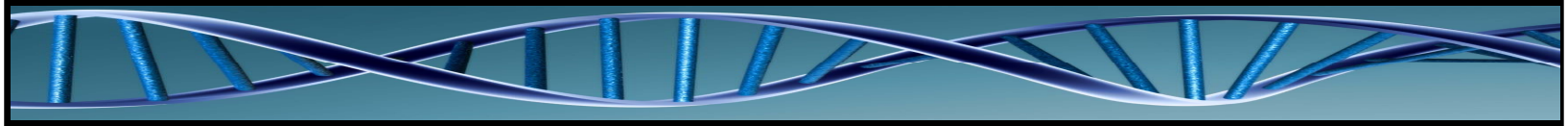
Application of DNA-based tools for algal bloom monitoring



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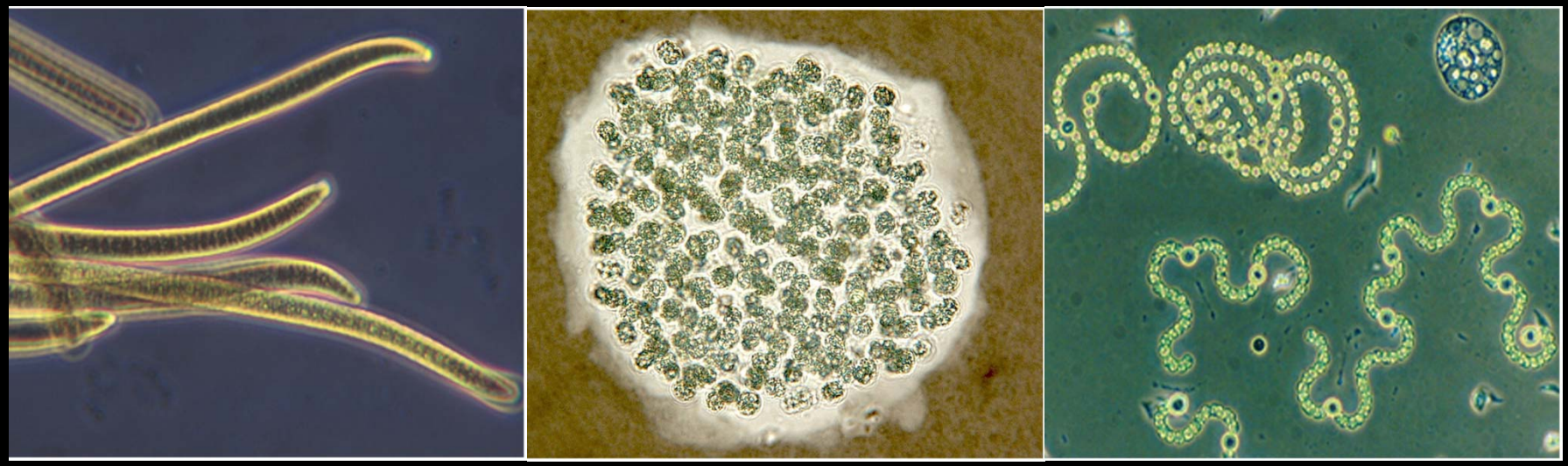
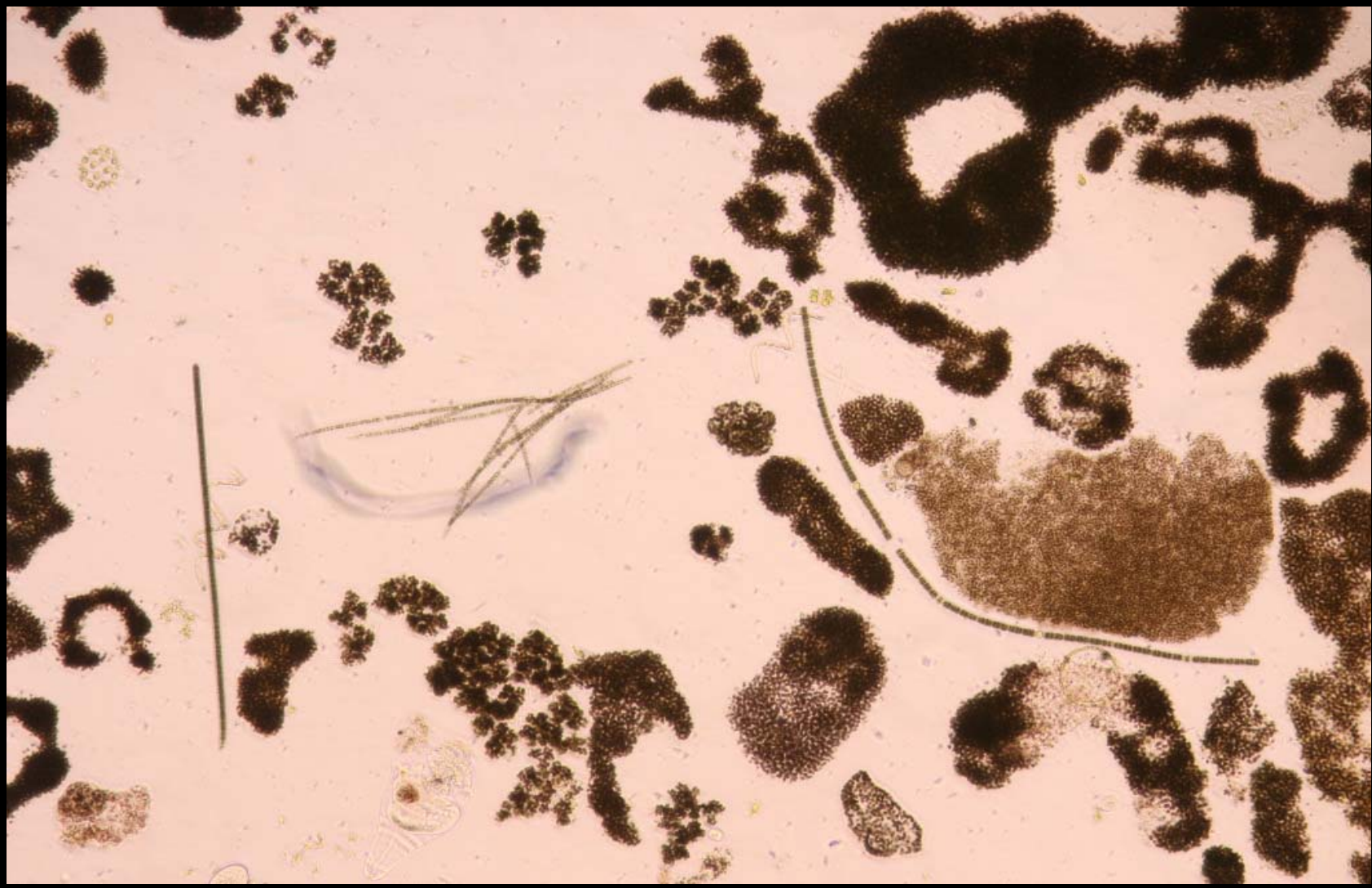
Presentation overview

