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Multiple stressors on the potential toxicity of Heterosigma akashiwo, a fish-killing flagellate in the Salish Sea.

Julia Matheson
Western University, juliamath22@gmail.com

William Cochlan
San Francisco State University

Charles Trick
Western University

Julia Matheson
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Primary Email: juliamath22@gmail.com

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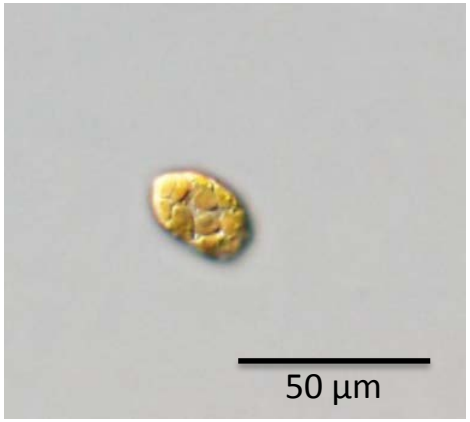
Julia R. Matheson
Department of Biology, Western University, London, Ontario, Canada

Charles G. Trick
Department of Biology, Western University, London, Ontario, Canada

William P. Cochlan
Romberg Tiburon Center for Environmental Studies,
San Francisco State University, Tiburon, California, USA

Physical Characteristics of *Heterosigma akashiwo*

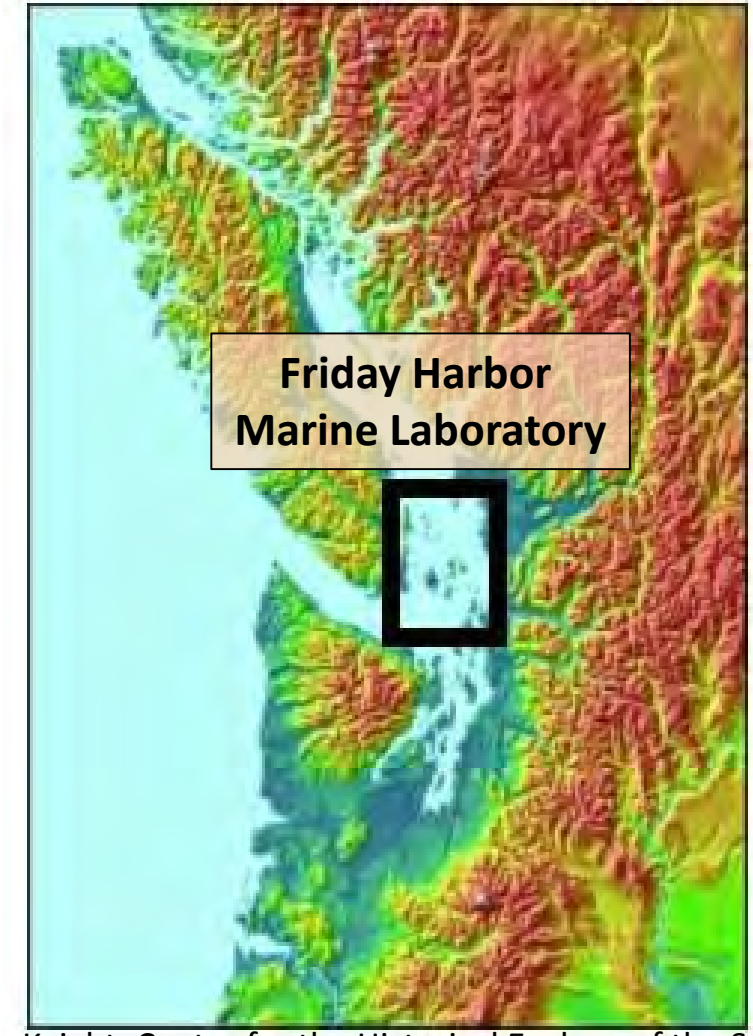
- Responsible for massive fin-fish mortality in aquaculture operations worldwide including the Salish Sea
- Estimated $2 million USD in losses per blooming event in Puget Sound.
- Environmental conditions that promote variably ichthyotoxic cells is not clearly understood
- Environmental conditions within the Salish Sea are unique – upwelling zone + anthropogenic inputs



