Single-cells breaking off of the *Microcystis*?
Cyanos to know.... “Annie, Fannie and Mike”.... What about “Pike”?  

**Aphanizomenon**

Anabaena  Microcystis  Picocyanobacteria

Lake Attitash

40x
Picocyanobacteria

*Aphanocapsa*, potential producer of microcystins

Brazil dialysis (Domingos, 1999 and Azevedo, 2001)

Picoplankton forming colonies in the presence of grazers? Passoni, 2000
Plankton defined by size

Whole lake water
- Net plankton (> 50 μm)
- Nanoplankton (2-50 μm)
- Picoplankton (0.2-2.0 μm)

If picocyanobacteria (such as *Synechococcus* spp.) are toxic, then filtrates and filters from “fractionation” of whole lake water could be analyzed to target different sizes of cyanobacteria.

Filters and filtrates could be processed and analyzed for pigments and cyanotoxins.
A potential method for monitoring cyanobacteria/microcystins from already established methods (chlorophyll filtration <0.45 um).

This study revealed that a high percentage of microcystins in New England Lake water were in the dissolved fraction (in the form of extracellular cyanotoxins).

Extracellular toxins can occur due to natural senescence; due to light, grazers, and/or time of bloom degradation.

Typically, extracellular MCs will persist in the water column longer than intracellular toxins.
Picocyanobacteria are a more likely source of food for zooplankton than net colonies or filaments of cyanobacteria that are difficult to filter.

Are picocyanobacteria potentially toxic too?
Autofluorescence
Techniques in Epifluorescence

The microscope has two filter sets for epifluorescence; each includes an emitter, dichroic mirror and exciter:

1.) The “green” cube excites at 435 nm and excites the “green window”, which includes a broader range of the emitted chlorophylls.

2.) The “PC” cube excites the cells with a wavelength of 572 and emits in the range of 605-630 nm.

These cubes were especially chosen to target the autofluorescence of photosynthetic picoplankton. Therefore, the first cube allows for viewing a wide range of chlorophyll and the second cube allows for viewing those with phycobilin pigments, phycocyanin and phycoerythrin.
The use of phycocyanin for determining cells of cyanobacteria

Overestimates due to contribution of single cells (not counted because went through the net (50um))? 

Comparing net counts to fluorescence signals...

Overestimates due to CDOM?
Equipment and techniques that aid in determining relative concentrations of cyanobacteria by measuring phycocyanin:

1. YSI (600 OMS) phycocyanin probe
2. YSI EXO sonde (correction for FDOM)
3. Turner hand-held Aquafluor (RFU of chlorophyll and phycocyanin)
YSI has many design to choose from. Other companies have versions of this as well.

YSI 6600 or 600 (equipped with PC probe) does not correct for CDOM and cause an artificially high level if color is significant in the water (stained lakes will have higher levels)

Potential “quench factor”
CDOM or blooms
Hand-held Devices

Turner offers a hand-held device for PC and Chl. The small cuvette only allows for about 3 ml of water. Not much sample is needed and a Relative Fluorescent Unit (RFU) is reported. Cheaper and convenient. Good, quick method for determining relative concentrations of cyanobacteria. Reasonable and easy to use for lay monitors.
Citizen-based Cyanobacteria Monitoring Program

Sampling protocols and services provided by the Center for Freshwater Biology (UNHCFB CCMP):

- Cyanobacteria Lake Monitoring
- Cyanotoxins in Drinking Water

The Center for Freshwater Biology at the University of New Hampshire initiated a Citizen-based Cyanobacteria Monitoring Program (CCMP) in 2010, providing assistance to Lake Associations and Drinking Water Facilities in the tracking of cyanobacteria and microcystins in various bodies of water. Protocols and services can be accessed by clicking the links at the left of the panels.

Please contact Amanda Murby for questions or assistance.
1. Monitoring Lake Water

2. Monitoring Drinking Water
Monitoring Lake Water:

1. Lake Water Quality
2. Bloom Watches

Samples analyzed for Phycocyanin and Microcystins
Monitoring Drinking Water:
1. Untreated (raw)
2. Treated (specific to facility)

Samples analyzed for Phycocyanin and Microcystins

UNH CFB Protocol for the Monitoring of Cyanobacteria & Microcystins in Drinking Water:

1. Water collections should be sampled from both treated and untreated (raw) water. You may also choose to sample water from other stages of the treatment if desired.
2. Rinse the HDPE bottle (1 liter) with a small amount of sample water before collection and clearly label each bottle.
3. The HDPE sample bottle should be filled ¾ to allow for expansion when frozen.
4. Place the samples on ice and in the dark until delivery to UNH CFB lab.
5. Freeze the sample if delivery/drop-off time exceeds 12 hours.

