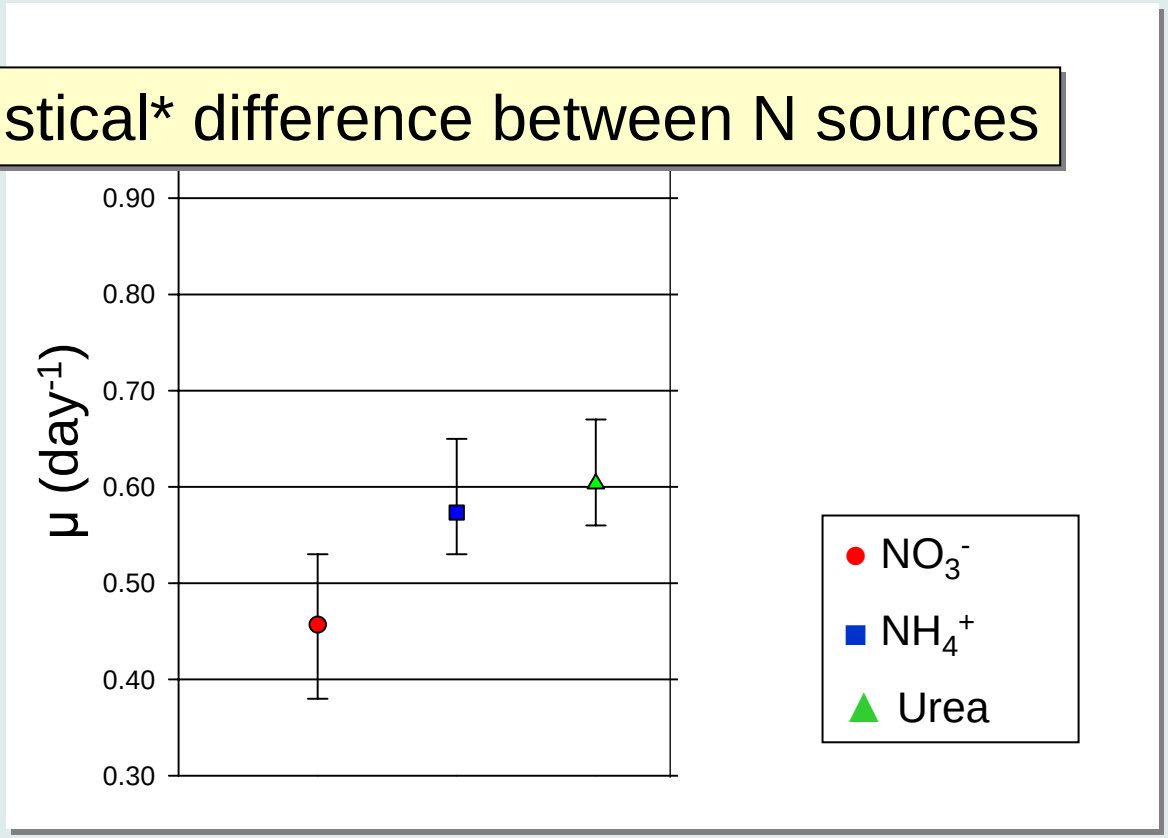


Strain (CCMP 1912) isolated from Kalaloch, WA (R. Horner)

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.