Heterosigma akashiwo

- **How *H. akashiwo* responds to nutrient-rich, acidified ocean conditions projected for future coastal zones is unknown.**

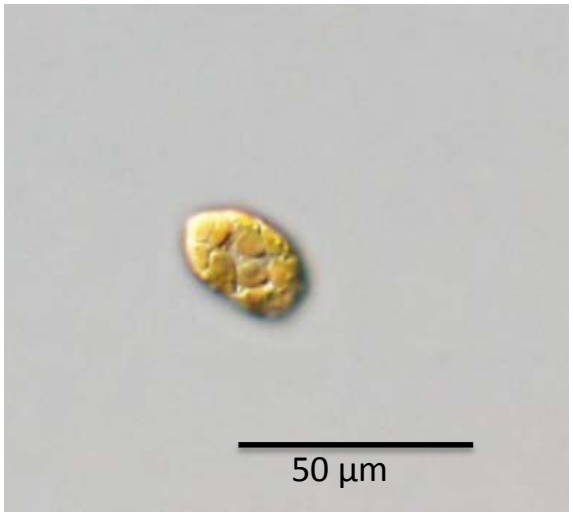
Salish Sea Study Site

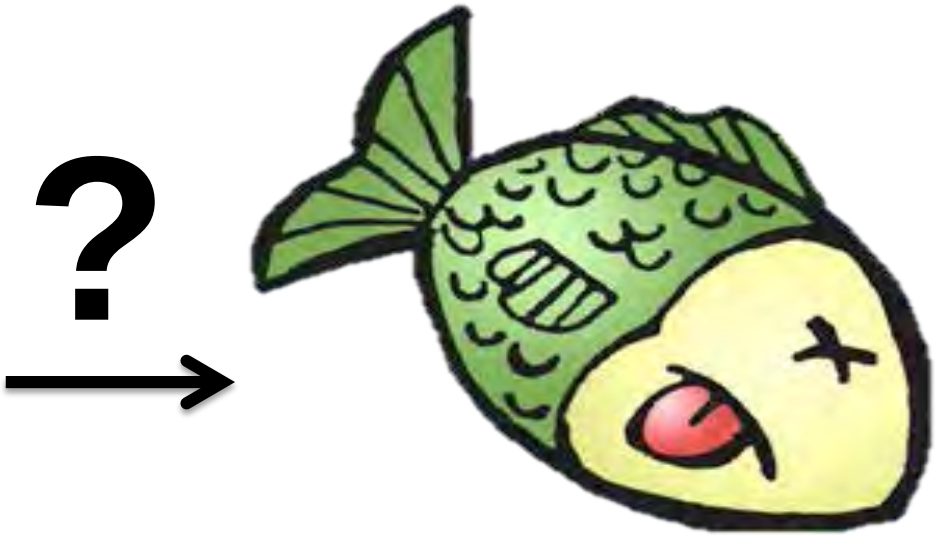


Kaiaht: Center for the Historical Ecology of the Salish Sea

My Research Question

Will future coastal waters promote the formation of **toxic** HABs of *Heterosigma akashiwo*?





My Hypothesis

I hypothesize that *Heterosigma akashiwo* can remain a potential HAB species under new ocean conditions.

