# Case studies: Application of DNA-based tools for cyanobacterial monitoring

June 29, 2017

Bend Genetics

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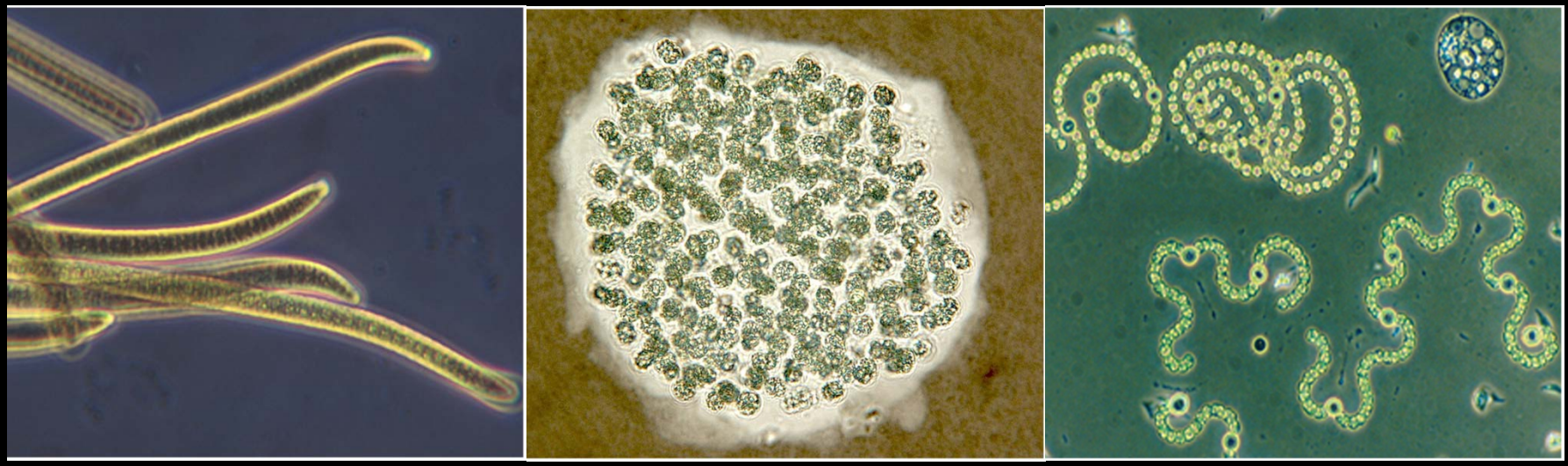
# Presentation overview

Brief overview of cyanobacterial harmful algal blooms (CHABs) and their ecological and human health effects

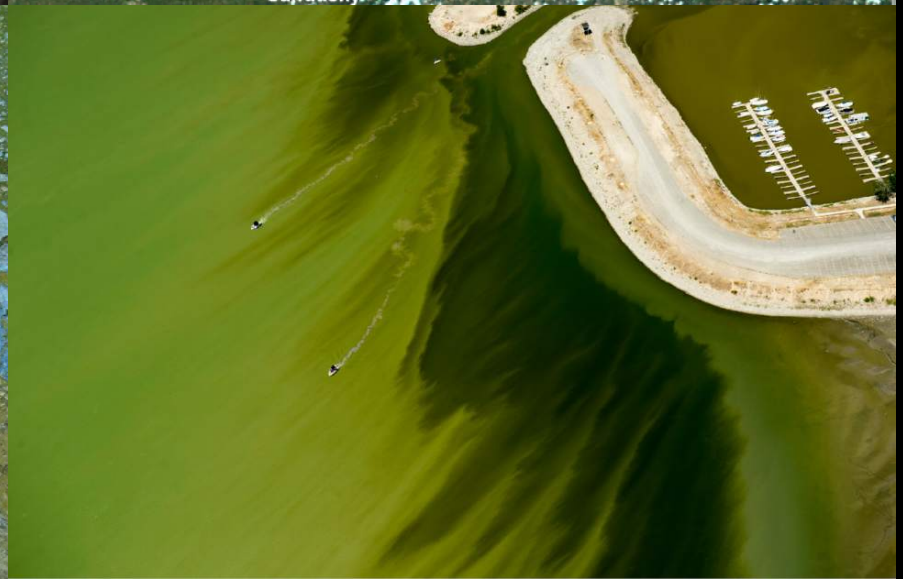
Principle of real-time quantitative polymerase chain reaction

Examples of QPCR as part of a tiered monitoring framework

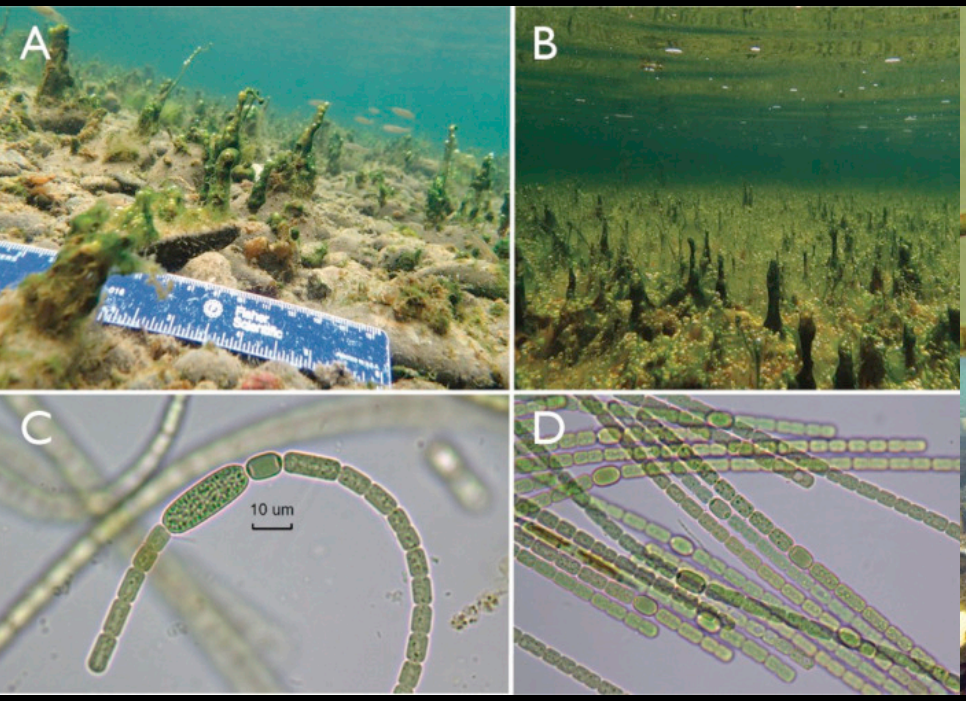
Sample collection procedures and the Pros & Cons of QPCR