Case studies to illustrate how different molecular tools can be used for monitoring and risk assessment and interpretation of these types of data

- Real-time quantitative PCR (QPCR)
- Amplicon sequencing (DNA barcoding)
- Shotgun metagenomics



Variable risks associated with certain CyanoHAB taxa or from mixed assemblages



Overview of common genetic tools

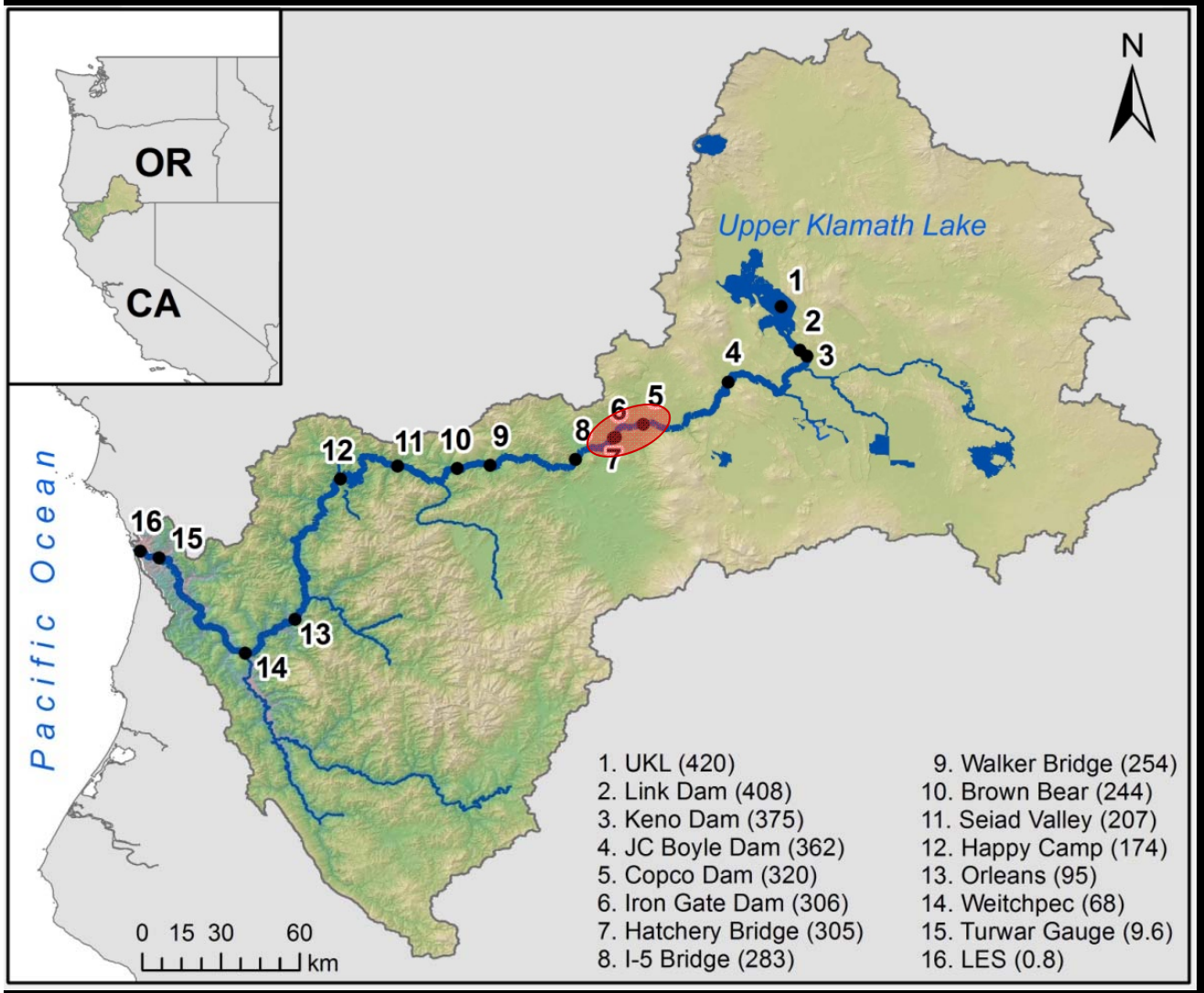
- Polymerase Chain Reaction (PCR) – the amplification of specific DNA sequences using complementary synthetic DNA molecules (primers)
  - Sequence information is required in order to design assays
  - Assays can be designed to be strain-specific or universal

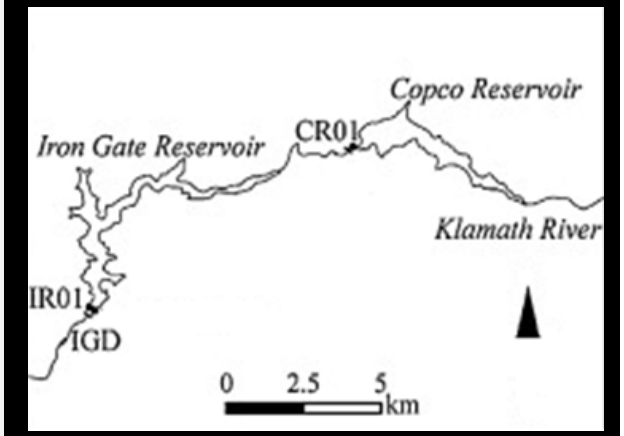
- Amplicon Sequencing – PCR products (amplicons) are barcoded (indexed), then deeply sequenced in parallel
  - Used to estimate the relative abundance of targeted organisms in each sample
  - Not a practical method for public health monitoring, but great for research

