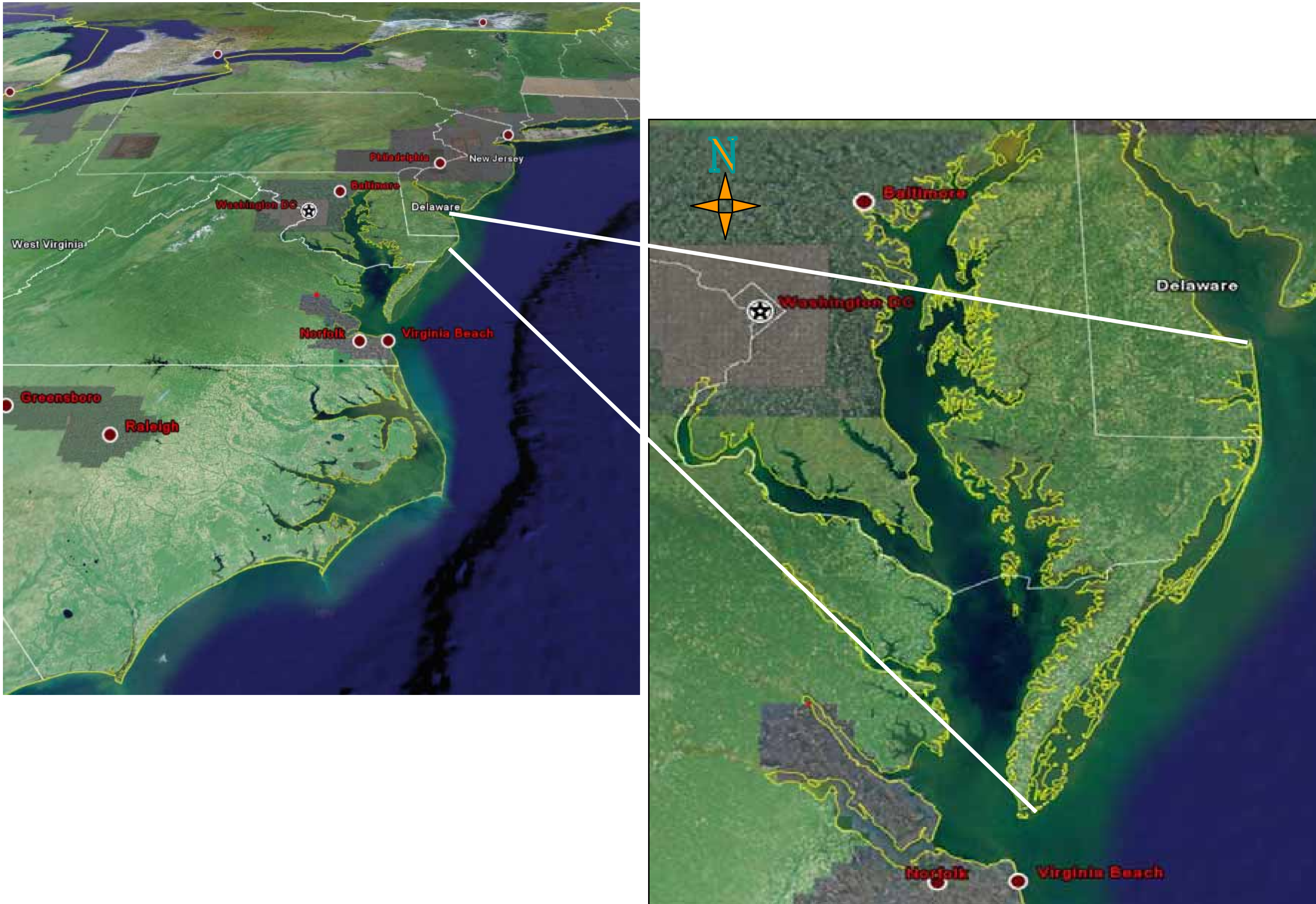
Characterization of nitrogen uptake by *Heterosigma akashiwo* grown in turbidostat culture under two light intensities

D Johns and

PM Glibert

Raphidophytes in Maryland and Delaware

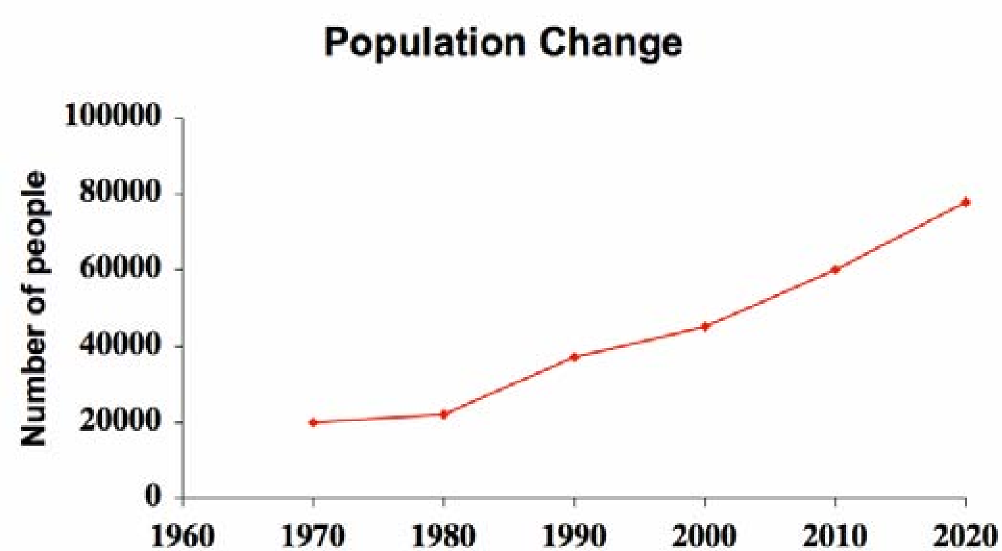


Maryland northern coastal lagoons are eutrophic systems

- Classified as highly susceptible to eutrophication (Bricker et al. 1999, 2007)
- Extensive poultry farming and agriculture within watershed
- Shallow poorly flushed bays