# CyanoHABs are an increasingly common occurrence in many freshwater systems

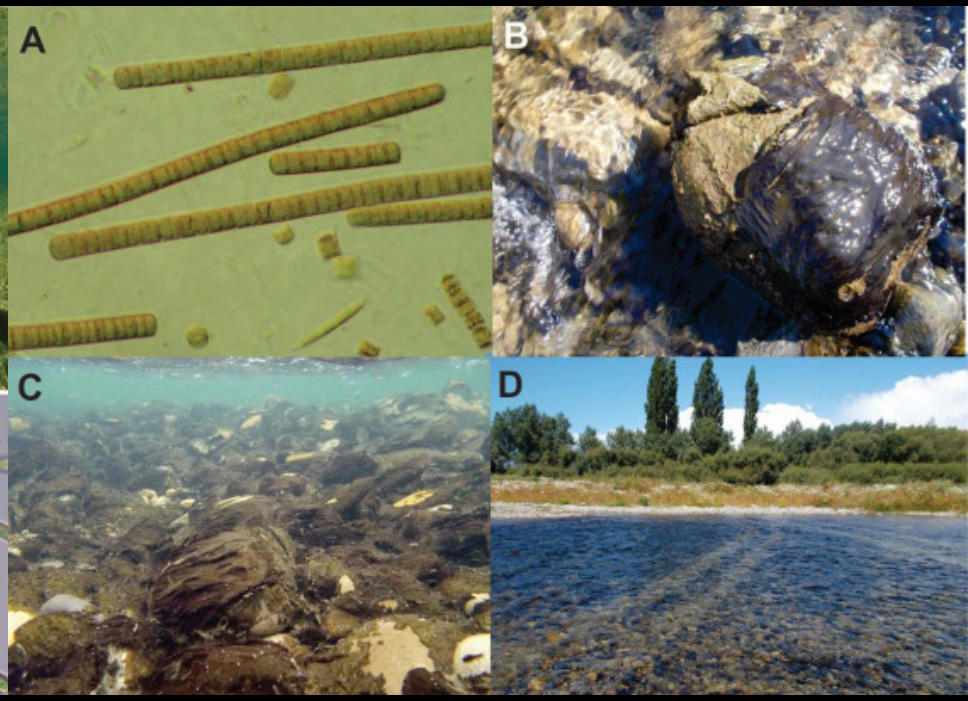



# Benthic & periphytic CyanoHABs




Benthic *Anabaena* sp. – Eel River, CA

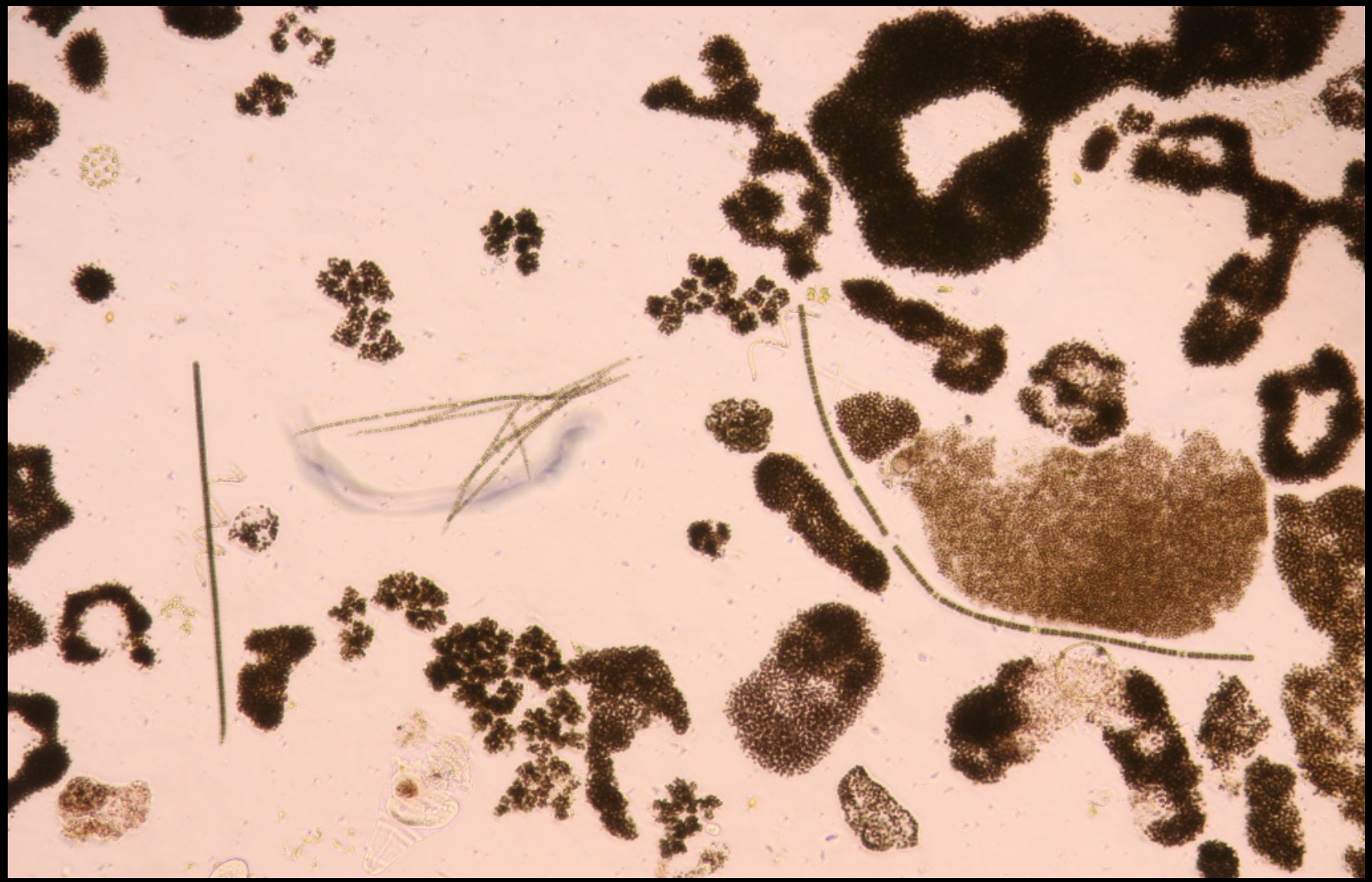
Bouma-Gregson et al., 2017.
Harmful Algae 66:79-87




Benthic *Phormidium* sp. – New Zealand

McAllister et al., 2016.
Harmful Algae 55:282-294.