- Real-Time Quantitative PCR (QPCR) – same concept as regular PCR, but includes a fluorescent dye or probe allowing for absolute quantification of gene copies
  - Used in a tiered monitoring framework; high throughput & quick time to results
  - Assumes gene copies/mL equivalent to cells/mL for single copy genes targeted

- Shotgun metagenomics – DNA molecules are fragmented and massively sequenced without any amplification step. The generated sequences are assembled into larger fragments of individual genomes.
  - Longer time required for analysis, but useful for assigning specific functions (e.g., toxin or taste-and-odor production) to specific organisms → Culture-free method

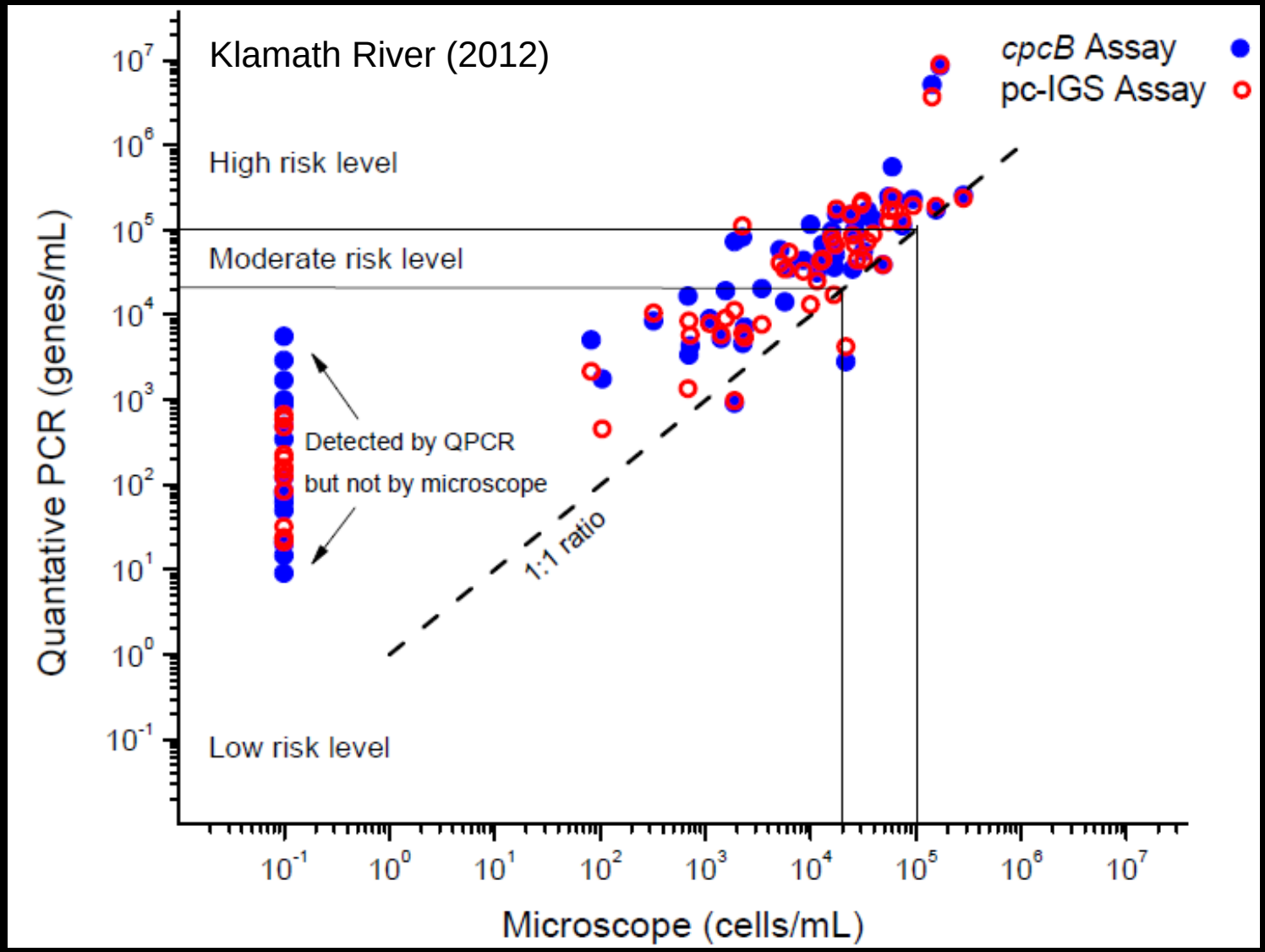
Genetics of Klamath River *Microcystis* blooms