Public Landing, MD is associated with annual brown tide blooms

Human population has doubled since 1980



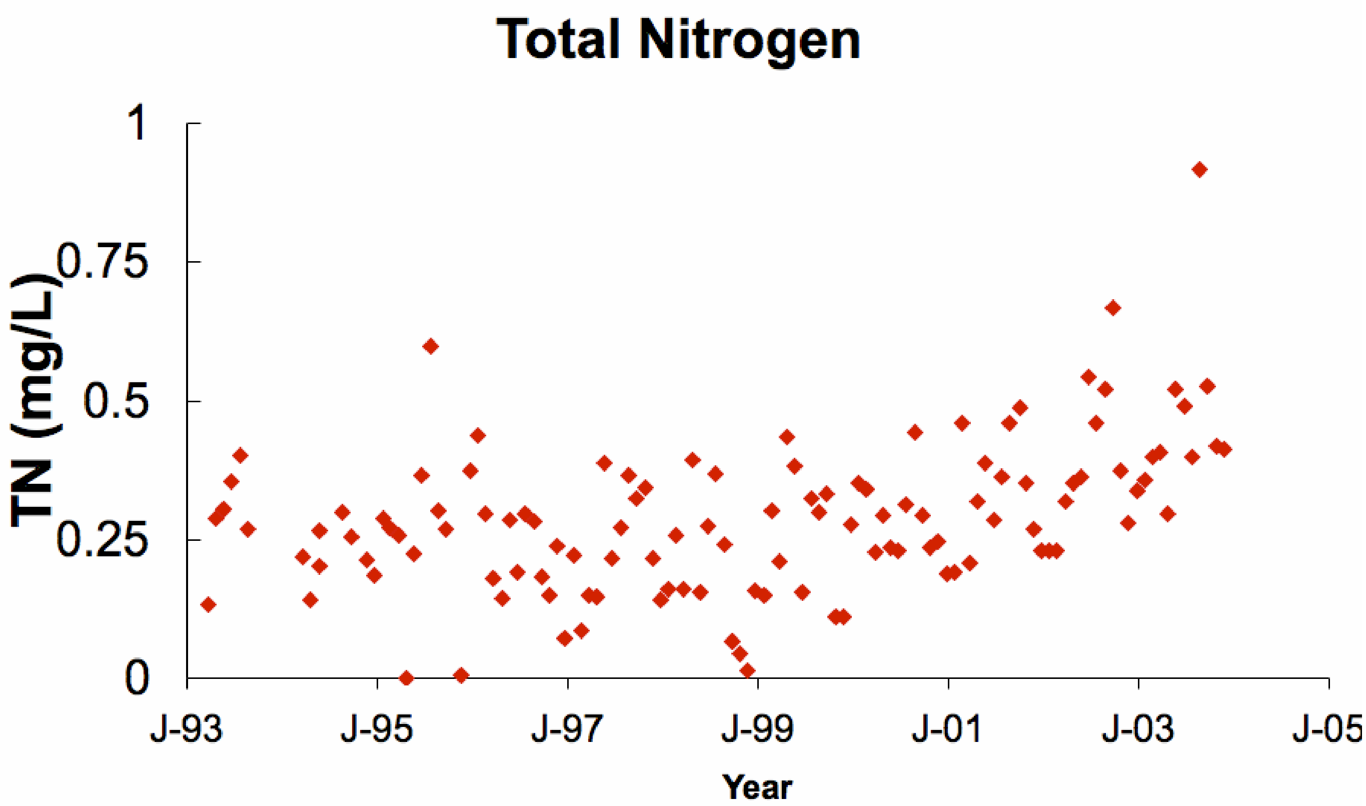
Increase in population in Ocean City and surrounding areas since 1970

Dense housing developments surround lagoons and canals

Glibert et al. 2007

Nutrient increases since mid-1990s (Glibert et al. 2007):