Heterosigma akashiwo growth rates as a function of light and nitrogen source

No statistical* difference between N sources

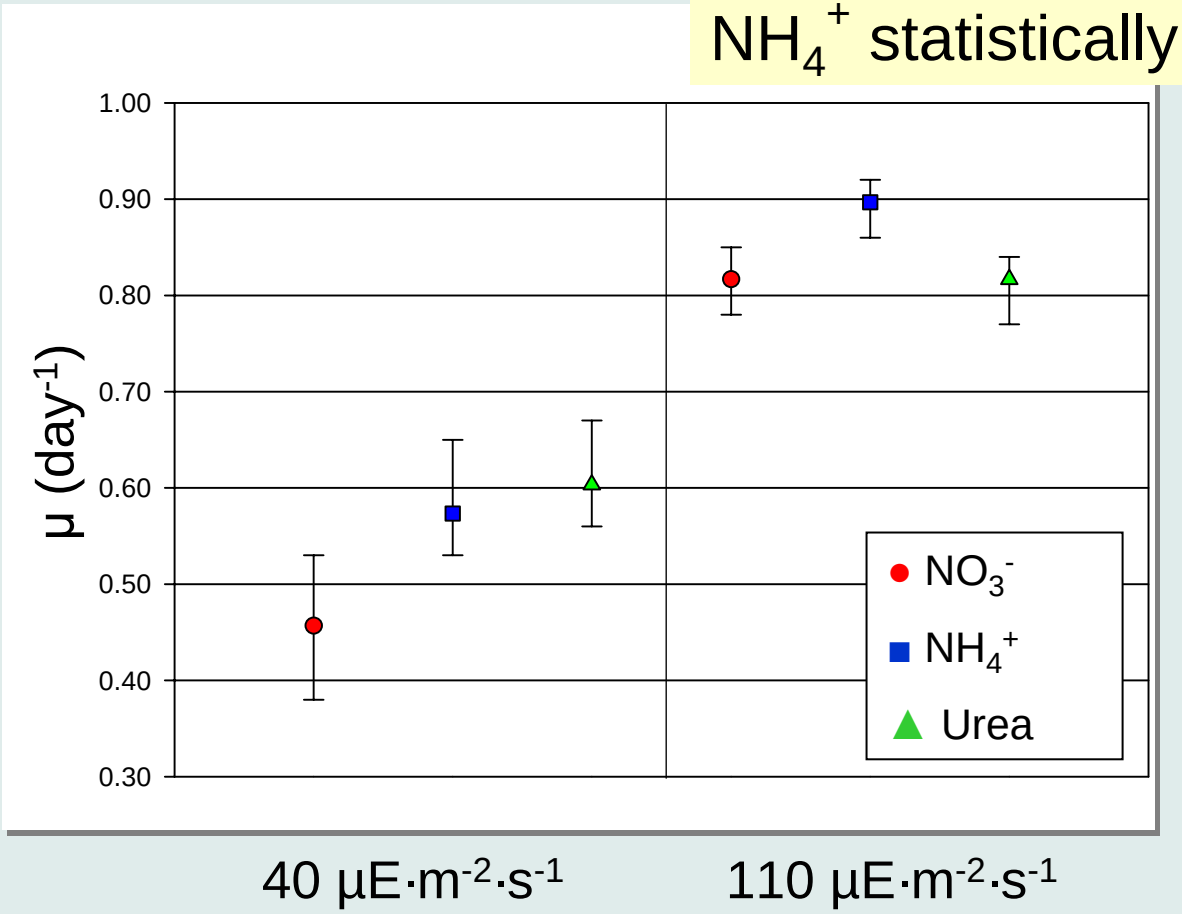


40 μE·m^{-2}·s^{-1}

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

NH_4^+ statistically faster

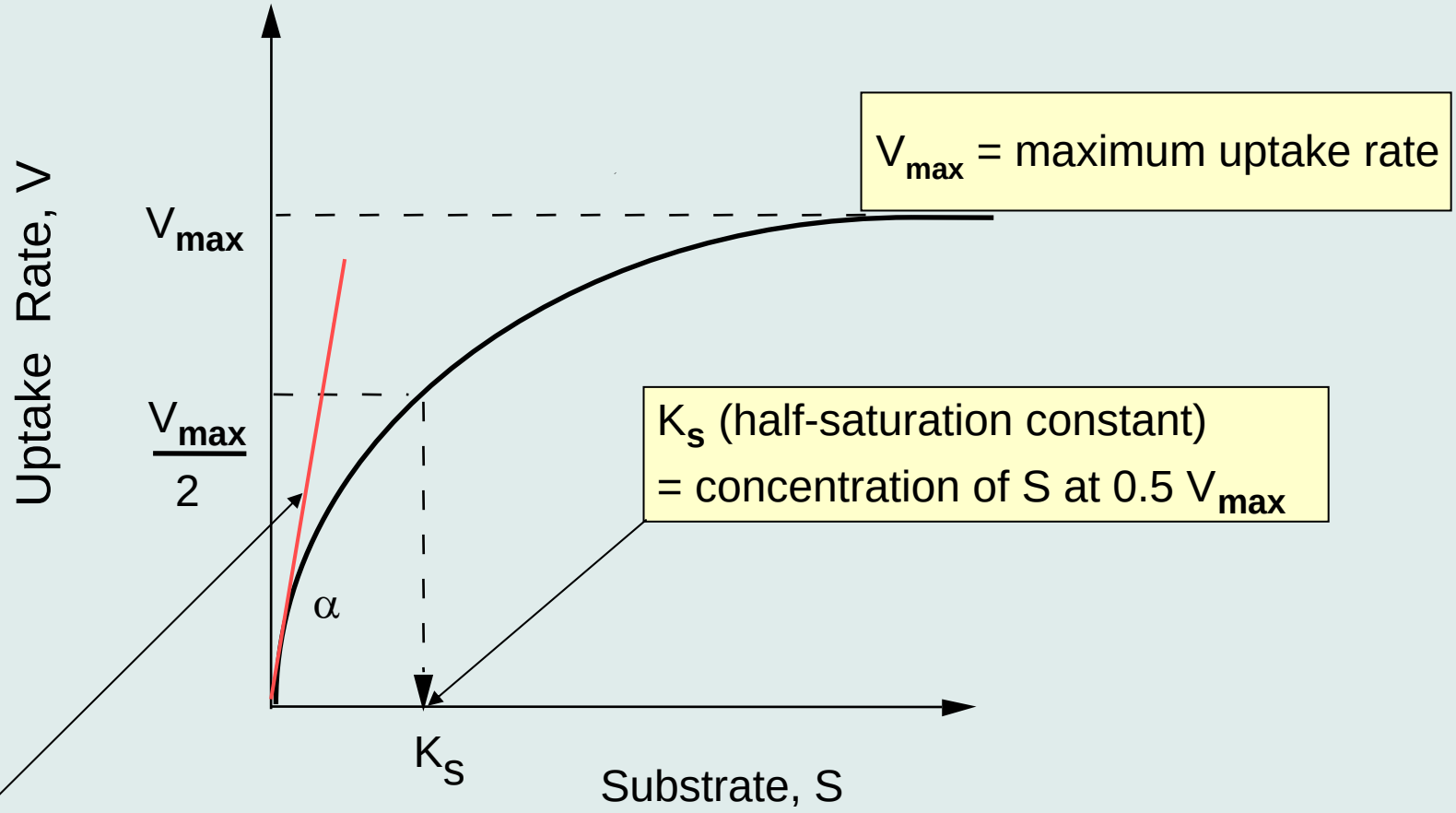