Visual evidence for co-occurring strains

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



All samples were 0.5 m grab samples

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

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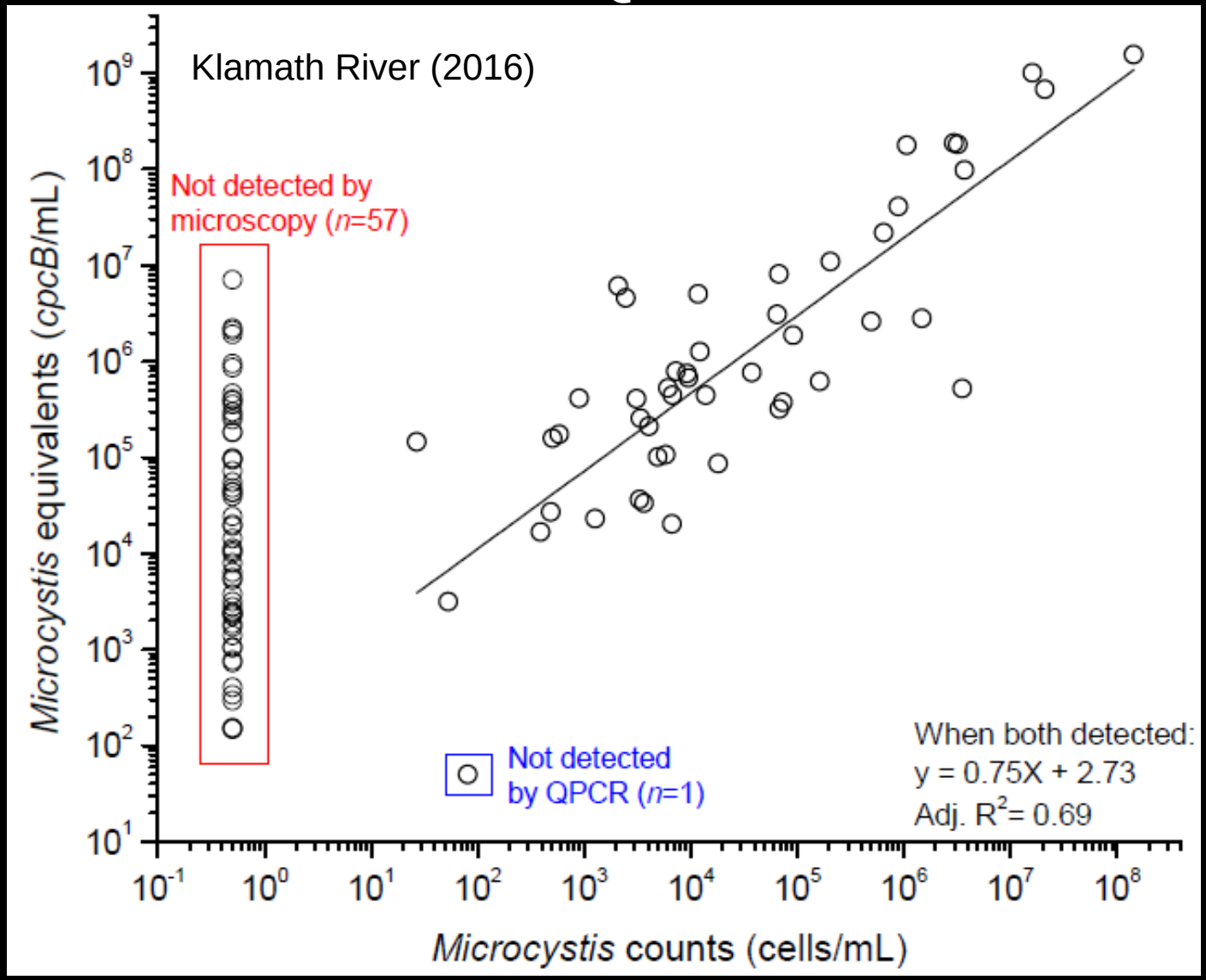

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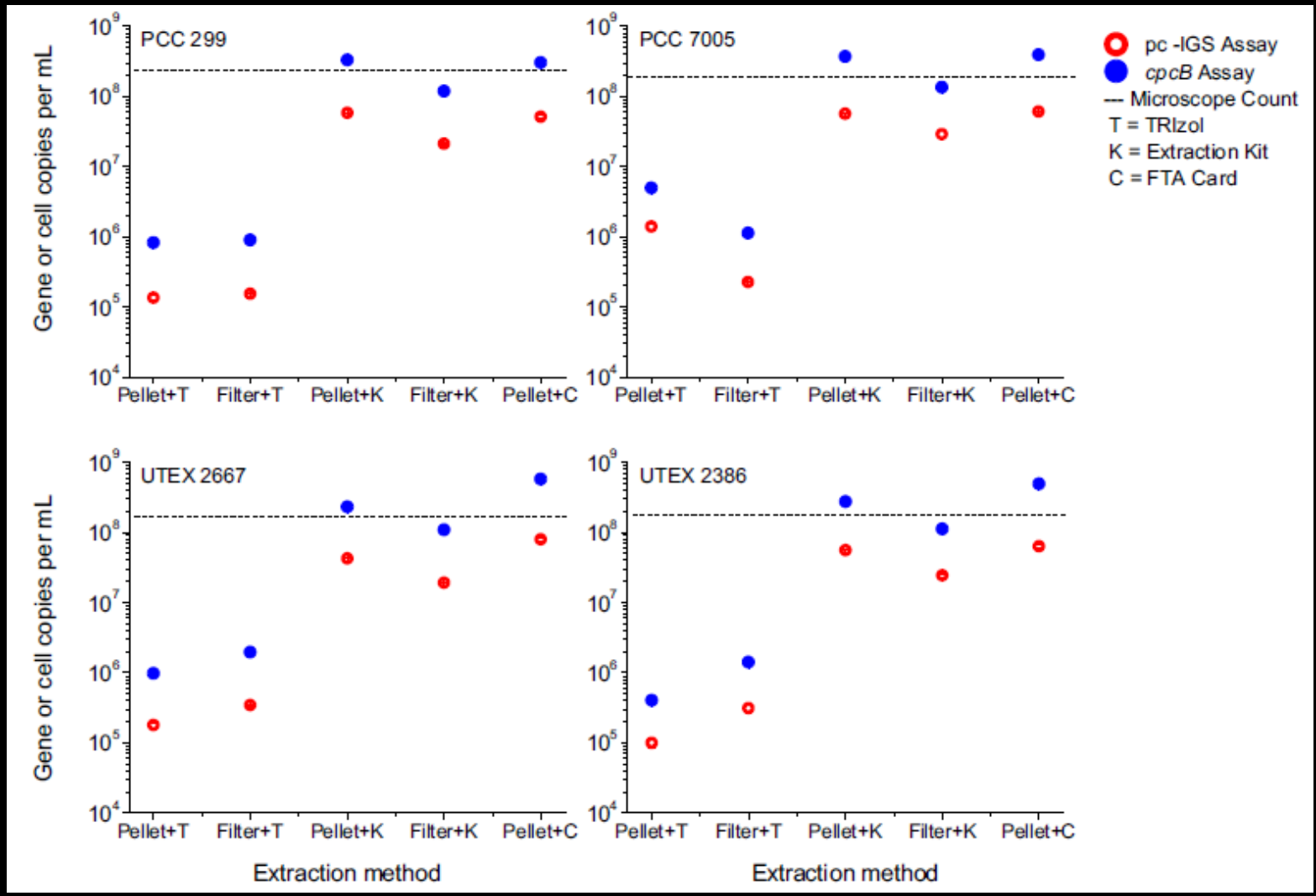
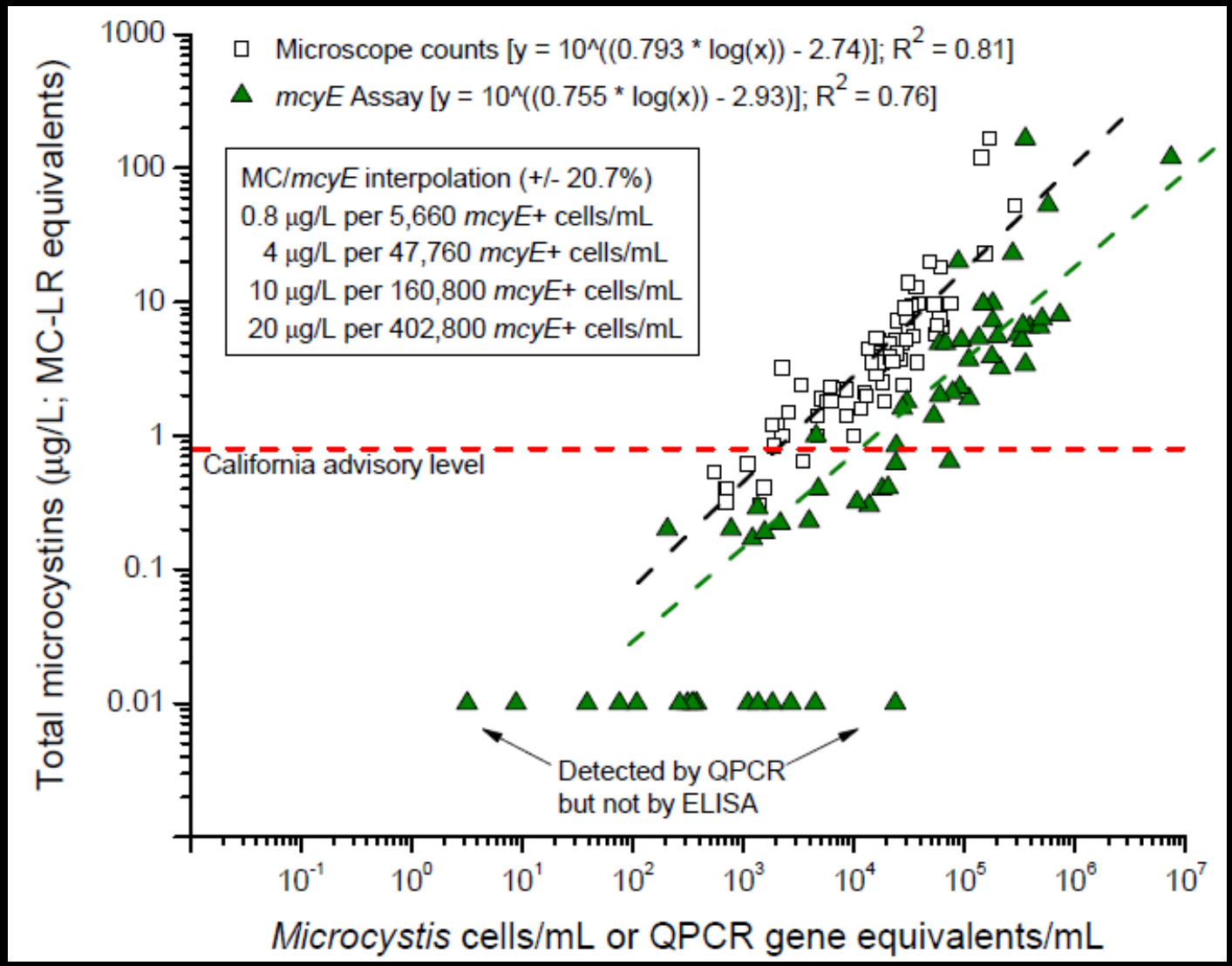