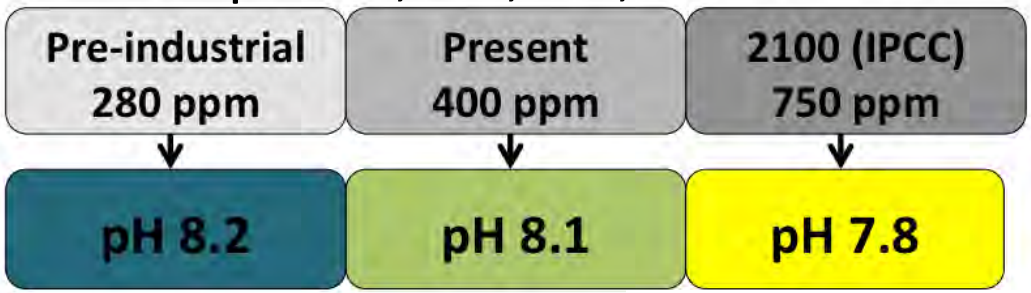
Heterosigma akashiwo

✓ Growth Rates

✓ Toxicity

Methodology

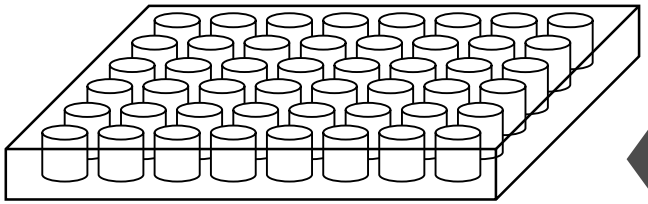
- Batch cultures of *H. akashiwo* (isolate 513):
  - ESAW salts enriched with f/2 nutrients
    - titrated to pH 8.2, 8.1, 7.8, 7.4



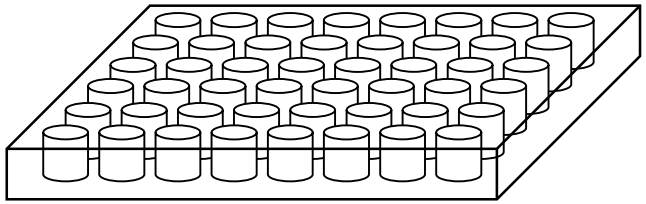
- Growth Rates:
  - Cell counts using the flow cytometer
  - Validated with hemocytometer counts
- Toxicity:
  - Rainbow trout gill cell assay (RTgill-W1)

Gill Cell Assay

Rainbow trout gill cells
150,000 cells/mL
Pre-incubated; 72 h at 19 °C

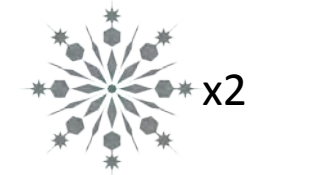


Incubated 24 h,
at 19 °C

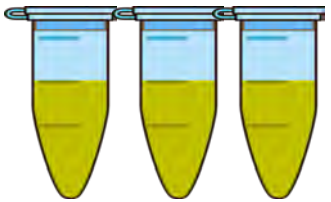


Incubated 2 h,
in the dark

Culture is removed and
Alamar Blue is added

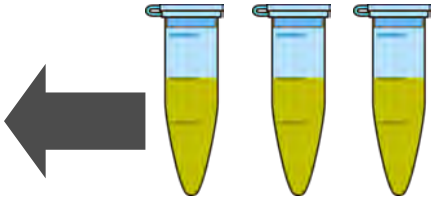


Cells are lysed



Gill Cell Assay samples

200,000 cells/mL



$$\%\ of\ control = \frac{\left(RFU_{ex.\ cells} - RFU_{ex.\ no\ cells}\right) x\ 100}{\left(Average\left[RFU_{control} - RFU_{control\ no\ cells}\right]\right)}$$