Symbols denote means, error bars are the range of replicates (n=3), growth rates determined at 15˚C.

Michaelis-Menten formulation for Nitrogen Uptake Kinetics

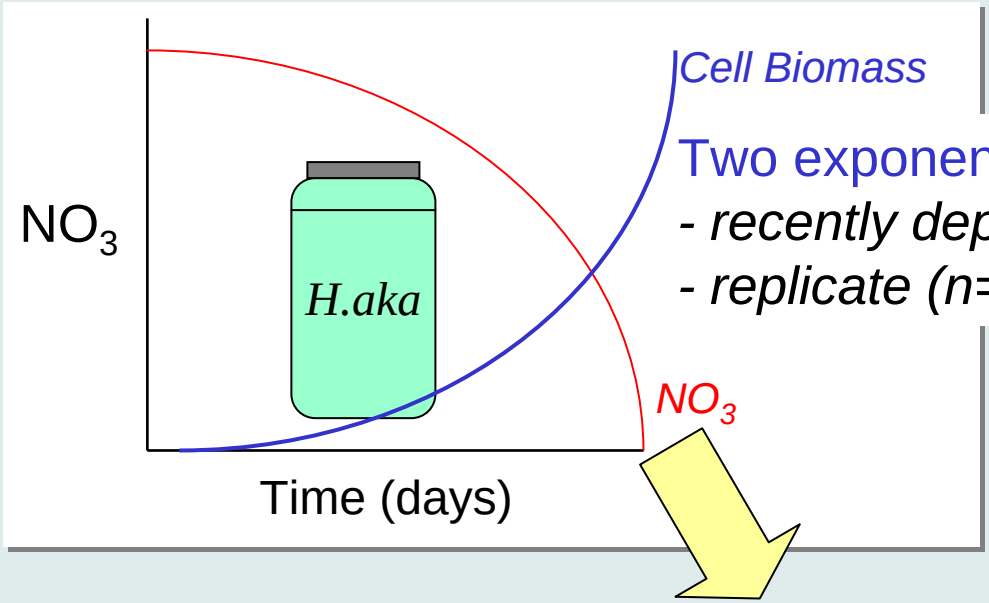
Dugdale (1967); MacIsaac and Dugdale (1969)

$$V = V_{max} \bullet S / (K_s + S)$$



α = initial slope, substrate affinity at low S (Healey,1980; Cochlan and Harrison, 1991)