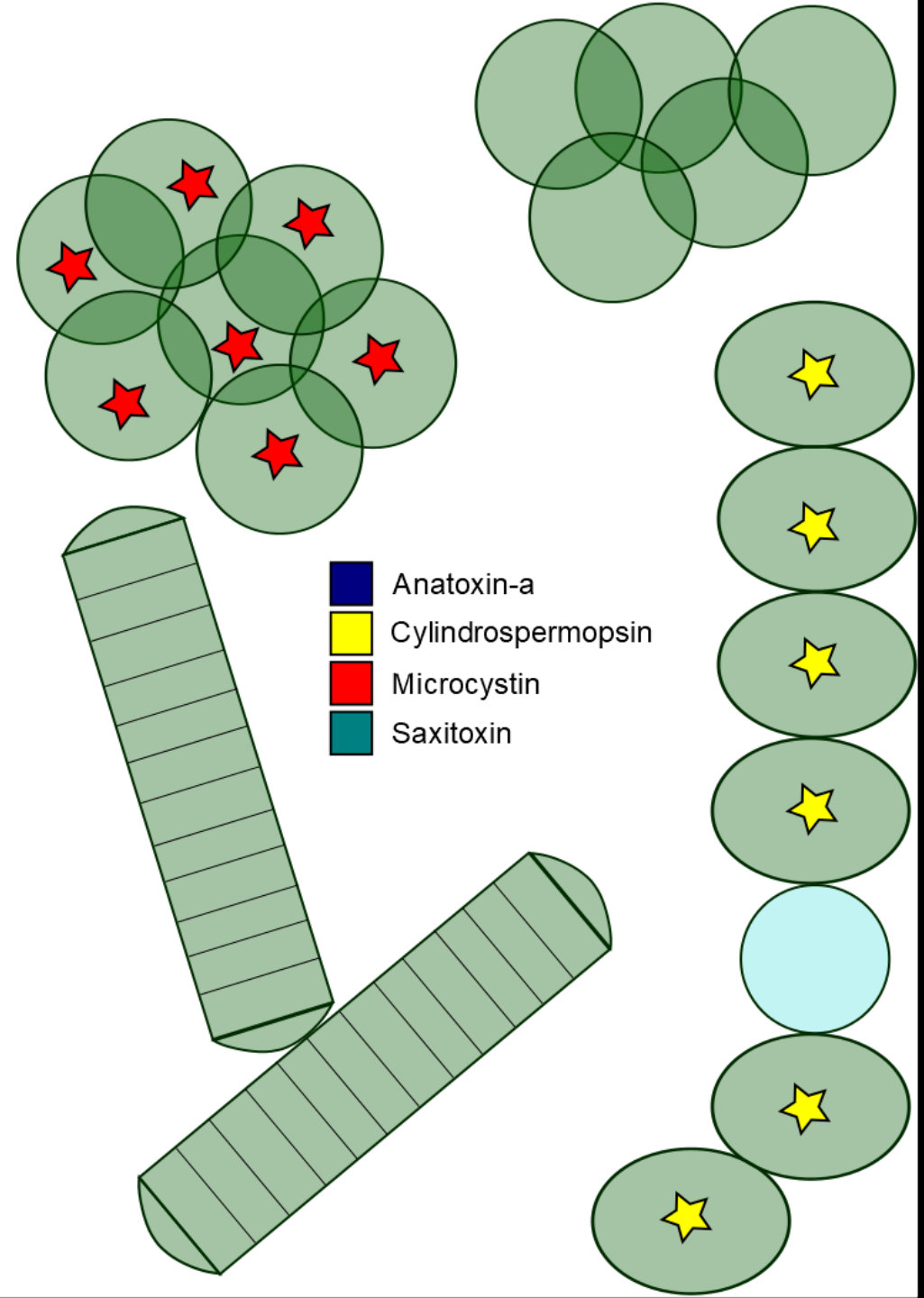
# Different CyanoHAB taxa present different cyanotoxin risks



# Potential toxins produced by common cyanobacterial genera

| Cyanobacterial Genera | Anatoxin-a | Cylindrospermopsin | Microcystin | Nodularin | Saxitoxin |
|---|---|---|---|---|---|
| *Aphanizomenon* | X | X | | | X |
| *Anabaena/Dolichospermum* | X | X | X | | X |
| *Cylindrospermopsis* | X | X | | | X |
| *Fischerella* | | | X | | |
| *Gloeotrichia* | | | X | | |
| *Lyngbya* | | | | | X |
| *Microcystis* | | | X | | |
| *Nodularia* | | | | X | |
| *Nostoc* | | | X | | |
| *Oscillatoria* | X | | X | | |
| *Phormidium* | X | | | | |
| *Planktothrix* | X | | X | | X |
| *Pseudanabaena* | | | | | |
| *Raphidiopsis* | X | X | | | X |