Analyses:
- Samples will be analyzed for the concentration of the liver toxin, microcystin, using the Envirotek, Quantiplate-ELISA Kit (Portland, Me) with increased sensitivity (UNH, CFB). Results will be reported as ng microcystins per liter.
- Phycocyanin fluorescence (a pigment characteristic of cyanobacteria) will be determined and converted to equivalent Microcystis aeruginosa cells mL⁻¹.

Deliver to:
Dr. Jim Haney, Center for Freshwater Biology
38 Academic Way, Spaulding G28 (mail) or Spaulding 116 (in person)
Durham, NH 03824

Contacts:
- cfb.unh.edu
- 603-862-2105: “Haney Lab”
- Dr. Jim Haney: jfhaney@unh.edu
- Amanda Murby: amurby@unh.edu
**General Procedure:**
1. Samples were received in 250 ml containers
2. Samples were integrated in the lab and mixed thoroughly
3. Fluorescence of phycocyanin was measured using a Turner Design hand-held Fluorometer (fluorescence was to determine a relative concentration of cyanobacteria)
4. Samples were frozen and thawed in triplicate to lyse cells
5. Water samples were concentrated 10-fold by lyophilization
6. Microcystin concentrations were determined using the Envirologix Quantiplate Kit for Microcystins, Portland, ME (tests results are equivalent to all variants of microcystin and nodularins*)
7. Detection range (with standards) between 25 and 2500 ng MC L-1
   
   (lower limit of detection for concentrated water (x10) is therefore 2.5 ng MC L-1)

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*What about other cyanotoxins?
CCMP Reports: Detailed reports to help explain the results

1. Summary
2. Specific results
3. Figures and tables to show data
4. References to guidelines on cyanobacteria and toxins (MCs)
**UNH Center for Freshwater Biology**
**LAKES LAY MONITORING PROGRAM**
**CYANOBACTERIA MONITORING DATA SHEET (2013)**

**MONITOR NAME:** __________________________
**DATE:** __________________________
**LAKE NAME:** __________________________
**SITE NAME:** __________________________
**SAMPLE DEPTH:** __________________________
**SAMPLE VOLUME:** __________________________
**SAMPLE METHOD:** __________________________

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Pigment analyses for relative concentrations of primary productivity (cyanobacteria and other phytoplankton):

**YSI Multi-parameter probe (6600 M V2/ 600 OMS)**
**Last Calibration:** __________________________

**Turner Design™ Aquaflour Hand-held Device**

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<th>Replicates</th>
<th>Phycocyanin (cells/mL)</th>
<th>Chl α (μg/L)</th>
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*Phycocyanin Fluorescence (equivalent to UTEX #2385, Microcystis aeruginosa cells ml⁻¹)*

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<th>Replicates</th>
<th>Sample</th>
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### Potential Toxicity

Is it good enough for now??  Good to know??

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<tr>
<th>Genus</th>
<th>Microcystin (Hepatotoxin)</th>
<th>Anatoxin-A (Neurotoxin)</th>
<th>BMAA (Neurotoxin)</th>
<th>Dermatotoxin</th>
<th>Cyanobacteria in sample (Yes/No)</th>
<th>Abundance (#/mL)</th>
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****This is not a complete list of cyanobacteria and their potential toxins****  * Indicates unknown
Steps taken for water samples: CFB group 2013

1. Receive complaint and water sample
2. Record location and methods for sampling
3. Measure the phycocyanin and chlorophyll levels (YSI probes, Turner Hand-held, Algae Torch)
4. Microscopic identifications
5. Photos of cyanobacteria
6. Notes on other plankton or parameters
7. Addresses to potential toxicity
Services and Education

- Responding to concerned lake users
- Training students to identify and analyze cyanobacteria
- Integration of cyanobacteria abundance and diversity with lake water quality monitoring
- Outreach and education on the potential problems associated with cyanobacteria

Goals

- Address public concerns on cyanobacteria and lake water quality
- Determine long term trends of cyanobacteria for specific lakes to better understand the ecology of the system
- Future advances in technology that allow for rapid *in situ* determination of cyanotoxins on a variety of scales.
Best feature of the Algae Torch is that it gives rapid, relative percentages of cyanobacteria in the water. It can be used in the field along the shore or in the lab with a water sample.
Summary

• There must be a service for analyzing potentially toxic cyanobacteria

• Outreach to public is important for recognizing and being aware of cyanobacteria

• Cyanobacteria must be monitored with other lake water parameters to track changes and trends within specific lakes

• Cyanobacteria coupled with lake water quality enhances our understanding of lake ecology and overall water quality

• There are many approaches or methods, identification (Size and Morphology) and abundance is important in understanding the potential cyanobacteria issues in any given water body.