Discrepancy between environmental counts and QPCR not likely explained by ploidy

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

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



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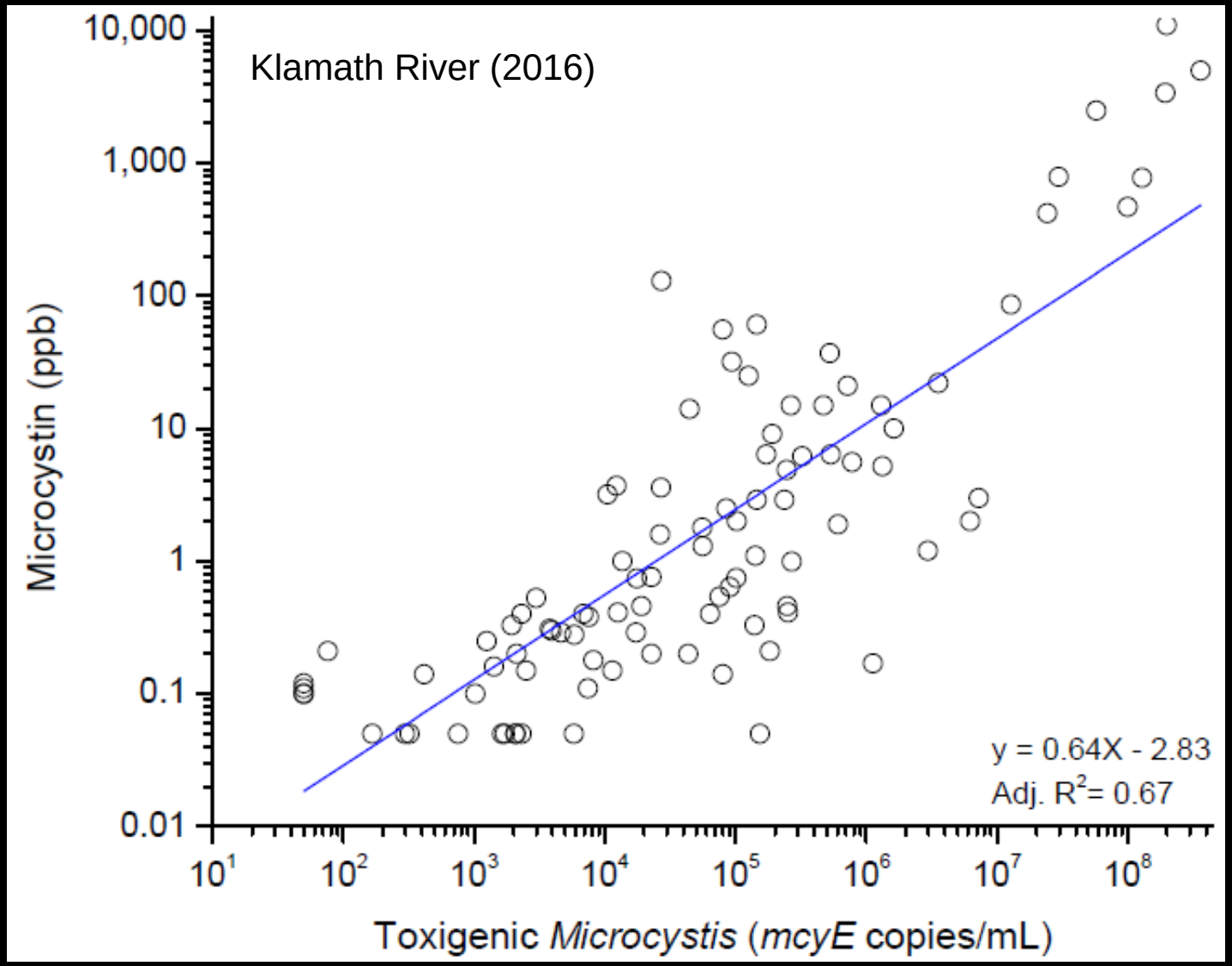