# QPCR “peers” into a cell’s genome

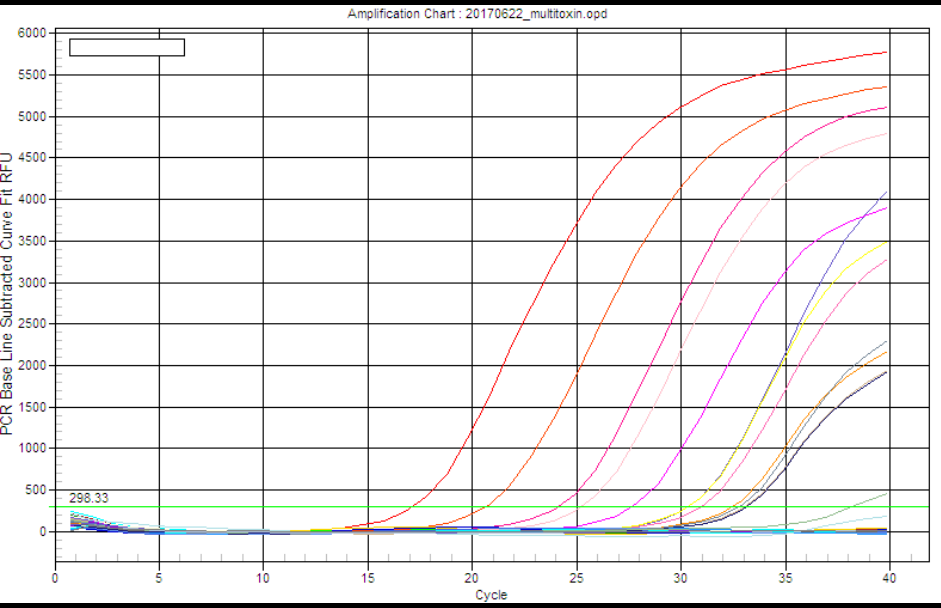


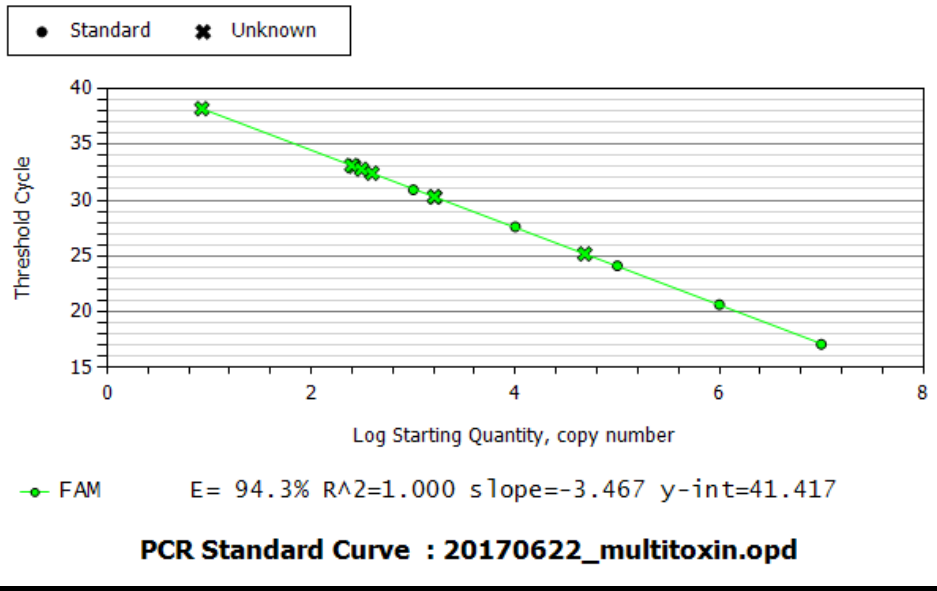

- Toxicity is a strain-specific trait
- Only cells with toxin genes can produce toxin
- Cells with toxin genes tend to use them (i.e., expression stays turned on)
- QPCR can be used to quantify cyanotoxin gene concentrations
- Because the majority of toxin occurs intracellularly, gene abundance correlates well with cyanotoxin concentration

# Overview of PCR-based tools

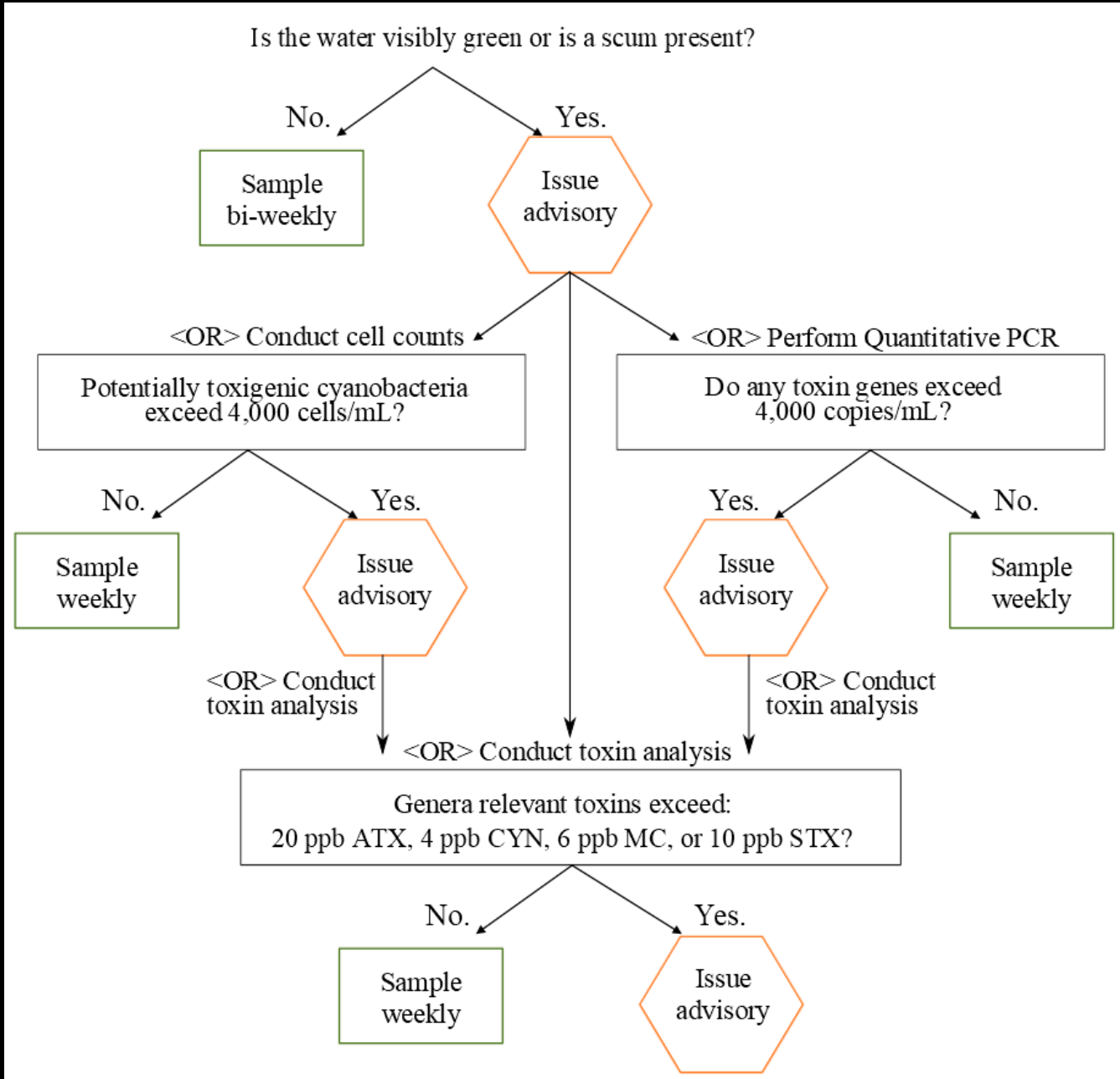
- Polymerase Chain Reaction (PCR) – the amplification of specific DNA sequences using complementary synthetic DNA molecules (primers)
  - Sequence information is required in order to design assays
  - Assays can be designed to be strain-specific or universal