- NH_4^+ has increased ~4x
- DON has increased ~2x



Glibert et al. 2007

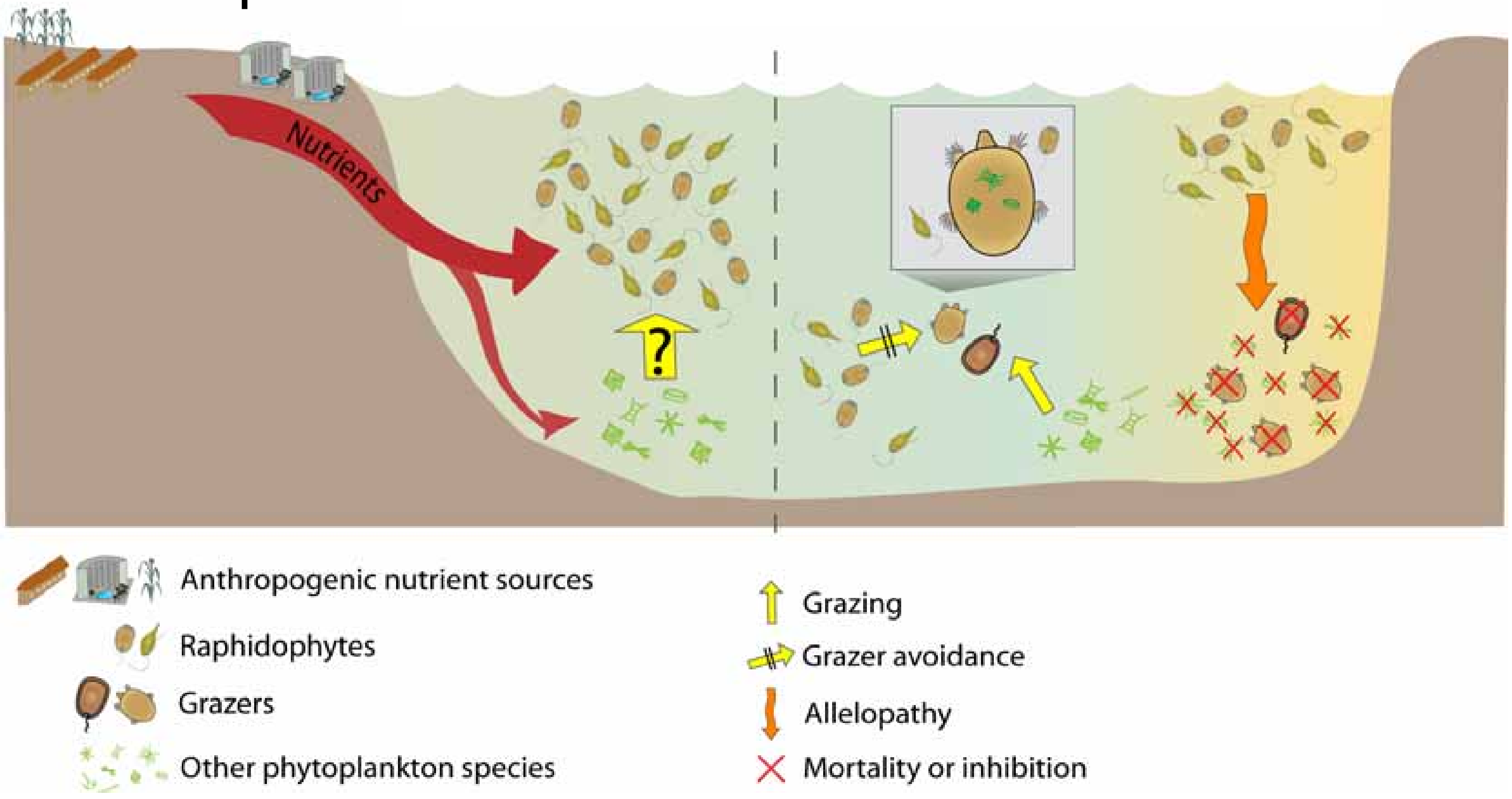
Raphidophytes are relatively new members of the phytoplankton

- Implicated in large fish kills from July-September 2000 in Delaware Inland Bays
- First documented in Maryland 2001
- Annual blooms since 2000
- Species include:
  - *Heterosigma akashiwo*
  - *Chattonella subsalsa*
  - *C.* cf. *verruculosa*
  - *Fibrocapsa japonica*

What factors are enabling raphidophyte blooms in these lagoons?

Increased nutrients or changing nutrient composition?

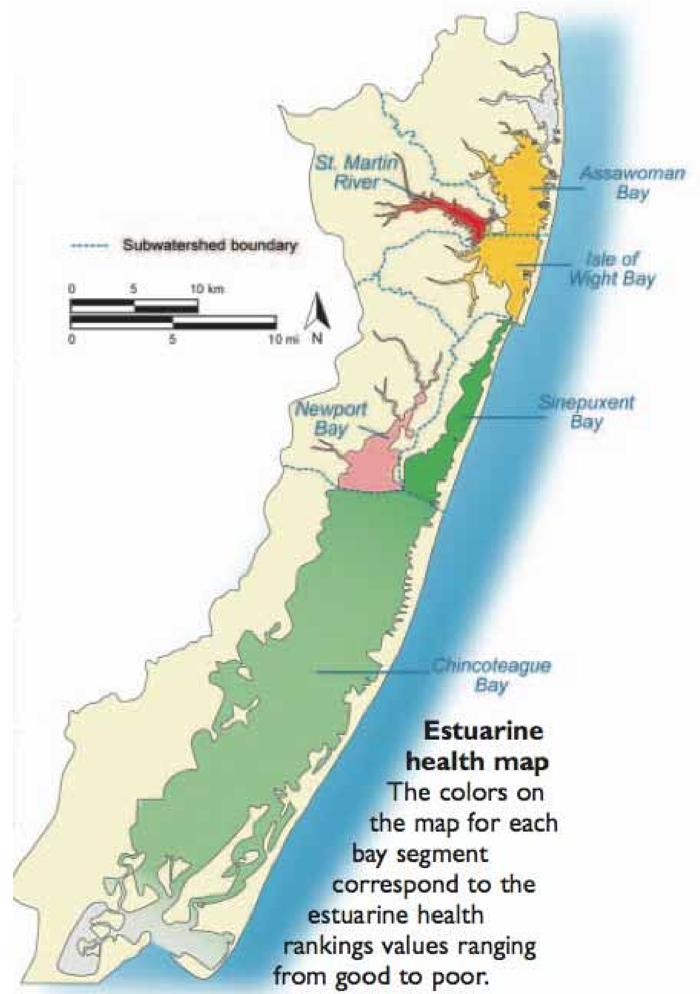
Allelopathy?