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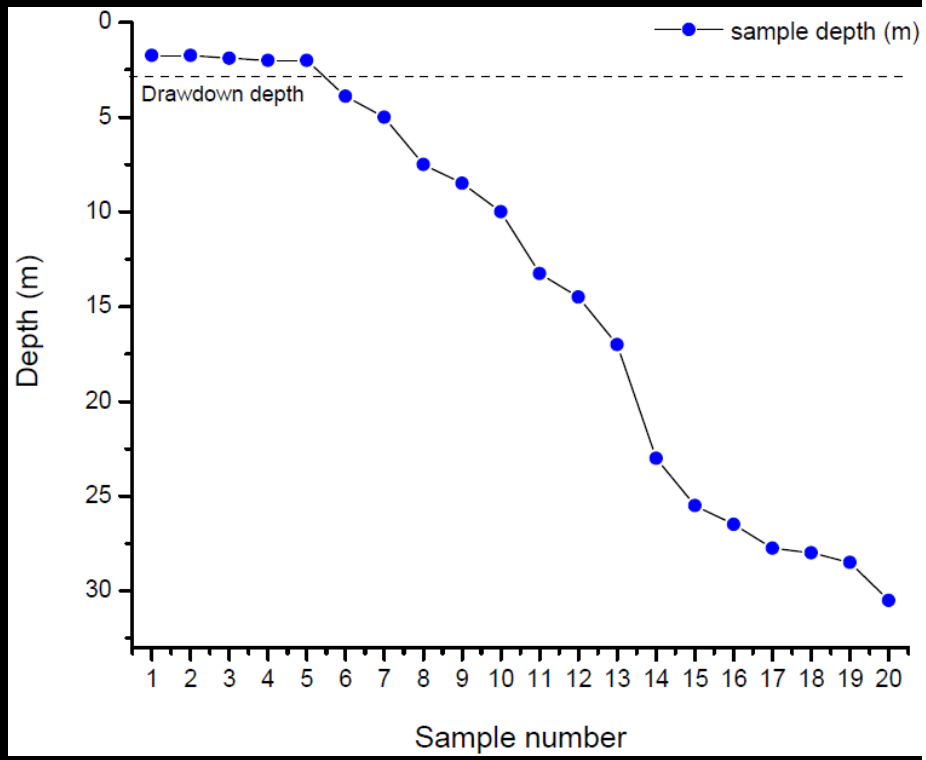
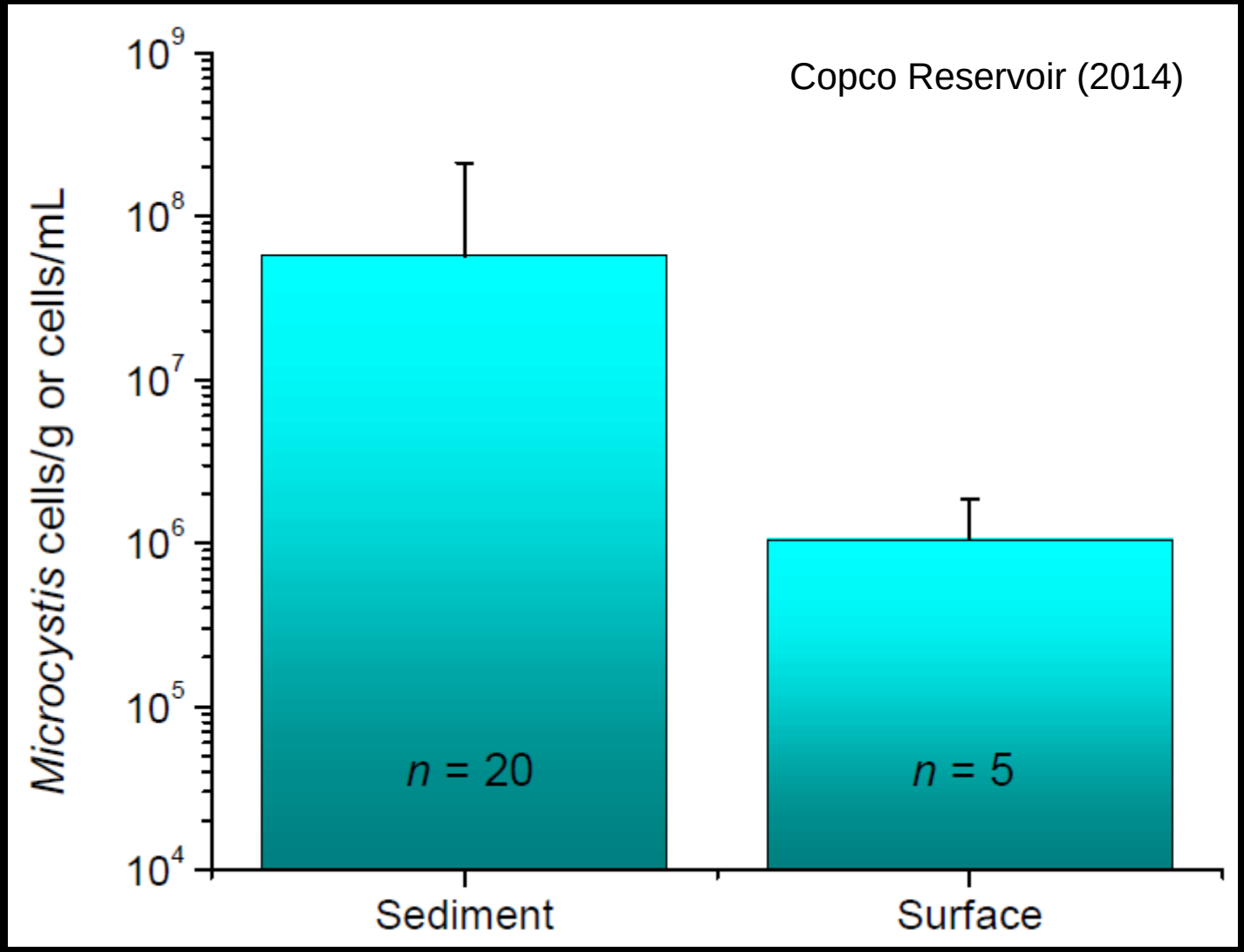
Sediment sample collection