- Real-Time Quantitative PCR (QPCR) – same concept as regular PCR, but includes a fluorescent dye or probe allowing for absolute quantification of gene copies
  - Assumes gene copies/mL equivalent to cells/mL for single copy genes targeted

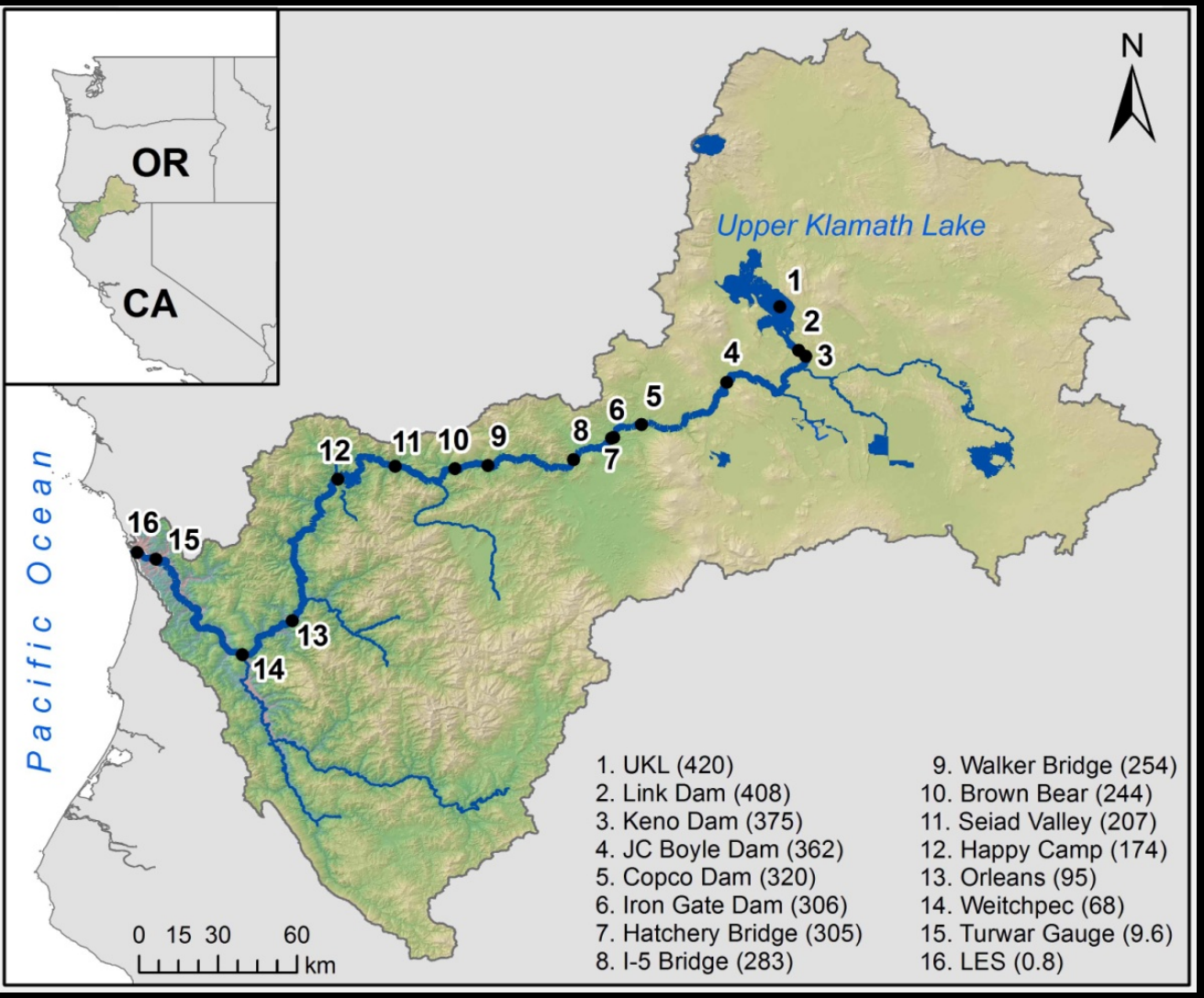


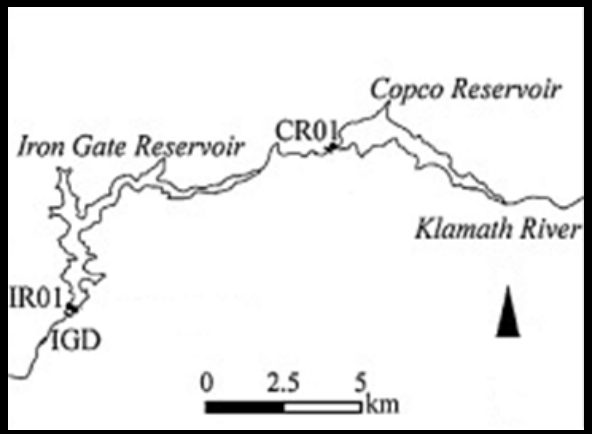