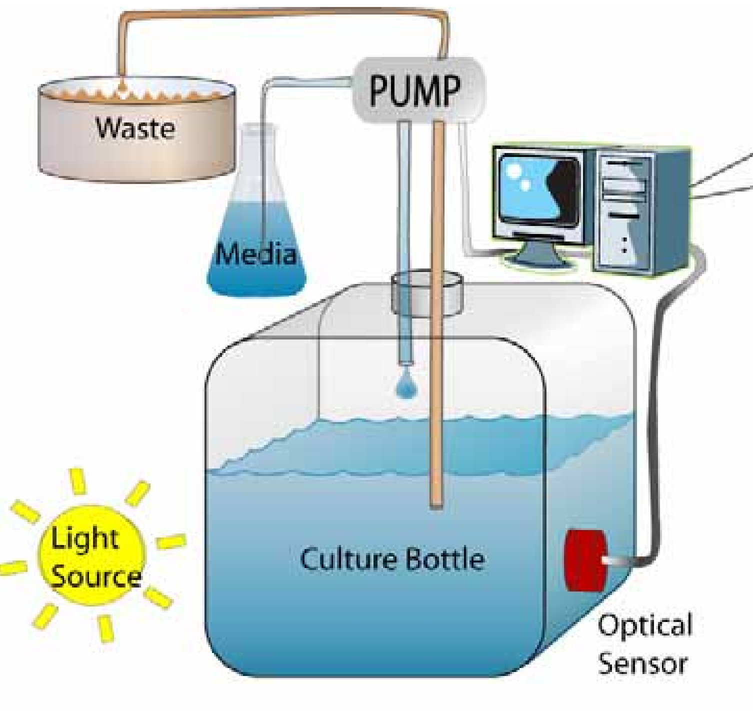
Does increasing eutrophication drive raphidophyte blooms in Maryland lagoons?

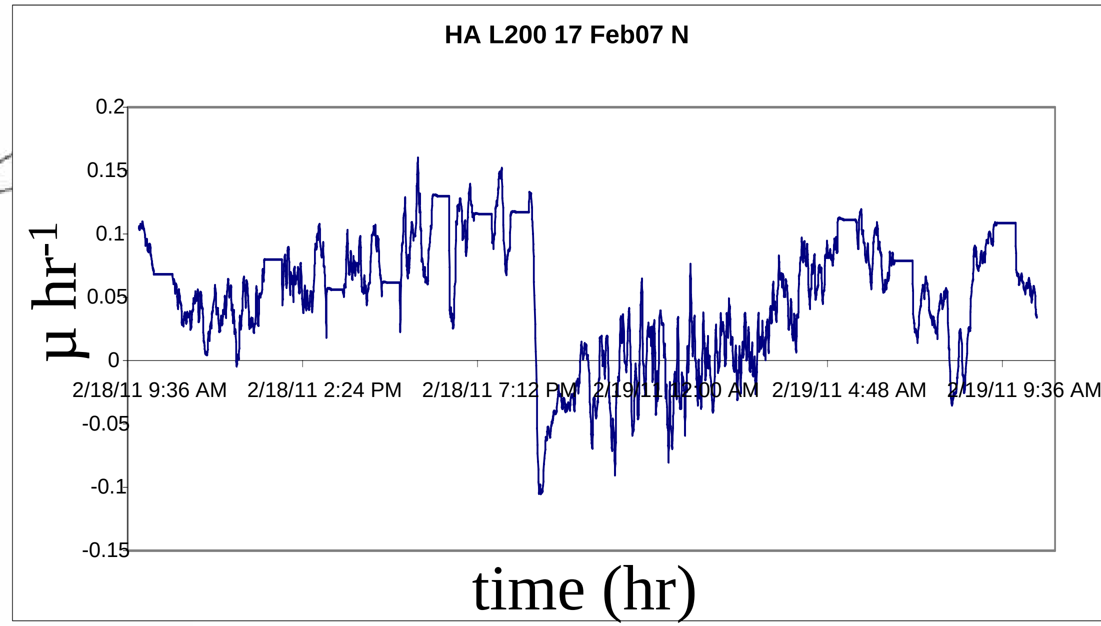
- Pigment record shows *C*. cf. *verruculosa* most frequently in Newport Bay
- Reports of other raphidophyte species by state agencies: Saint Martin River, Ayers Creek, Trappe Creek

  * Both of these areas are classified as in poor, or very poor estuarine health.