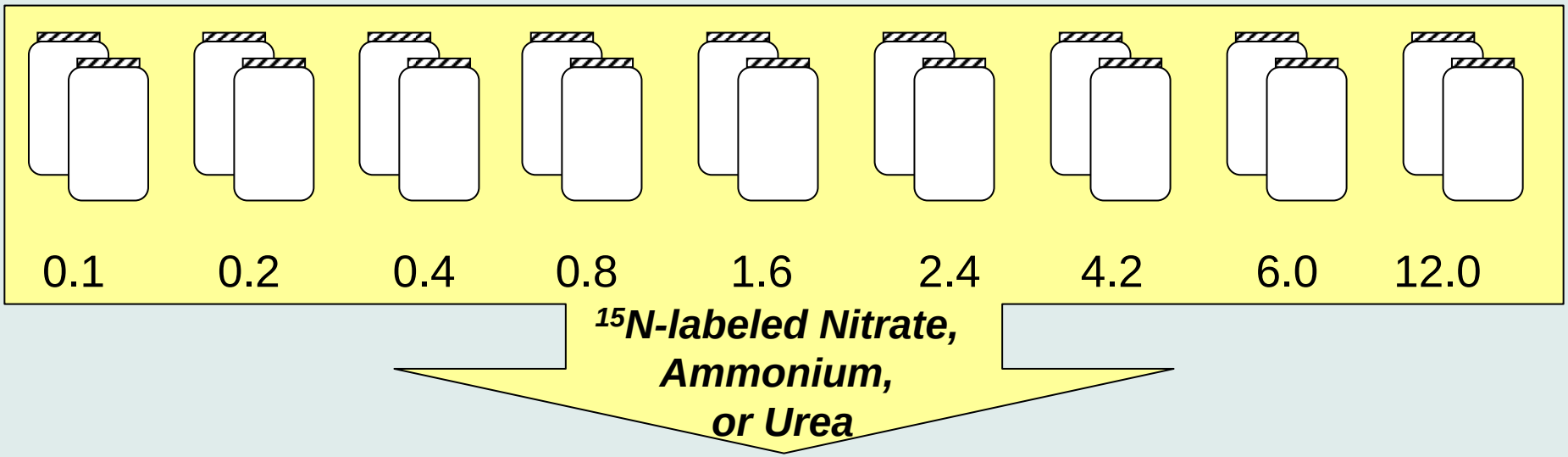
Nitrogen Kinetic Experiments



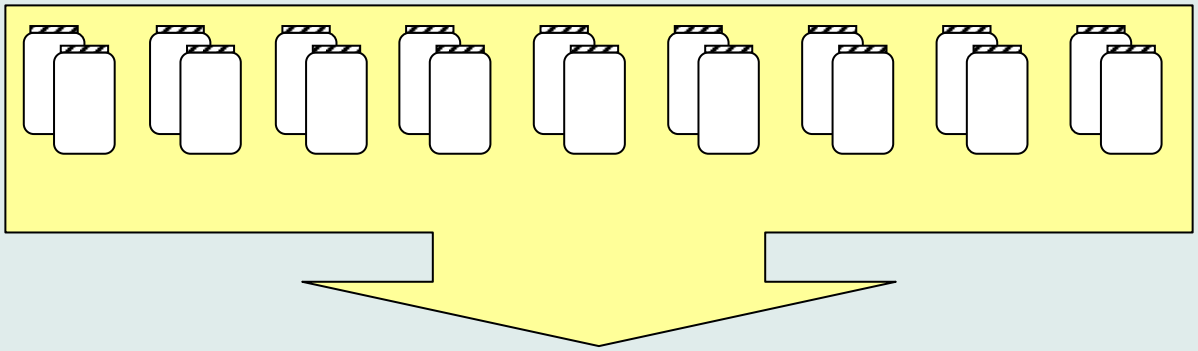
Two exponentially growing cultures of *H. akashiwo*