# QPCR as part of a tiered monitoring approach

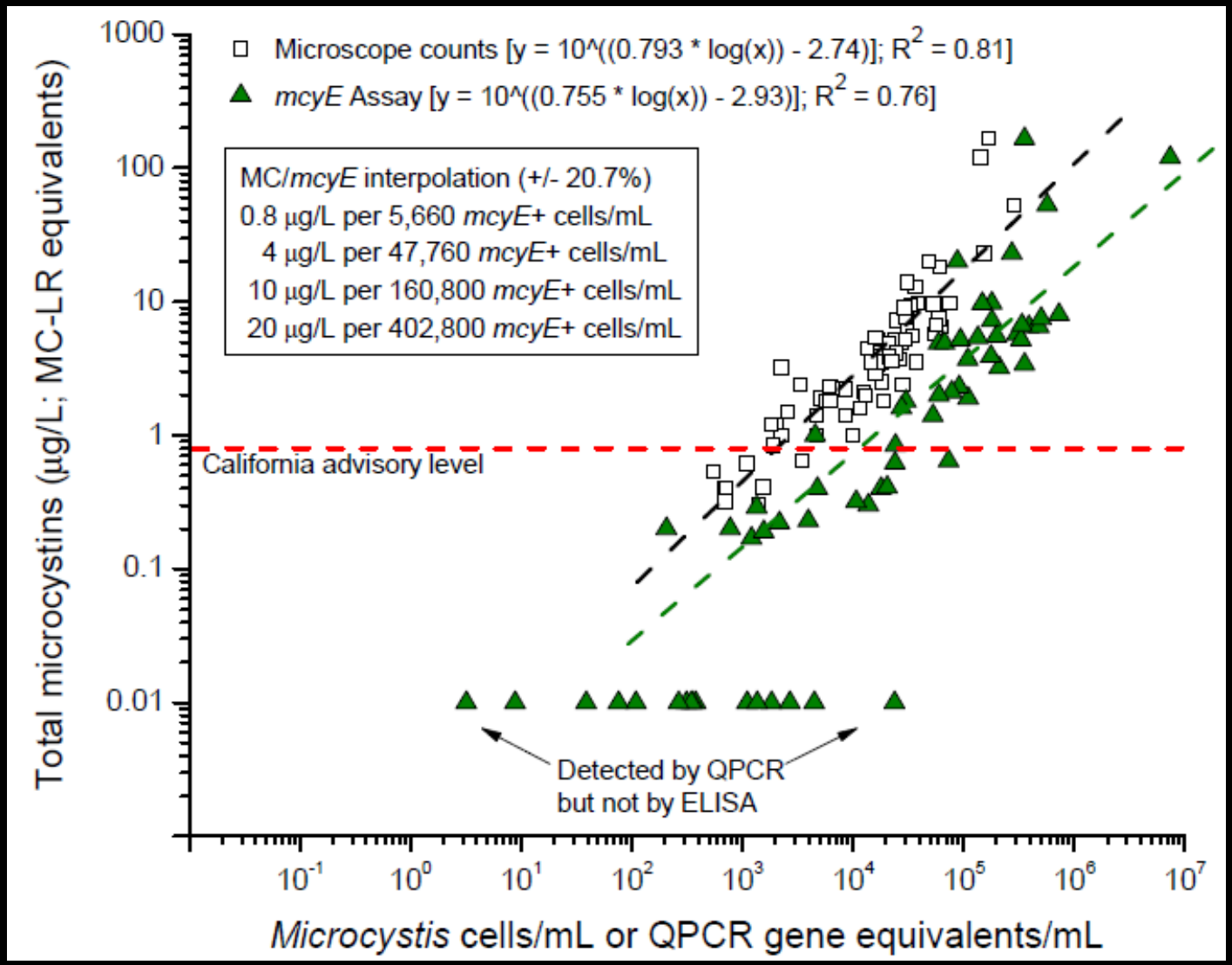


# Use of QPCR to assess the toxicity and distribution of Klamath River *Microcystis* sp. blooms



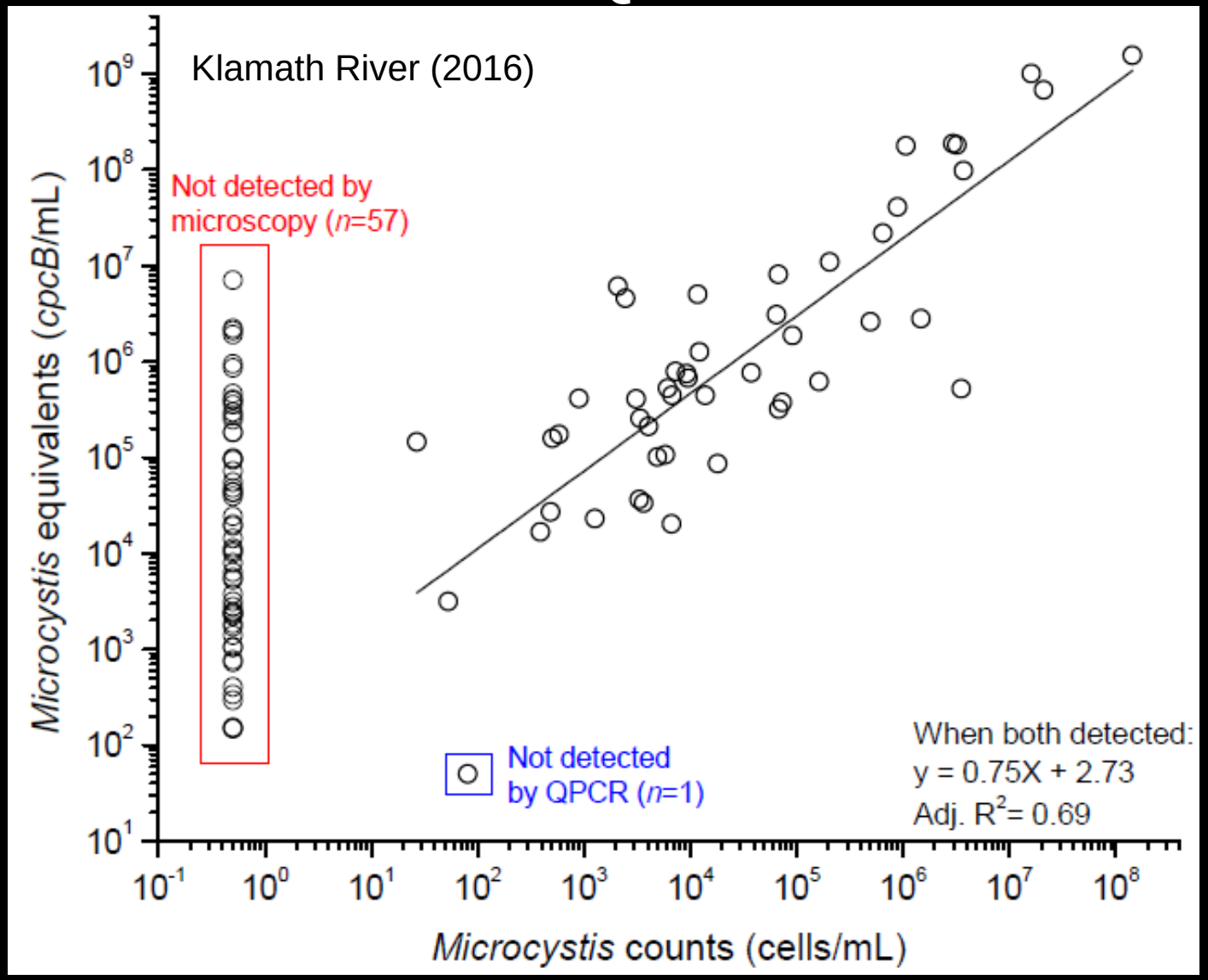
# Comparison of methods - Microcystins vs QPCR (*mcyE*) estimates