H. akashiwo was grown in turbidostat culture





H. akashiwo grown on enriched Indian River Seawater

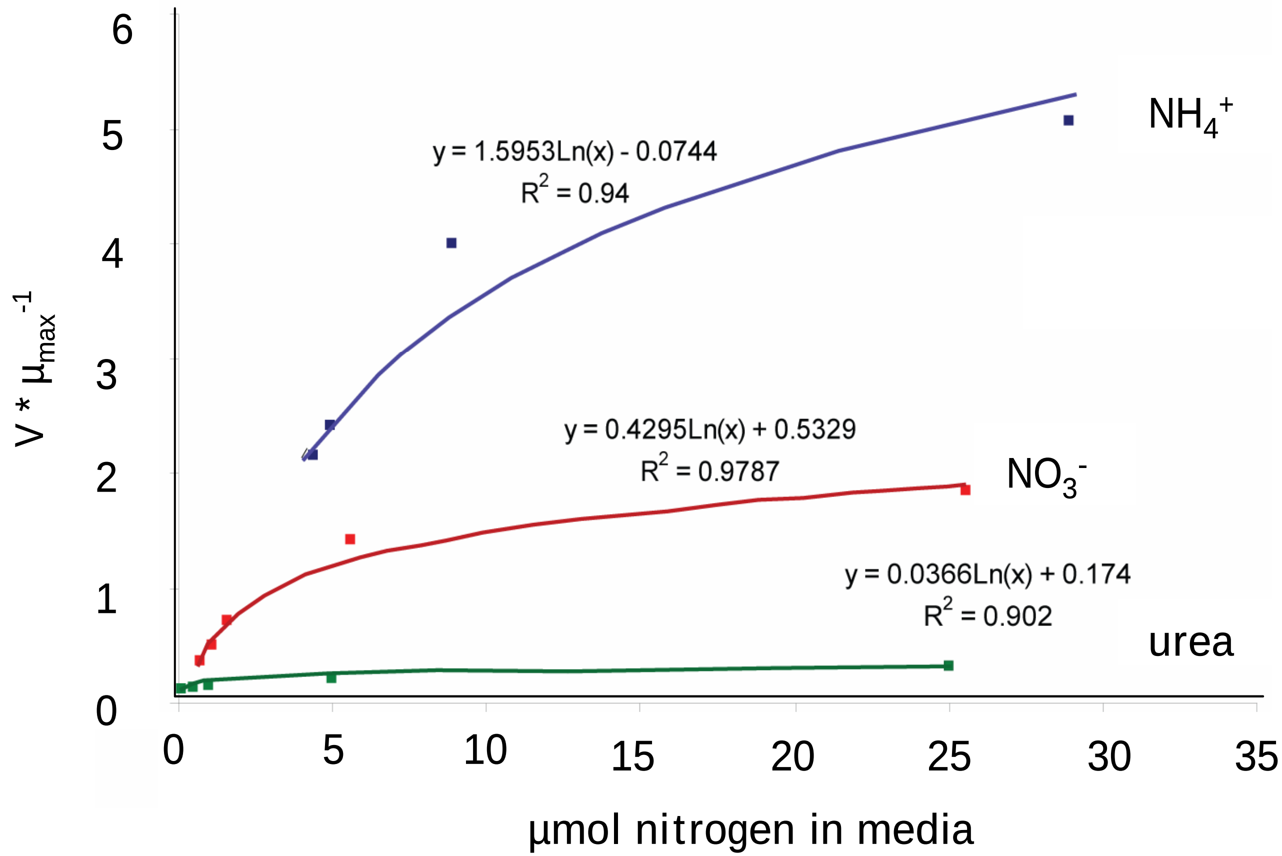
- grown at 100 and 200 µmol photons m^{-2} s^{-1}
- nutrient delivery ceased ~12 hours before initiation of experiment

Experimental Design

- ^{15}N addition of NH_4^+, NO_3^-, and urea
  - 0.1, 0.5, 1, 5, 25 µmol addition
- Sampling at 1, 10, 30 minutes
  - Total uptake
  - Trichloroacetic acid addition

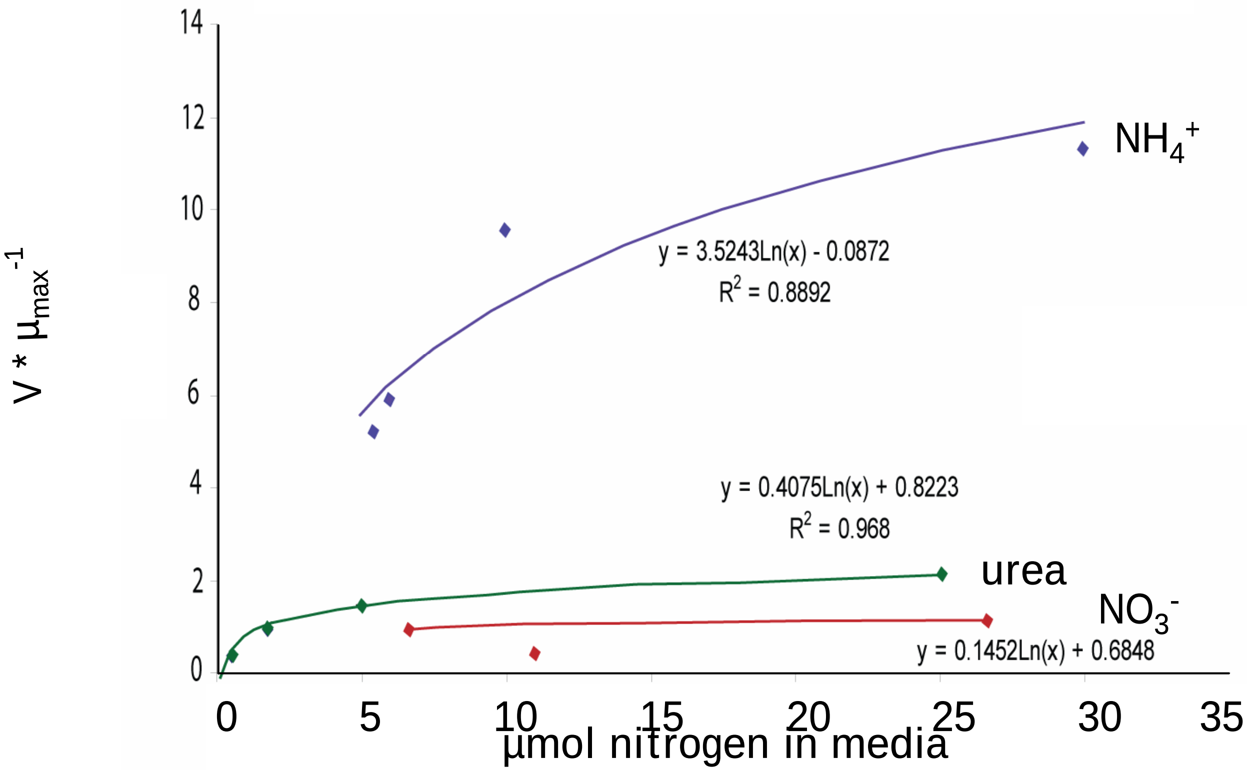
- Total N uptake and incorporation into protein measured by mass spectrometry

