Lyngbya wollei, a Toxic Bloom-forming Cyanobacterium Present in Florida Springs

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Outline

• What is *Lyngbya wollei*?
• What toxins are associated with *Lyngbya*?
• Research Questions
• Necessary research
  – Bioassays *in situ*
  – Molecular characterization of *Lyngbya wollei*
• Conclusions
• Future Research
**Lyngbya wollet**

- *L. wollet* is a freshwater benthic cyanobacterium
- found in lakes, rivers, springs, and water supply reservoirs throughout southeastern US
- capable of forming thick nuisance blooms
- “nitrogen fixer”
  - can convert N$_2$ to a biologically available form of N (NH$_3$)
- proliferates in eutrophying waters
  - a potentially useful bioindicator of eutrophication
Lyngbya Bioactive Compounds

Dermatotoxic Alkaloids
• lyngbyatoxin-a
• aplysia toxin
• debromoaplysia toxin

Neurotoxins
• saxitoxins
Lyngbya
Human Health Aspects

- Cutaneous inflammation with signs of erythema, blisters and desquamation within 12 hrs of exposure
- Severe oral and gastrointestinal inflammation
- Skin tumor promoters and protein kinase C activators

Rash associated with *Lyngbya majuscula* bloom in Australia
Florida Distribution of *Lyngbya*

- Salt Spring
- Silver Glen Spring
- Juniper Spring Run
- Juniper Spring
- Fern Hammock Spring
- Silver Spring
- Crystal River/Kings Bay
- Withlacoochee River
- Rainbow Spring
- Homosassa Spring Run
- Chassahowitzka Spring Run
- Weeki Wachee Spring Run
+ Ichetucknee Spring
Lyngbya
Florida Toxins

✓ Debromoaplysiaatoxin
✓ Lyngbyatoxin-a

Others?

Courtesy: Glen Shaw & Nick Osborn / National Research Centre for Environmental Toxicology, PO Box 594, Archerfield, Qld., 4108, Australia.
Research Questions

1. Is *Lyngbya wollei* a bioindicator of eutrophication?

2. Which nutrients strongly influence the growth of *Lyngbya wollei*?

3. Is there a way to quickly identify toxic strains of *Lyngbya wollei*?
Bioassays

Bioassays

• Bioassays in situ indicate which nutrient limits growth in the organism’s natural environment.

• Potential limiting nutrients are:
  - N, P, Ca++? Fe? in L. wollei
  - P, Fe, and Ca in L. magiluscula (marine analog)

• Important for future management and decision strategies.

Bioassays in situ indicate which nutrient limits growth in the organism’s natural environment.
**L. wollei bioassays in situ**

- additions and dilutions of nutrients ($\pm N$, $\pm P$, $\pm NP$, $\pm Ca$, $+Fe$)
- primary productivity ($\Delta$ biomass, $\Delta$ chlorophyll $a$)
- acetylene reduction experiments to measure nitrogenase activity
Promoters of Lyngbya blooms

- Cowell and Botts (1994) found P limiting; Ca\textsuperscript{2+} promoted growth in *L. wolfei* cultures.
- Yin, Carmichael, and Evans (1997) found Ca\textsuperscript{2+} to promote *L. wolfei* growth and toxicity in culture.
The Bioassay Plan

- perform bioassays in Silver Glen Spring as well as Salt Spring
- begin by adding N, P, Ca$^{2+}$, and Fe; dilute P, Ca$^{2+}$
- 5 repetitions/nutrient alteration + control group
- 5 day incubation
- toxicity analyses of each nutrient treatment
Research Questions

1. Is *Lyngbya wollei* a bioindicator of eutrophication?

2. Which nutrients strongly influence the growth of *Lyngbya wollei*?

3. Is there a way to quickly identify toxic strains of *Lyngbya wollei*?
Purpose of Molecular Characterization

• to compare strains of *Lyngbya* to one another through use of the sequence of one or more genes

• *nifH* is a known gene with successful detection in *L. wollei* and other *Lyngbya* species (*L. lagerheimii, L. majuscula, L. aestuarii*)

• 16S rRNA gene is present in all bacteria
Why not use toxin genes to detect toxic species?