All samples were 0.5 m grab samples

Otten et al., 2015. Harmful Algae 46:71-81.

# Comparison of methods - *Microcystis* cell counts vs QPCR estimates




The half-life of DNA in surface water is ~12 hours

Otten, *in prep*.

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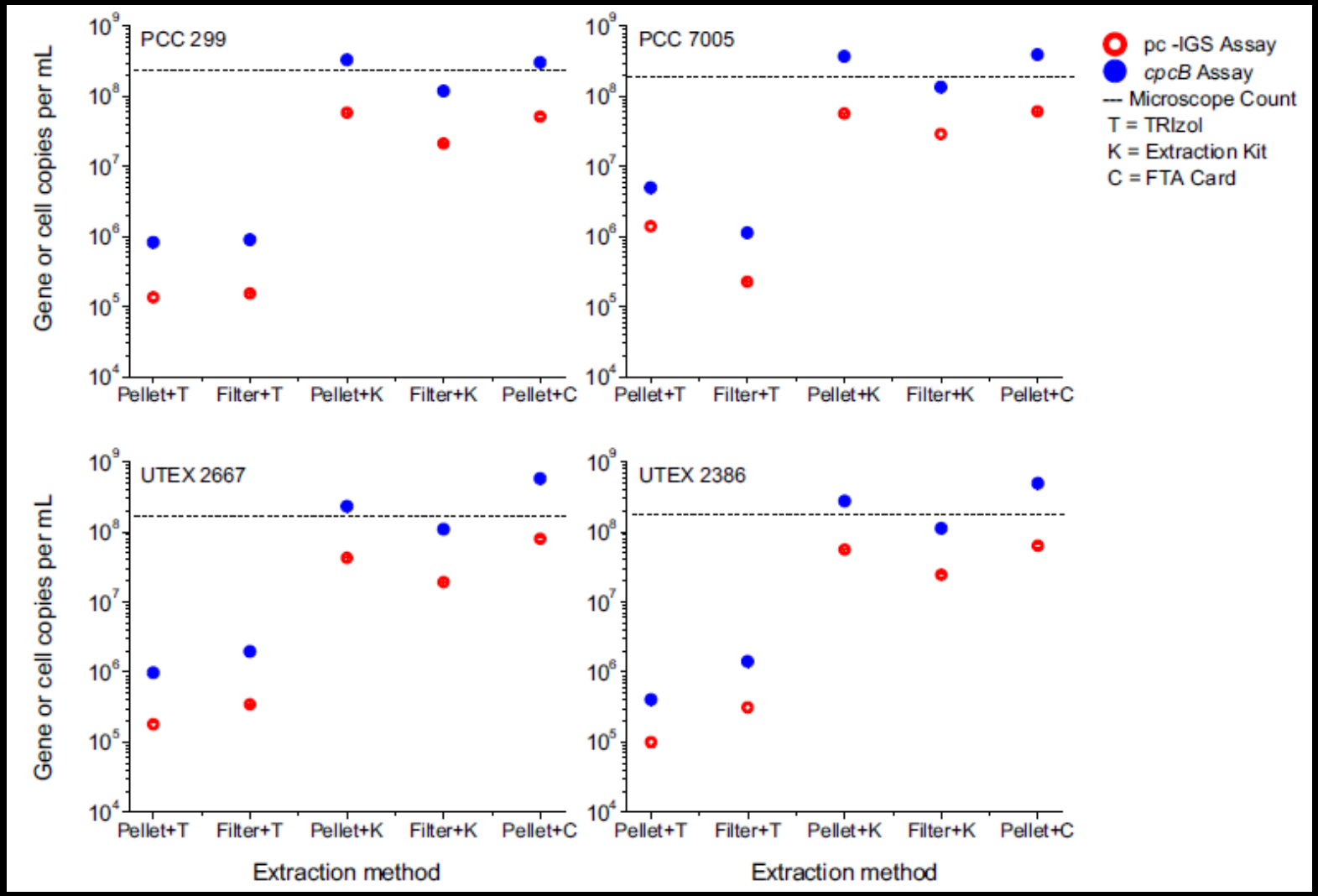


Discrepancy between environmental counts and QPCR estimates not likely explained by (i.e., genome copy number)

Otten et al., 2015. Harmful Algae 46:71-81.