Detection of fluorescence

- Excitation: 540 nm
- Emission: 590 nm

Determine % cell viability

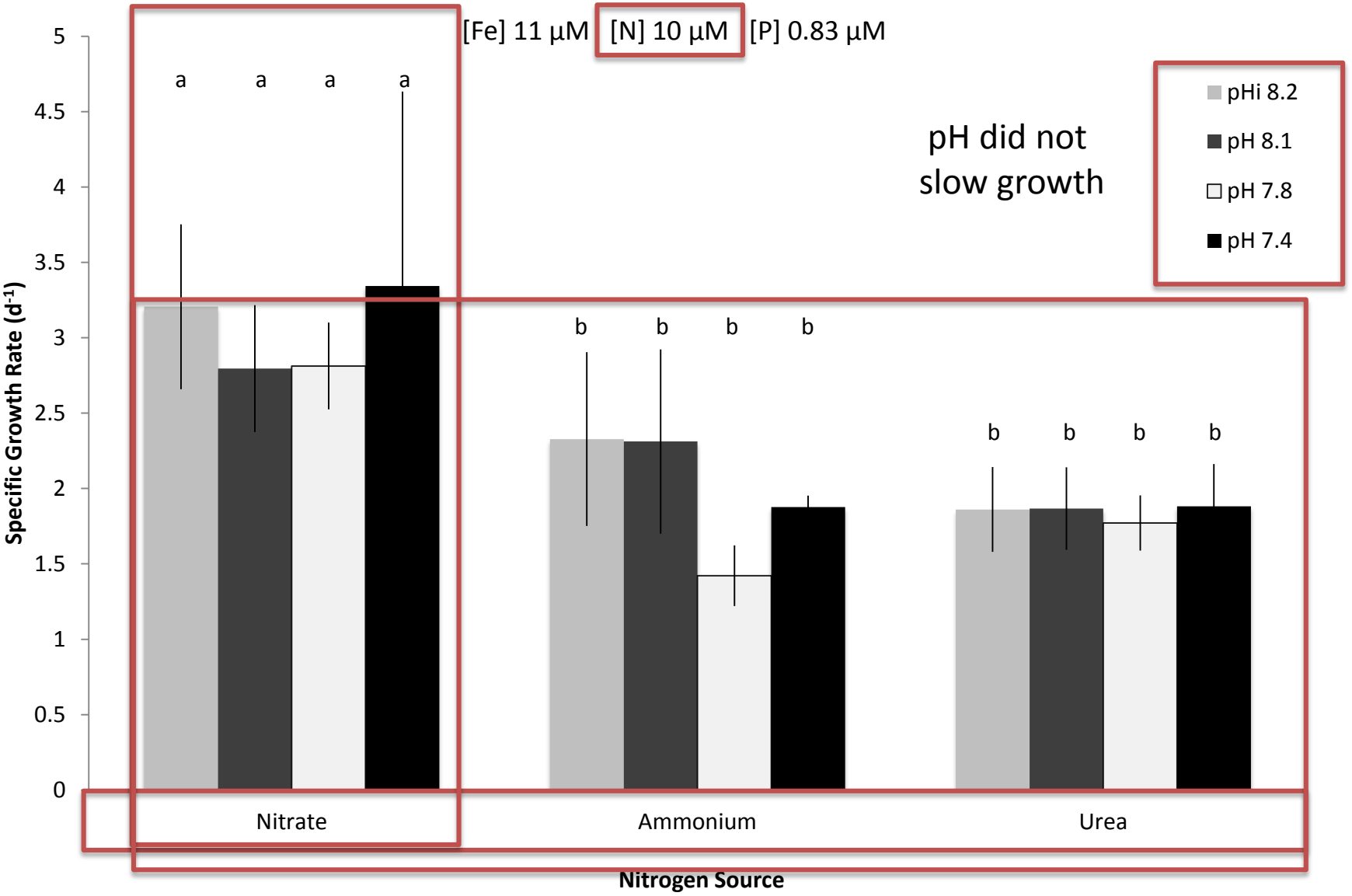
Modified from Chris Ikeda [from Schirmer et al., 1997; Dayeh et al., 2005; Dorantes et al., 2011]

GROWTH RATES

Will *Heterosigma akashiwo* maintain their presence in the future ocean?