Copco Cove during initial sampling (9/26/14)



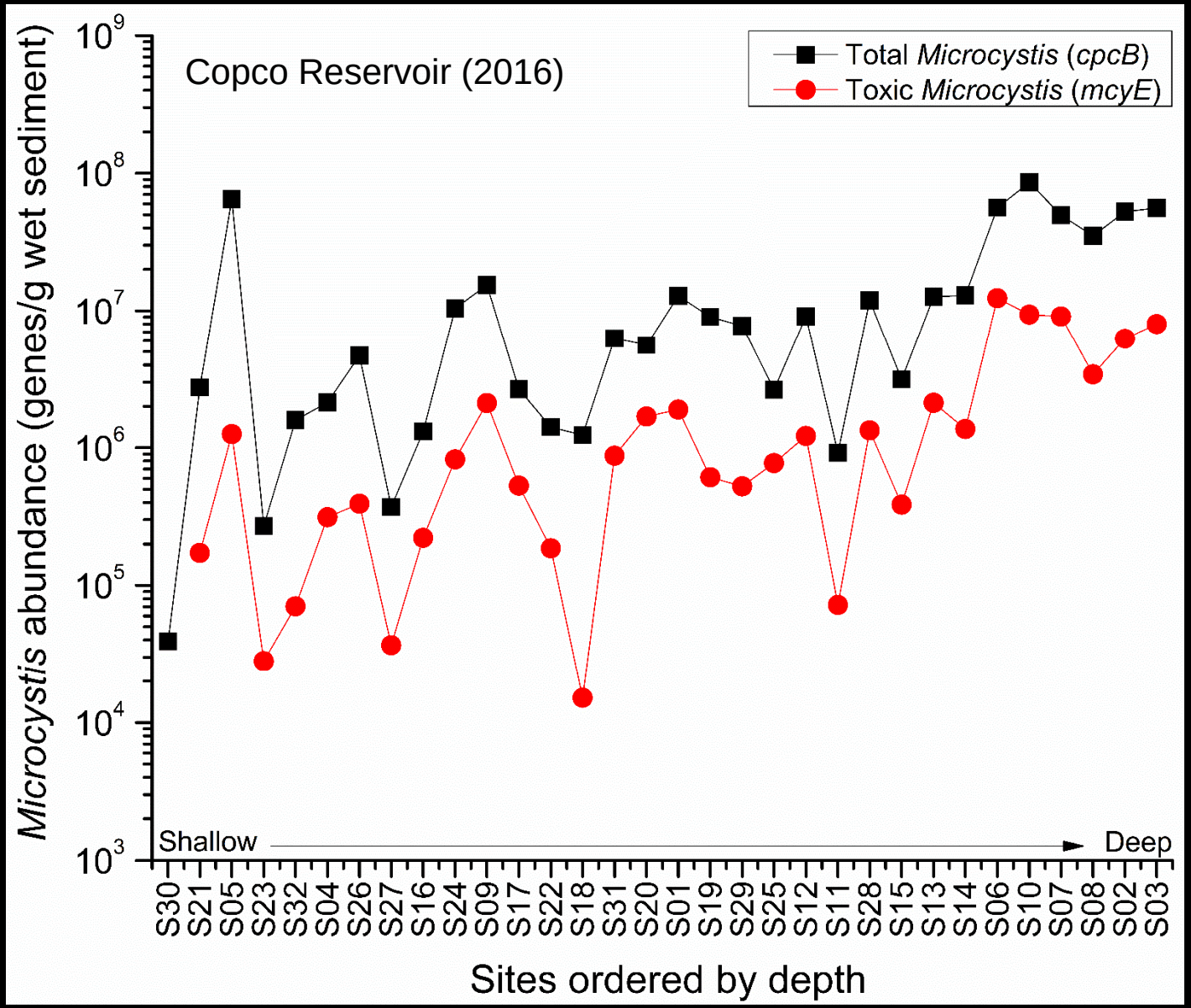
QPCR to assess *Microcystis* cell abundances



Surface samples (0.5 m) collected on 7/9/14, 9/23/14, 9/25/14, 10/20/14, 11/4/14 (CR01)