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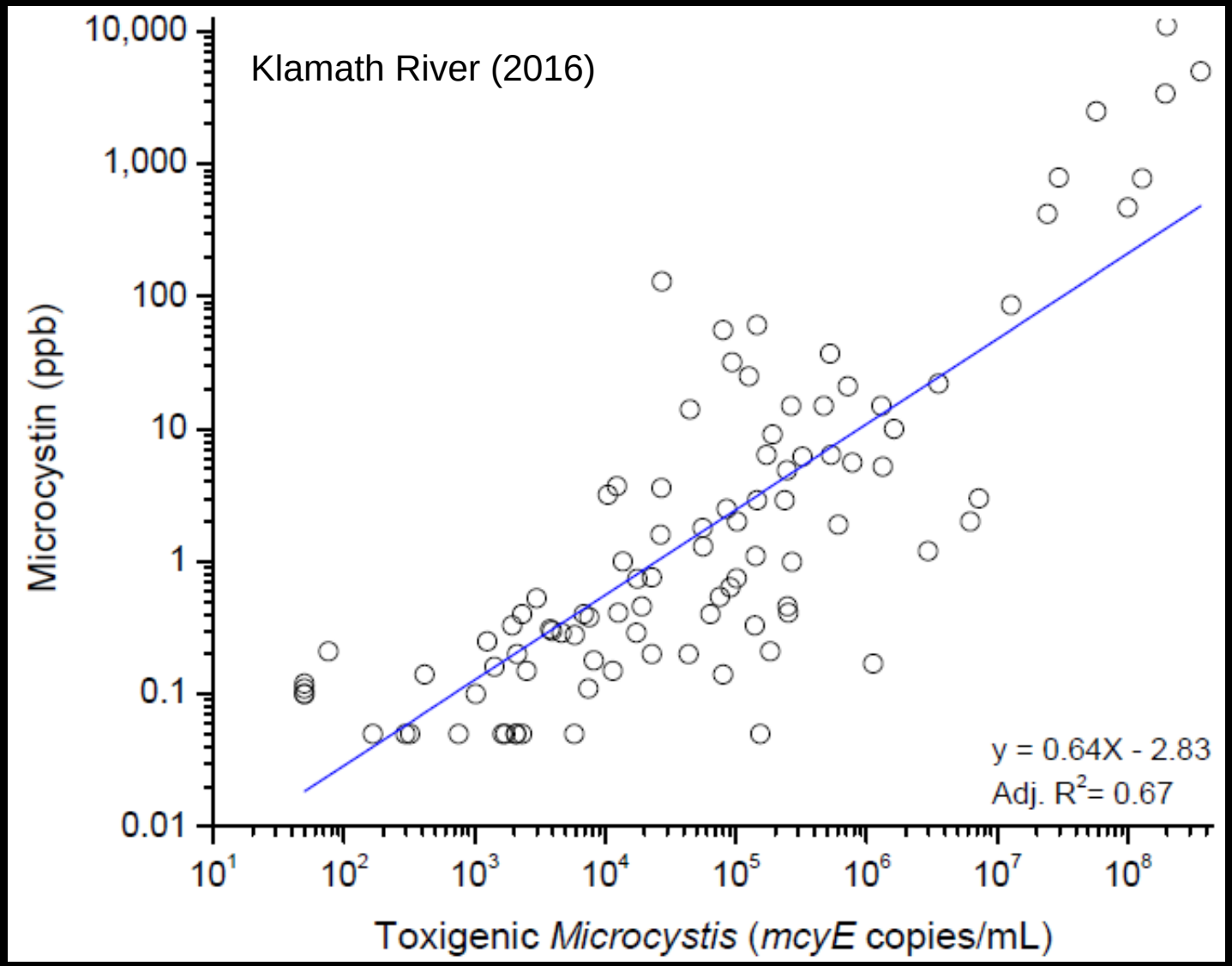



Otten, *in prep*.

# Sample collection & archival

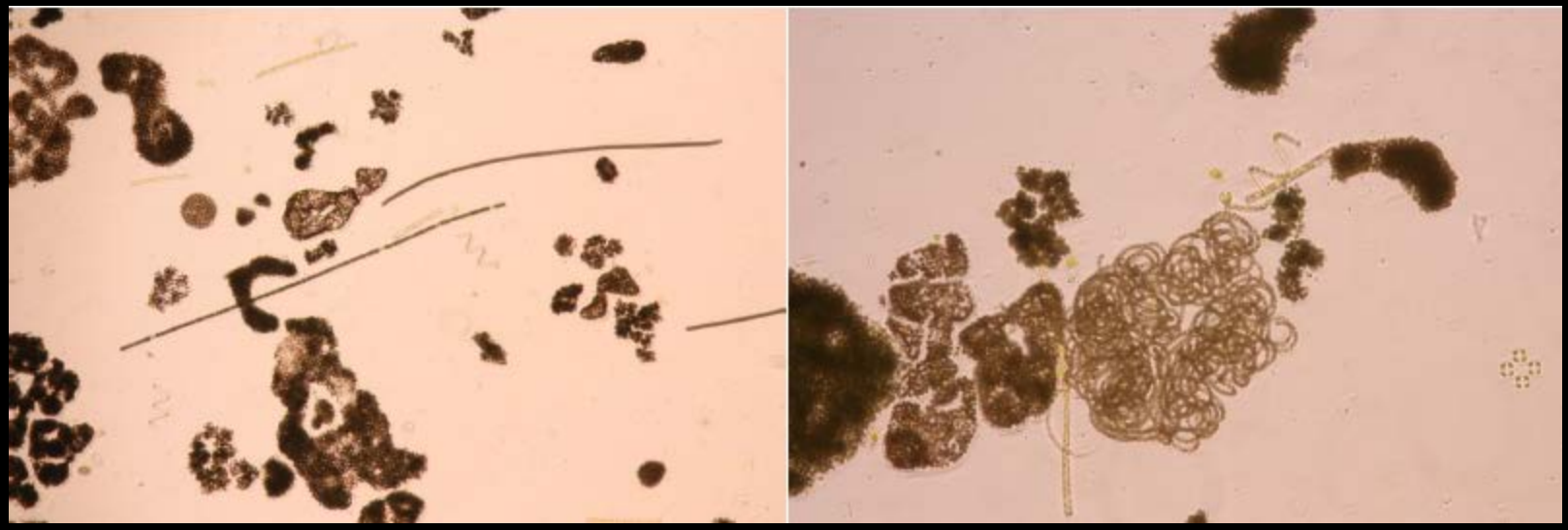
- Collect water sample and concentrate by vacuum filtration
  - Filter type is not critical, glass fiber or membrane filters work
  - Larger pore sizes (e.g., < 1 µm) will selectively retain cyanobacteria and other algae
  - Small pore sizes (e.g., 0.2 µm) retain all bacteria
- Don’t freeze water samples before filtering
- Record volume filtered, required for quantification
- Store filters in microcentrifuge tubes at -20ºC
  - Samples can be archived for years

# Pros & Cons of QPCR testing

- Pros
  - Faster than cell counting (2-3 hours from start to finish)
  - High throughput (40+ samples per analysis batch)
  - High sensitivity and specificity
  - DNA signal is amplified → good for early detection
  - Genes are better correlates of toxin than cell density
  - Cheaper than cell counting or toxin testing
  - Amenable to other targets (e.g, fecal bacteria)

- Cons
  - Not a true substitute for toxin testing → tiered strategy
  - Cells must be intact to collect their DNA
  - Not useful on finished drinking water
  - Requires specialized equipment and training

# Thanks for your attention!



Please feel free to contact me with any questions.

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