Heterosigma akashiwo

Growth Rates



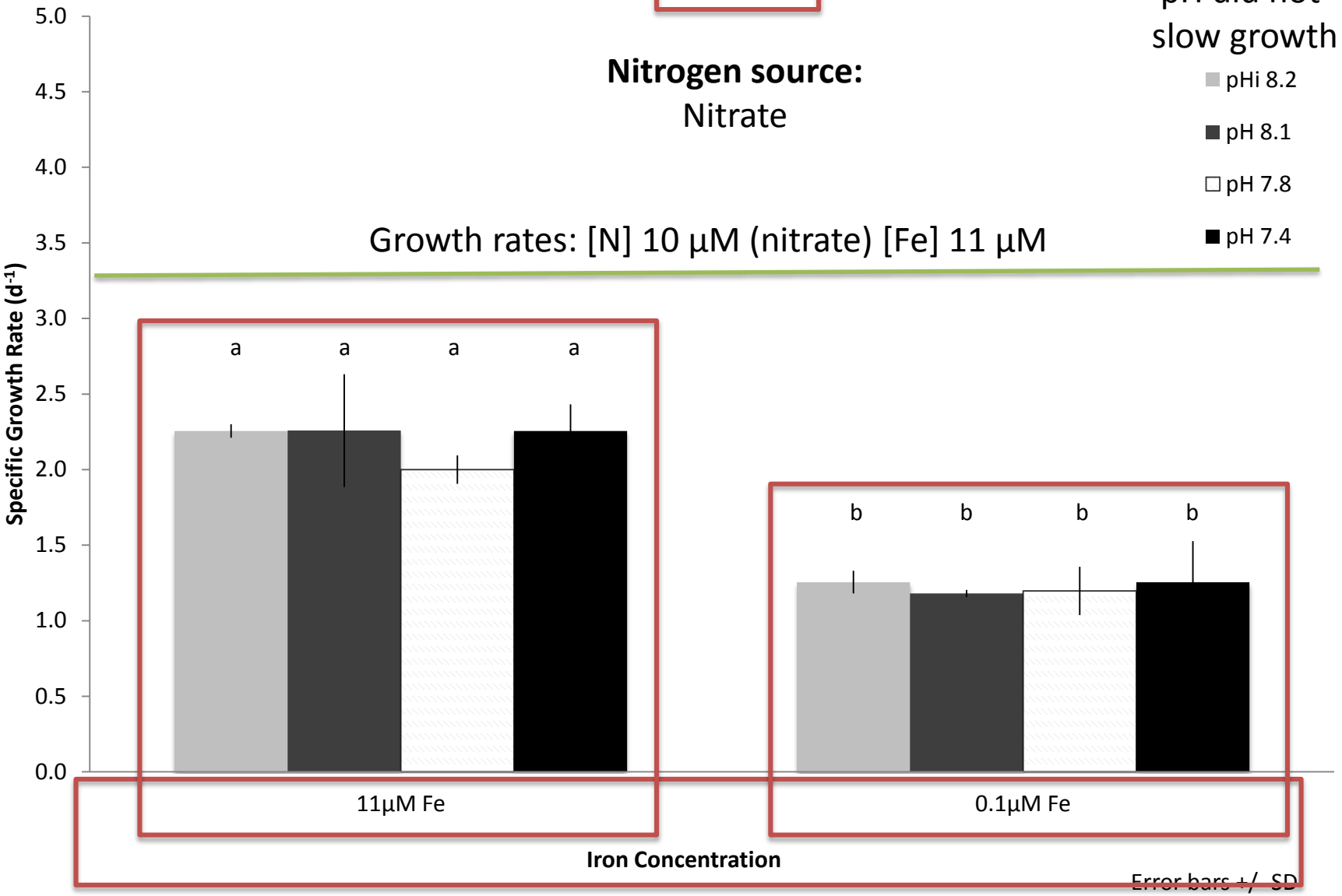
Error bars +/- SD
n=9
P < 0.0001

Growth Rates

[Fe] 0.1/11 µM [N] 880 µM [P] 37 µM

Nitrogen source:
Nitrate

pH did not slow growth



Error bars +/- SD
n=6
P < 0.0001

GROWTH RATES

Will *Heterosigma akashiwo* maintain their presence in the future ocean?