N uptake by *H. akashiwo* at 200 µmol photons $m^{-2}sec^{-1}$



- The ratio V: μ_{max}
  - NH_4^+ > NO_3^- > urea
  - NO_3^- uptake is >2x urea uptake

N uptake by *H. akashiwo* at 100 µmol photons m^{-2} sec^{-1}



- Relative to 200 µmol, V_{max}: μ_{max} for
  - NH_4^+ is ~2x that of higher light level
  - NO_3^- decreased slightly
  - Urea is ~2x greater

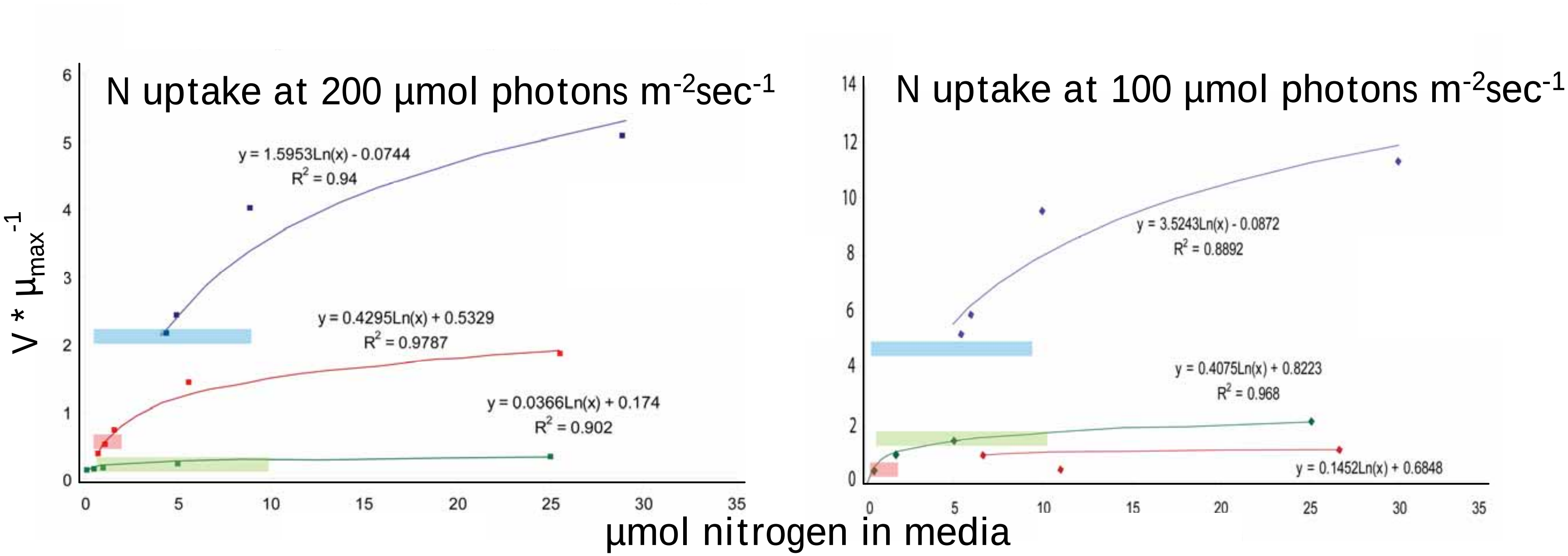
Can *H. akashiwo* meet growth demands on all substrates?