There are no known genes associated with toxins or toxin production in *Lyngbya*. 
Molecular characterization of *Lyngbya* species

- extract DNA from *Lyngbya* blooms
- compare samples from multiple sites based on *nifH* and 16S gene (conserved genes involved in the nitrogen fixation process and ribosomes respectively)
- compare sequences to toxin analyses of strains
Correlation between toxin-producing forms of *L. wolleii* and *nifH* and/or 16S sequence

- detection of toxic forms of *Lyngbya wolleii* possible within 2 weeks of sample collection
The Molecular Plan

- Molecular analyses on samples twice a year
- Toxin analyses on samples quarterly

Sample from 8 spring sites:
- Salt Spring
- Ichetucknee Springs
- Silver Glen Spring
- Silver Spring
- Rainbow Spring
- Crystal River Springs
- Homosassa Spring
- Weeki Wachee Spring
**nifH Sequences extracted from *Lyngbya* blooms**

(7) City Lake, High Point, NC *Lyngbya wollei*
(2) Brown’s Landing, St. Johns River, FL *Lyngbya wollei*
Murphy’s Island, St. Johns River, FL *Lyngbya wollei*
Silver Glen Springs, FL *Lyngbya wollei*
Silver Glen Sand Boil *Lyngbya wollei*
(2) Silver Spring, FL *Lyngbya wollei*
Rainbow Springs, FL *Lyngbya wollei*
(2) Withlacoochee River, FL *Lyngbya wollei*
(2) Kings Bay, FL *Lyngbya wollei*
Homossassa Spring Run, FL *Lyngbya wollei*
(2) Weeki Wachee Spring Run, FL *Lyngbya wollei*
Silver Glen Sand Boil, FL *Lyngbya wollei*

Guam
Australia
North Carolina
Florida
Other sequences

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*Lyngbya wollei* mat, Murphy’s Island, St. Johns River, FL
associated with Sheet forming *Lyngbya* Guam

*Lyngbya majuscula*, Australia
associated with *Lyngbya majuscula*, Australia
associated with *Lyngbya majuscula*, Deception Bay, Australia
associated with *Hormothamnion* Guam

*Lyngbya wollei* mat, Rainbow Springs, FL
associated with *Lyngbya wollei* mat, Chassahowitzka Spring Run, FL
associated with *Lyngbya wollei* mat, Silver Glen Springs, FL
associated with *Lyngbya majuscula*, Deception Bay, Australia
associated with *Hormothamnion enteromorphoides*, Guam

*Lyngbya majuscula*, Guam
associated with *Lyngbya majuscula* Guam
associated with Sheet forming *Lyngbya* Guam

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*Lyngbya langerheimii* GenBank
Anabaena species (outgroup)
Diverse *nifH* sequences

- Associated with *Hormothamnion enteromorphoides*, Guam
- Methanococcus jannesii
- Methanobrevibacter arboriphilicus
- Frankia sp., microaerophiles
- Microcoleus chthonoplastes
- Trichodesmium thiebautii
- Associated with *Lyngbya majuscula*, Guam
- Synechococcus sp.
- Gloethece sp.
- Nostoc commune
- Fischirella sp.
- Anabaena azollae
- Anabaena oscillarioides
- Lyngbya wollei, Silver Glen Spring Sand Boil, FL
- Lyngbya wollei, City Lake, NC
- Cyanothece sp.
- Phormidium sp.
- Nostoc commune
- Lyngbya majuscula, Guam
- Lyngbya lagerheimii
- Dermocarpa sp.
- Calothrix sp.
- Pleptonema boryanum
- Chromatium purpuratum
- Acauligenes faecalis
- Vibrio diazotrophicus
- Azotobacter vinelandii
- Azotobacter chroococcum
- Klebsiella pneumoniae
- Bradyrhizobium sp.
- Rhizobium melliloti
- Azotobacter vinelandii
- Rhodobacter capsulatus
- Rhodospirillum rubrum
- Clostridium pasteurianum
- Clostridium cellulosi
- Associated with *Lyngbya majuscula*, Deception Bay, Australia
- *Lyngbya* majuscula, Australia
- *Lyngbya* majuscula, Australia
- Associated with *Hormothamnion enteromorphoides*, Guam
- Associated with *Lyngbya majuscula*, Deception Bay, Australia
- Associated with *Lyngbya majuscula*, Australia
- Associated with *Lyngbya majuscula*, Australia
- Associated with *Lyngbya majuscula*, Australia
- Associated with *Lyngbya majuscula*, Australia
- Associated with *Lyngbya majuscula*, Australia
- Desulfovibrio gigas
- Desulfovibrio gigas
- Associated with *Lyngbya wollei*, Silver Glen Spring, FL
- Associated with *Lyngbya wollei*, Chassahowitzka Spring Run, FL
- Associated with *Lyngbya wollei*, Rainbow Spring, FL
- Associated with *Lyngbya wollei*, Murphy’s Island, St. Johns River
- Associated with *Lyngbya wollei*, Chassahowitzka Spring Run, FL
- *Desulfoverna limicola*
Conclusions

- *L. wollei* is present in Florida springs and contains toxins.
- In situ bioassays of *L. wollei* are necessary for wise management practices.
- Molecular characterization research will bring about quick detection of *L. wollei* and possibly of toxic strains.
Future Research

- Bioassays in Florida springs

- Molecular characterization of *L. wolleii* with *nifH* gene as well as 16S gene

- Compare analyses of toxins found in specific strains with the gene sequences
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