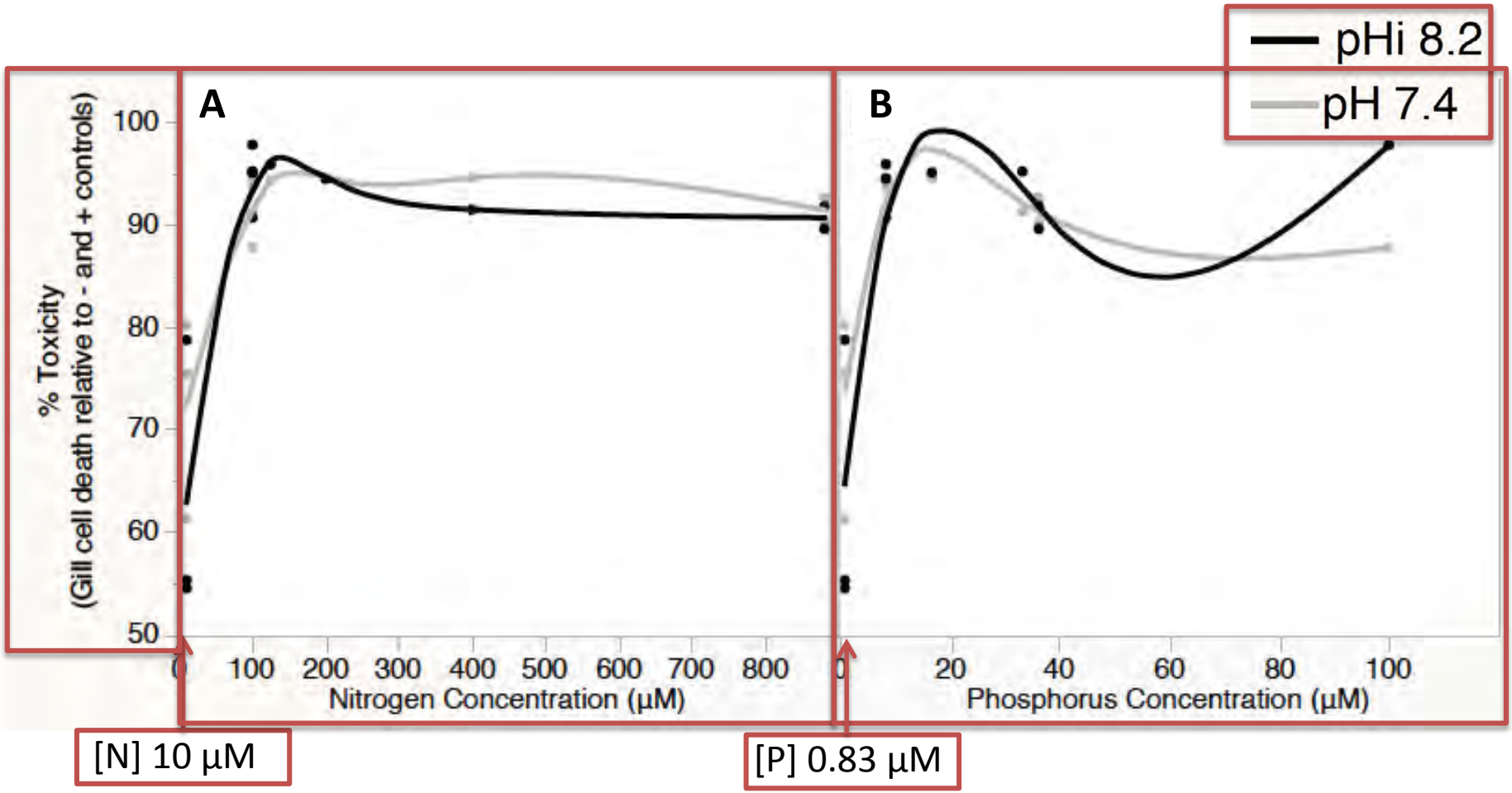
- Cells grew well on 3 forms of nitrogen
- **Resilient to changes in pH**
- Showed maintained growth rates across a range of N and P concentrations

ICHTHYOTOXICITY

Will *H. akashiwo* be toxic to fish?