- Uptake exceeds growth for NH_4^+ and NO_3^- regardless of light intensity
- Urea uptake may support growth at 100 µmol light level, but not at 200 µmol

	L100	L200
NH_4^-	6.19	3.15
NO_3^-	1.3	1.94
urea	1.56	0.26

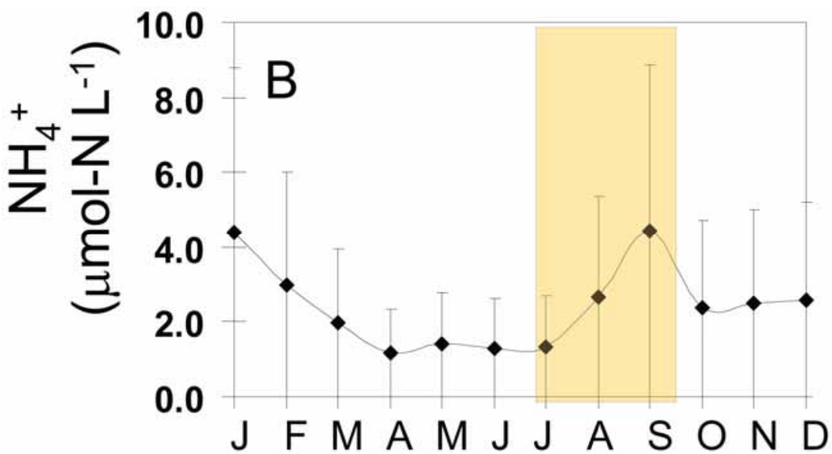
Comparison of V_{max} normalized to μ_{max} after 30 minute incubation at both light intensities.

What nitrogen forms does *H. akashiwo* encounter in coastal lagoons?

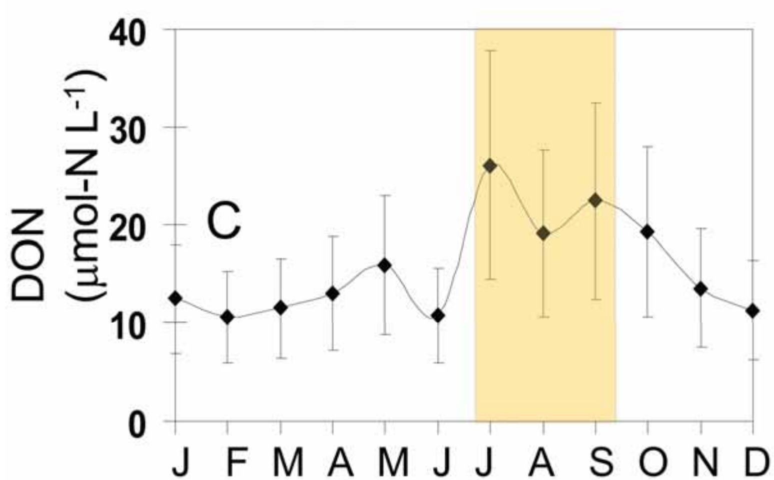


- Rapid uptake of NH_4^+ when available
- *H. akashiwo* is operating near V_{max} for urea at both light intensities
- NO_3^- uptake is not saturated in *H. akashiwo* at ambient conditions in coastal lagoons

What nitrogen forms does *H. akashiwo* encounter in coastal lagoons?