- recently depleted of nitrate

- replicate (n=18) sub-samples into 50-mL tubes

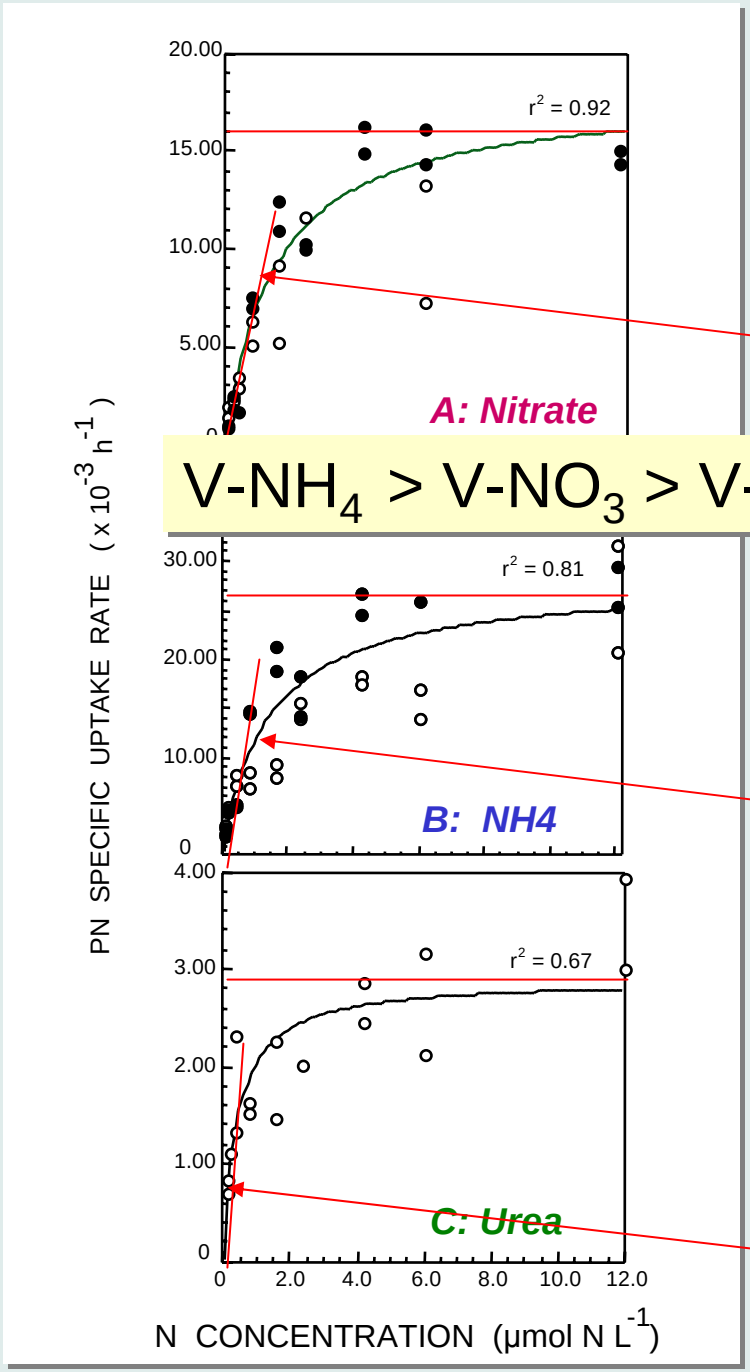


For: Nitrate, Ammonium and Urea:
duplicate incubations at all concentrations (0.1-12.0 µmol $N \cdot L^{-1}$)



- Short (10 min incubations)
- Filtration of cells (PN) onto 5.0 µm Ag filters
- $^{15}N/^{14}N$ 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



$V\text{-}NH_4 > V\text{-}NO_3 > V\text{-urea}$

- V_{max} = 18.0 x 10^{-3} h^{-1}
  –(std. error = 2.28)
- K_s = 1.47 µmol N L^{-1}
  –(std. error = 0.25)
- α_N = ***12.2***

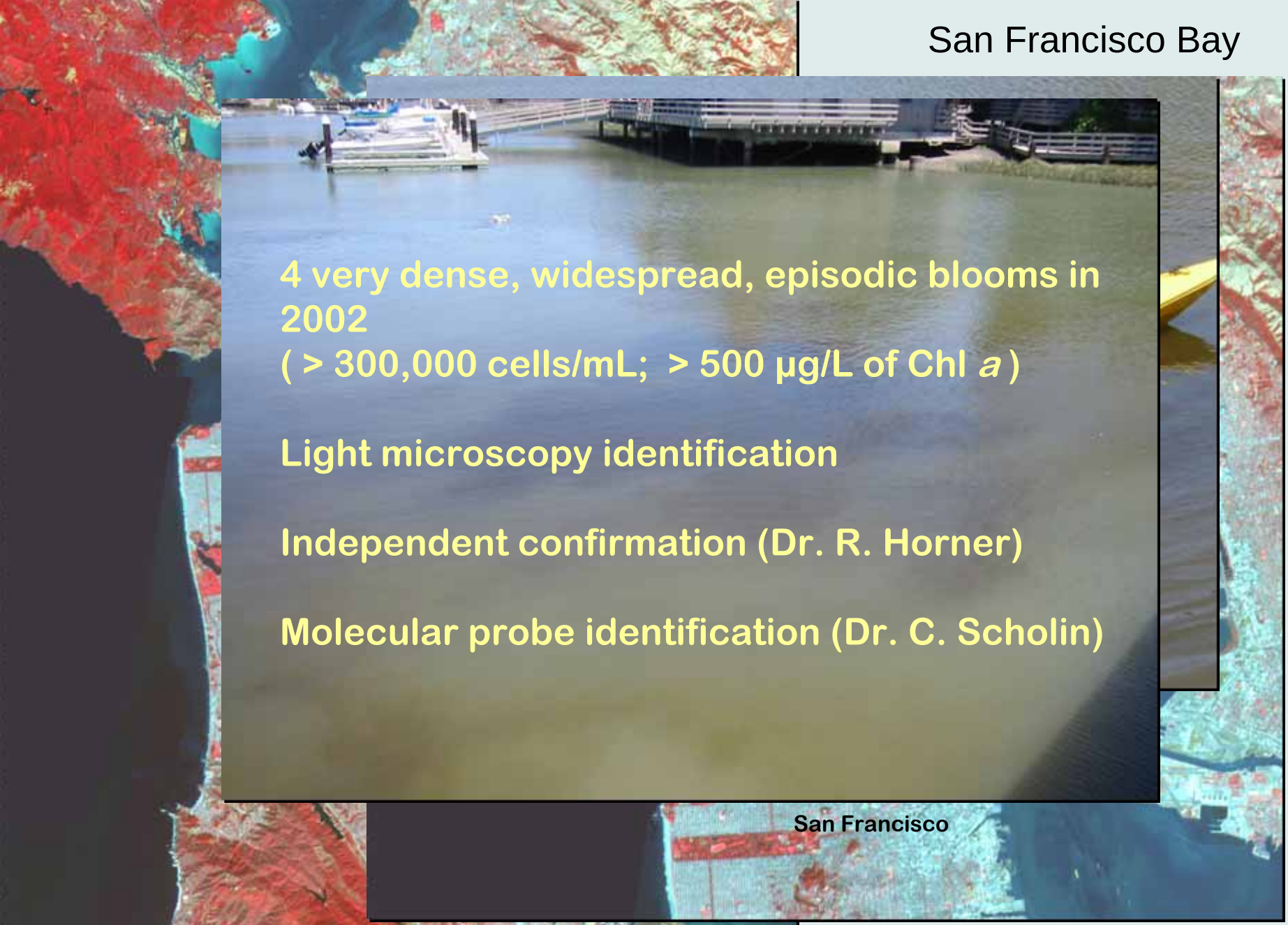
- V_{max} = 28.0 x 10^{-3} h^{-1} ✓
  –(std. error = 2.17)
- K_s = 1.44 µmol N L^{-1}
  –(std. error = 0.35)
- α_N = ***19.4*** ✓