Toxicity

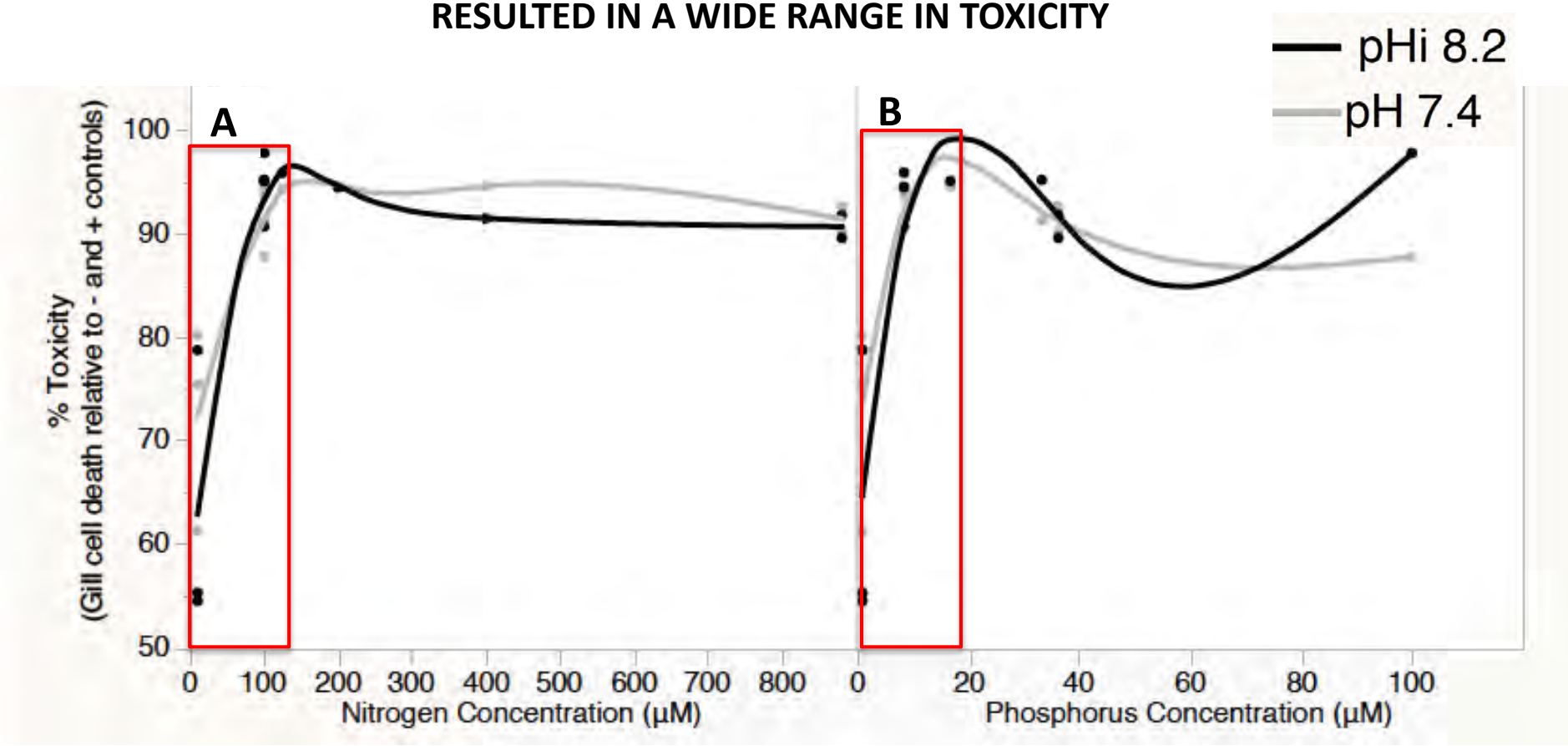
Toxicity is not enhanced at lower pH



λ of cubic spline = 0.05

Toxicity

A NARROW RANGE OF NUTRIENT CONCENTRATIONS RESULTED IN A WIDE RANGE IN TOXICITY



λ of cubic spline = 0.05

ICHTHYOTOXICITY

Will *H. akashiwo* be toxic to fish?

- Toxicity was not affected by pH
- **Showed increased toxicity across a narrow range of N and P concentrations**
- No change in growth rates despite enhanced toxicity

Conclusions

Nutrient concentration impacts toxicity; nutrients led to toxic cells.

- **A small shift in nutrient concentrations led to a wide range in toxicity**
  - A range of 0-100 µM N and 0 -20 µM P caused toxicity to increase by 50%.

***H. akashiwo* cells were resilient to changes in pH.**

- **Growth and toxicity were not impacted by lower pH**
  - For pH values tested in my study (7.4, 7.8, 8.1, 8.2)

Future conditions with high nutrients and low pH could result in continued blooms of *H. akashiwo* that are more toxic.

- Implications to the aquaculture industry (in upwelling-zones)

Acknowledgements

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Julia Matheson
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Primary Email: juliamath22@gmail.com