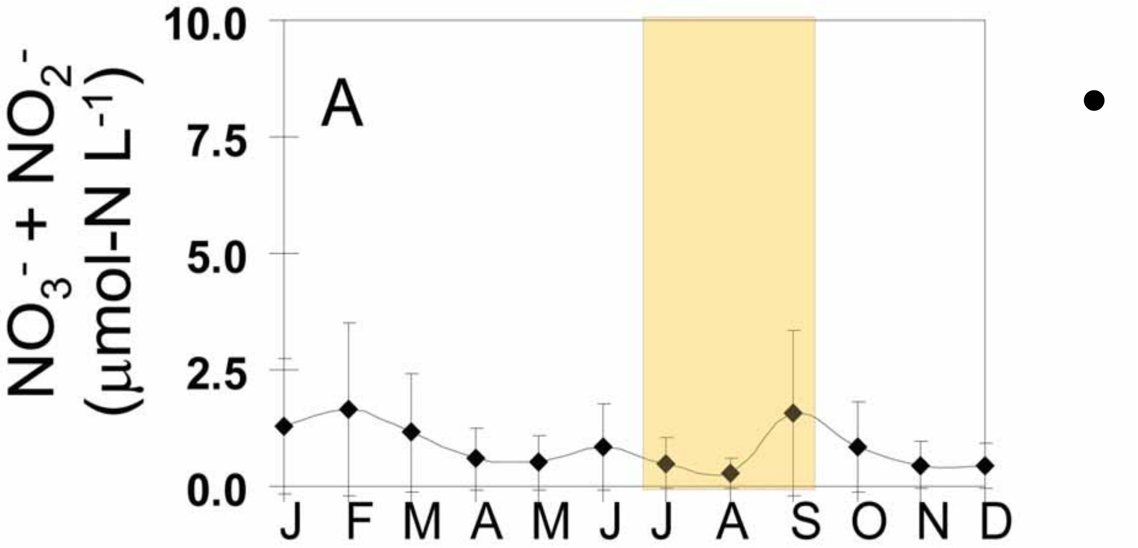


- NH_4^+ is significantly elevated



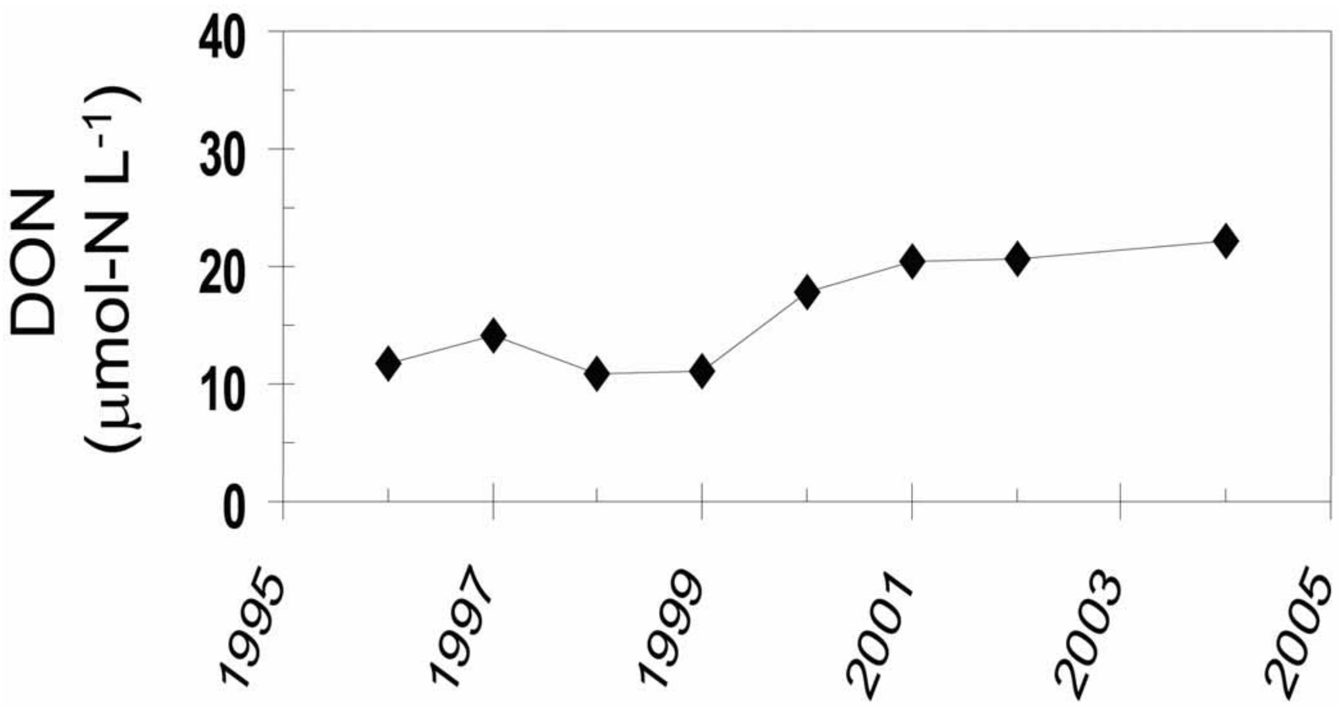
- DON increases sharply in July

Which Nitrogen Forms are Available when Raphidophytes Form Blooms?



- NO_3- is not a dominant form

DON Concentrations are Increasing Annually



In fact, it has doubled!

Glibert et al. 2007

Summary of Uptake and Growth Demands in *H. akashiwo*

- 100 µmol photons m^{-2} sec^{-1}:
  - *H. akashiwo* can meet growth demands on NH_4^+, NO_3^-, and urea

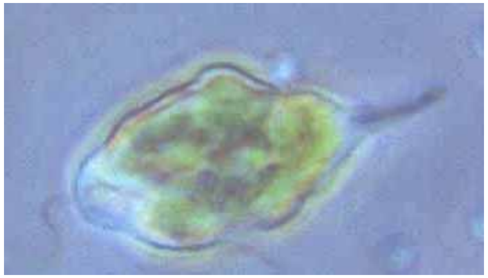
- 200 µmol photons m^{-2} sec^{-1}:
  - *H. akashiwo* can meet growth demands for N on NH_4^+ and NO_3^-

H. akashiwo in Maryland coastal lagoons

- *H. akashiwo* is well suited to take advantage of increasing eutrophication in these lagoonal embayments

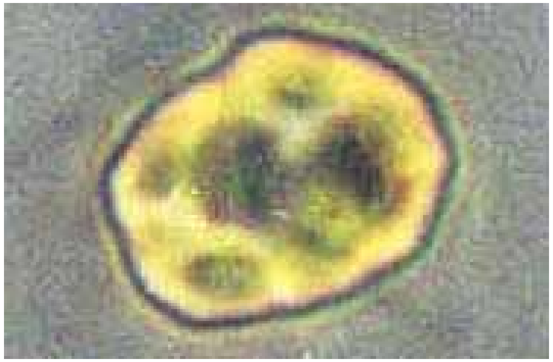
Future questions: Allelopathy

- Experiments to demonstrate allelopathy towards
  - Phytoplankton
  - Microzooplankton

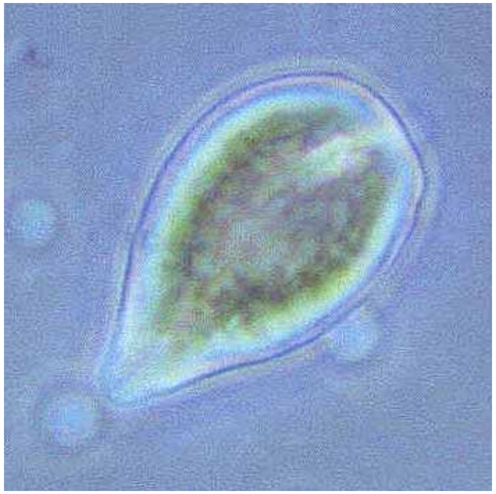


Chattonella cf. verruculosa

Fibrocapsa japonica



Heterosigma akashiwo



Chattonella subsalsa

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Comparison of α over both light levels and between substrates

	L100	L200
NH4+	0.45	1.01
NO3-	--	1.344
urea	0.45	1.037

* *Normalized to* μ_{max}