- V_{max} = 2.89 x 10^{-3} h^{-1}
  –(std. error = 0.24)
- K_s = 0.42 µmol N L^{-1}
  –(std. error = 0.25)
- α_N = ***6.87***

Field Study Site
Summer 2002

Northwestern Richardson Bay

San Francisco Bay



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

Light microscopy identification

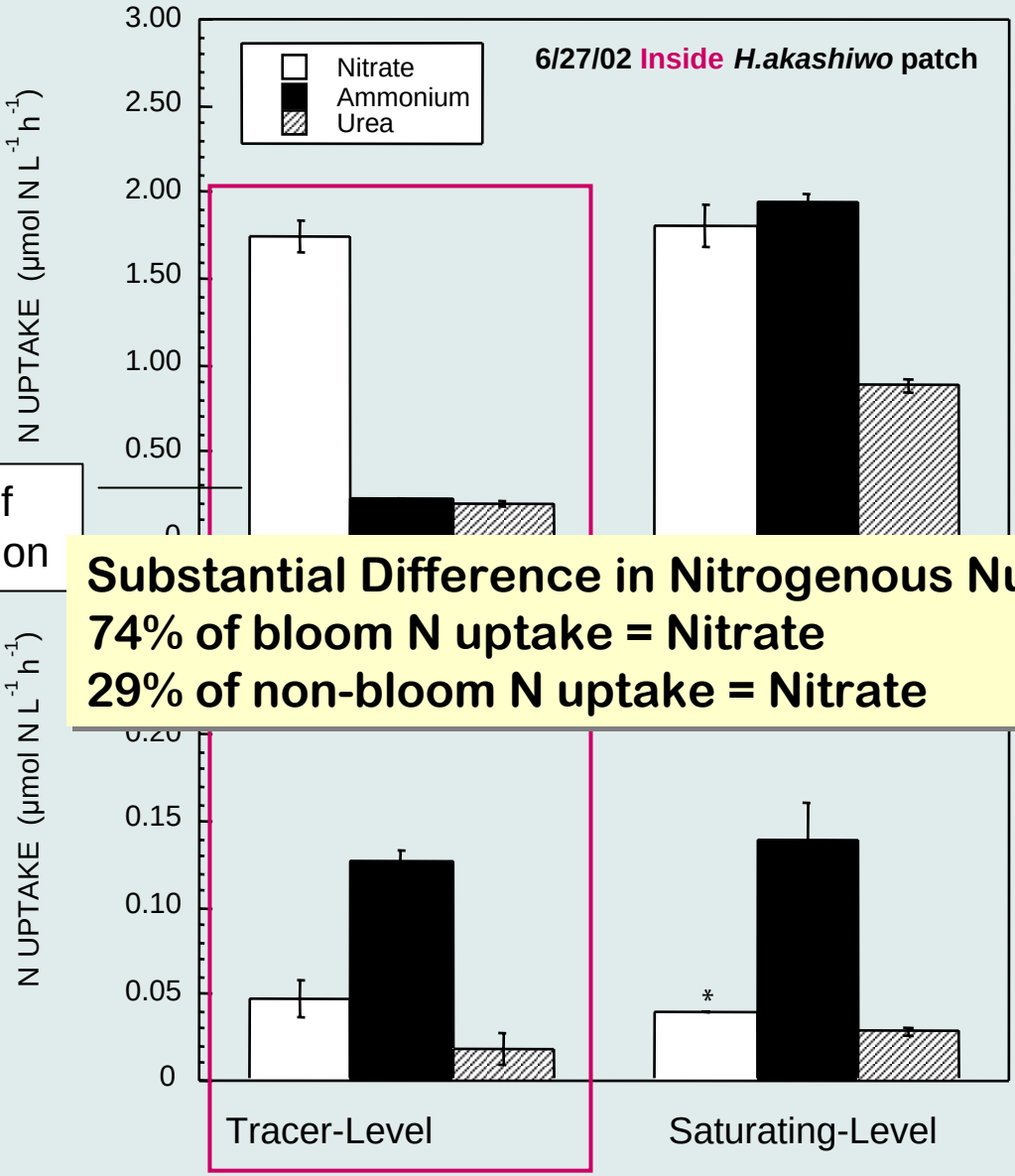
Independent confirmation (Dr. R. Horner)

Molecular probe identification (Dr. C. Scholin)

San Francisco

Richardson Bay Nitrogen Utilization

^{15}N-tracer methods



Indicative of *in situ* utilization

Substantial Difference in Nitrogenous Nutrition
74% of bloom N uptake = Nitrate
29% of non-bloom N uptake = Nitrate

IN BLOOM

NH_4: 0.39 µM

Urea: 0.31 µM

NO_3: 13.69 µM

NON-BLOOM

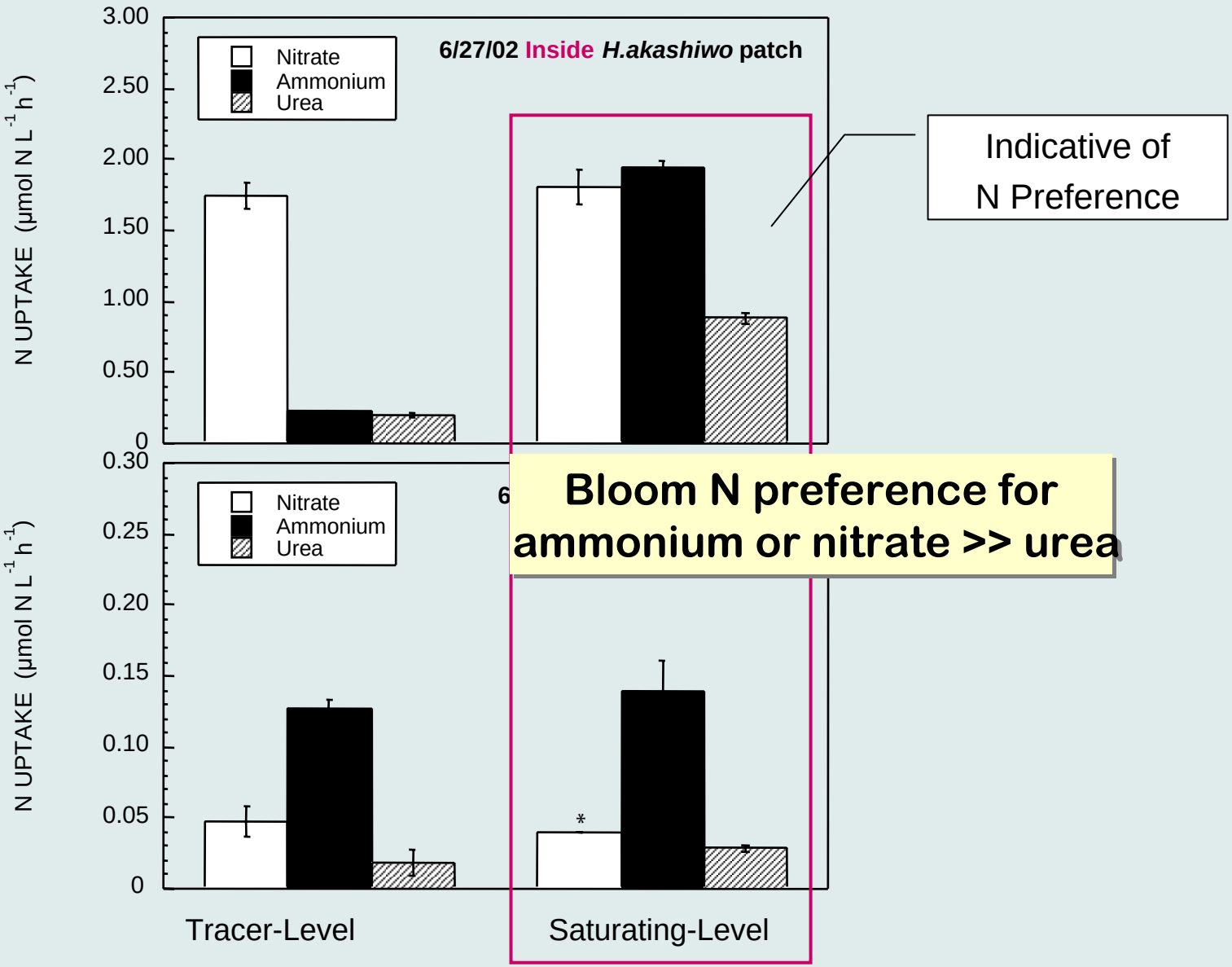
NH_4: 4.14 µM

Urea: 1.01 µM

NO_3: 15.65 µM

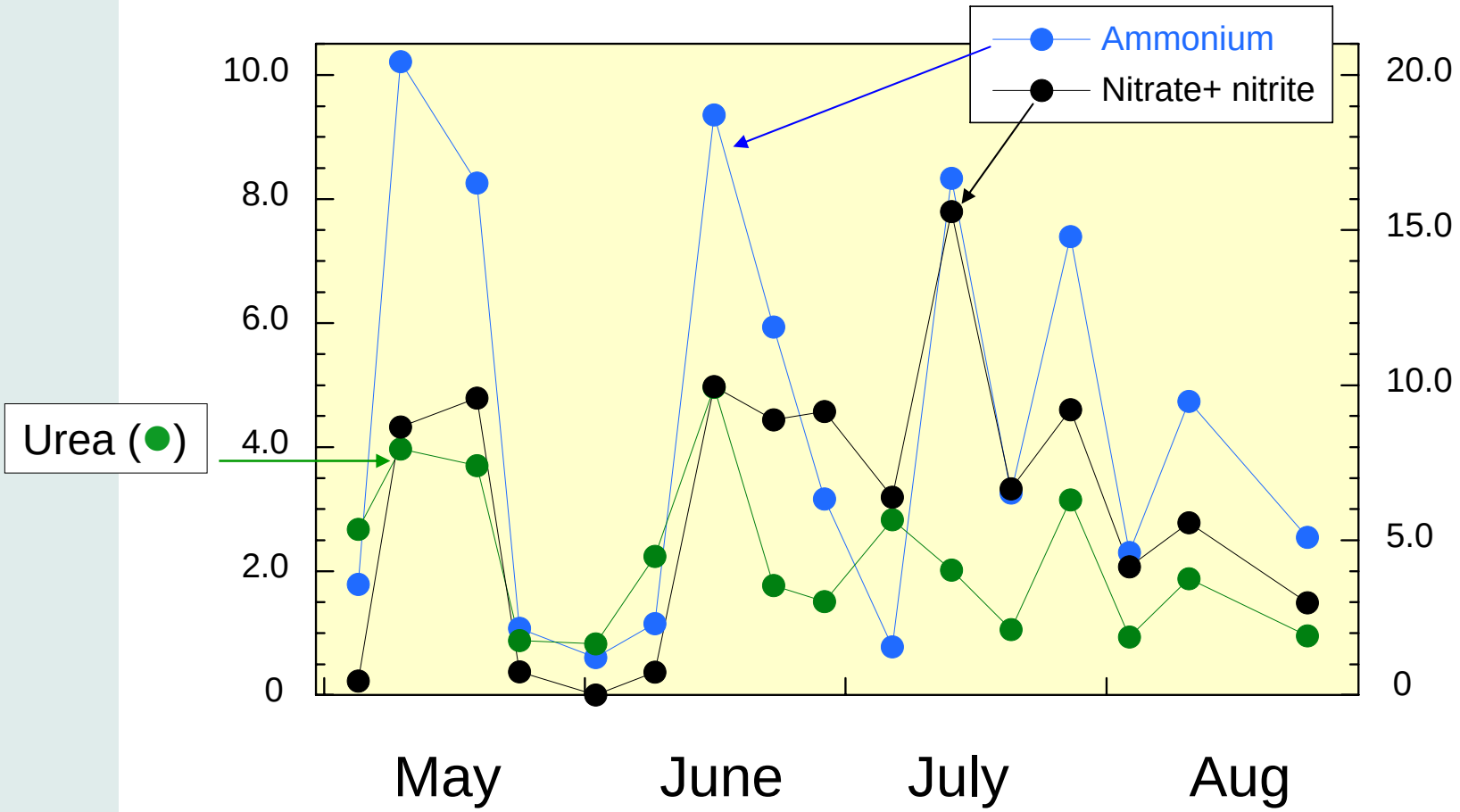
Richardson Bay Nitrogen Utilization

^{15}N-tracer methods



Richardson Bay Ambient Nitrogen Levels (0.5 m)

[all concentrations reported in μg-at $N \cdot L^{-1}$]



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 (α) 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 of 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.

Reprints of this study available at PICES:
Herndon & Cochlan. *Harmful Algae* 6 (2007): 260-270.

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