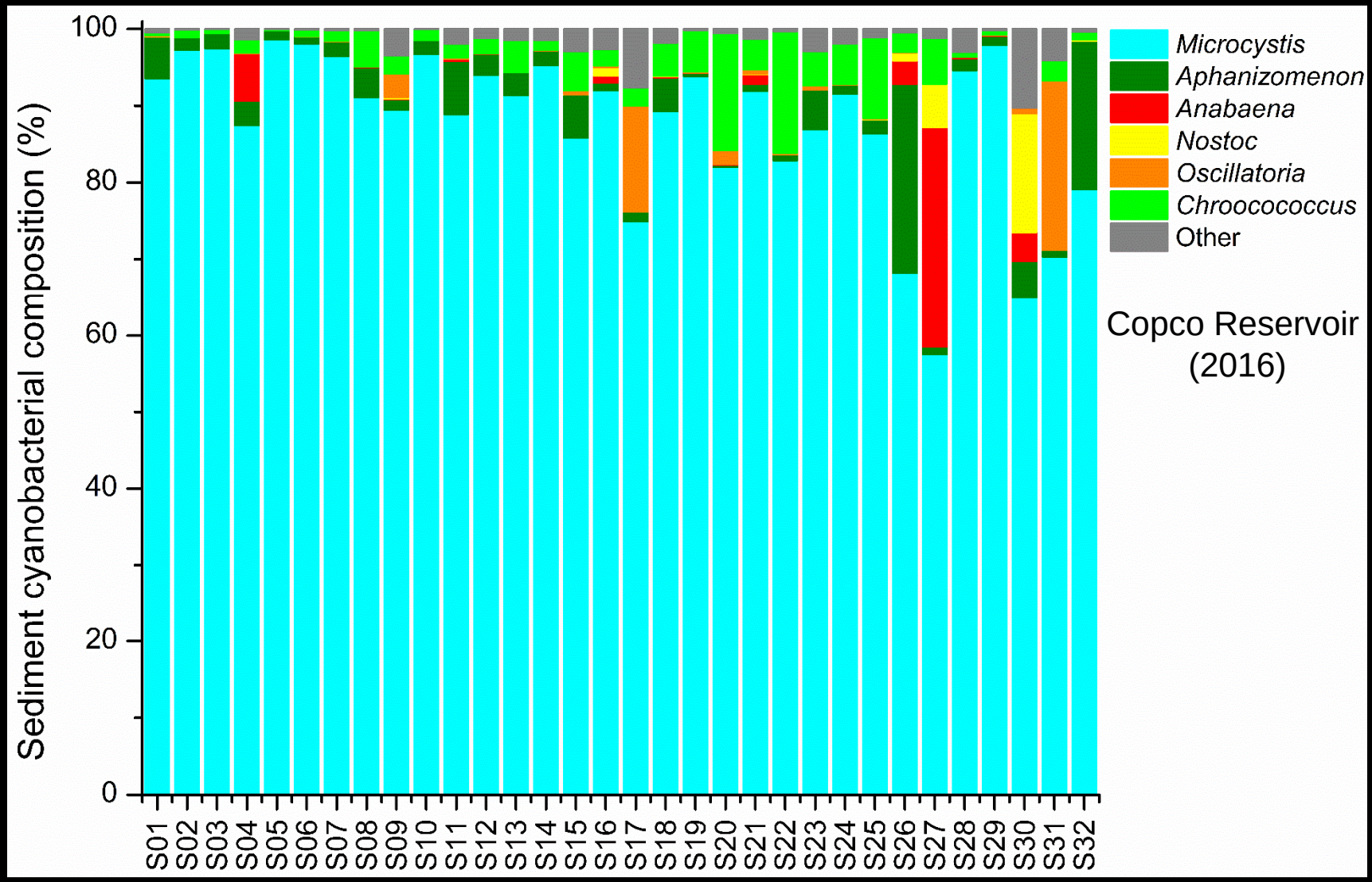
Otten, *in prep*.

Microcystis abundance increased with depth



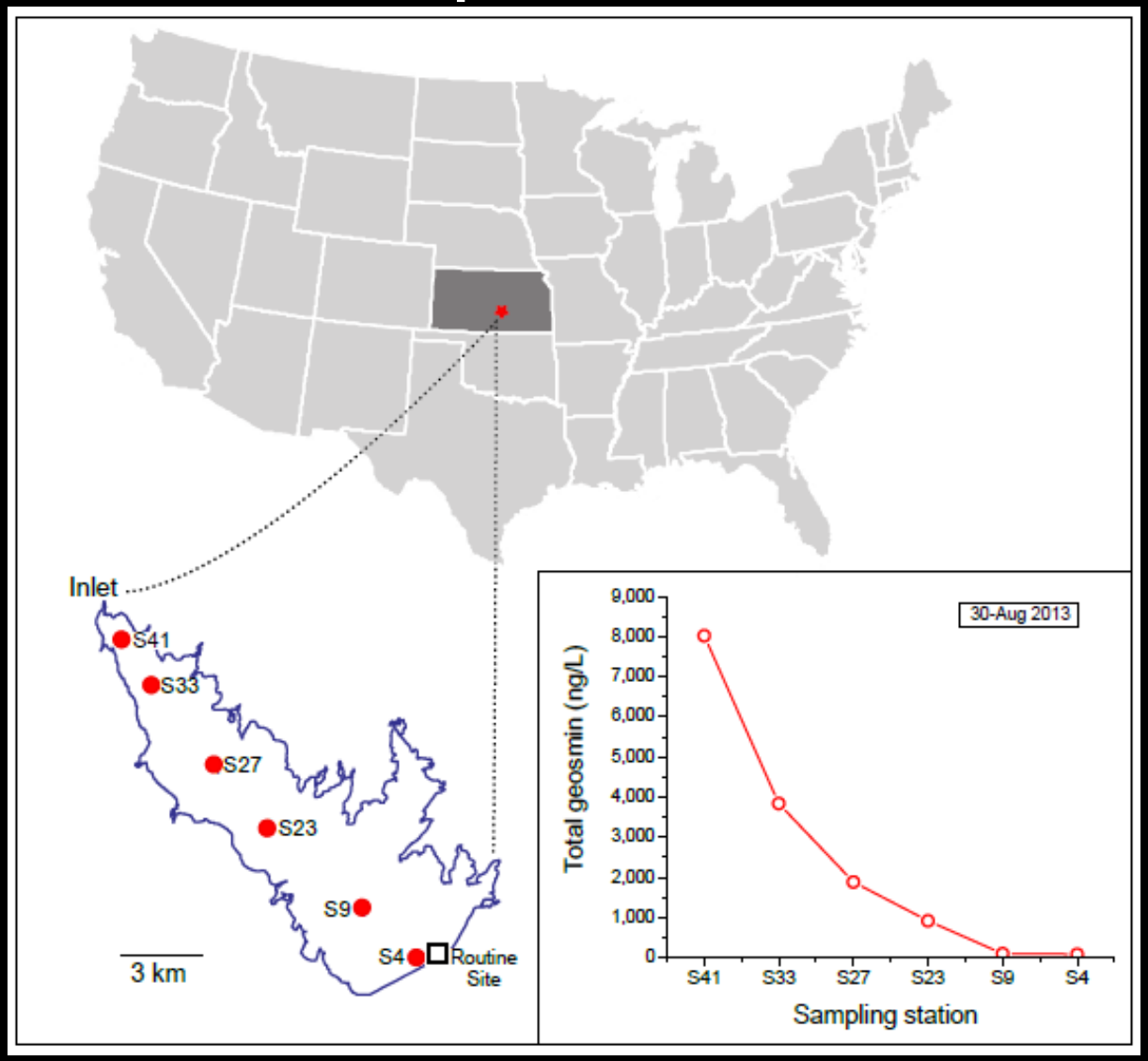
Otten, *in prep*.

Amplicon sequencing (cpcBA) to assess cyanobacterial community in sediments



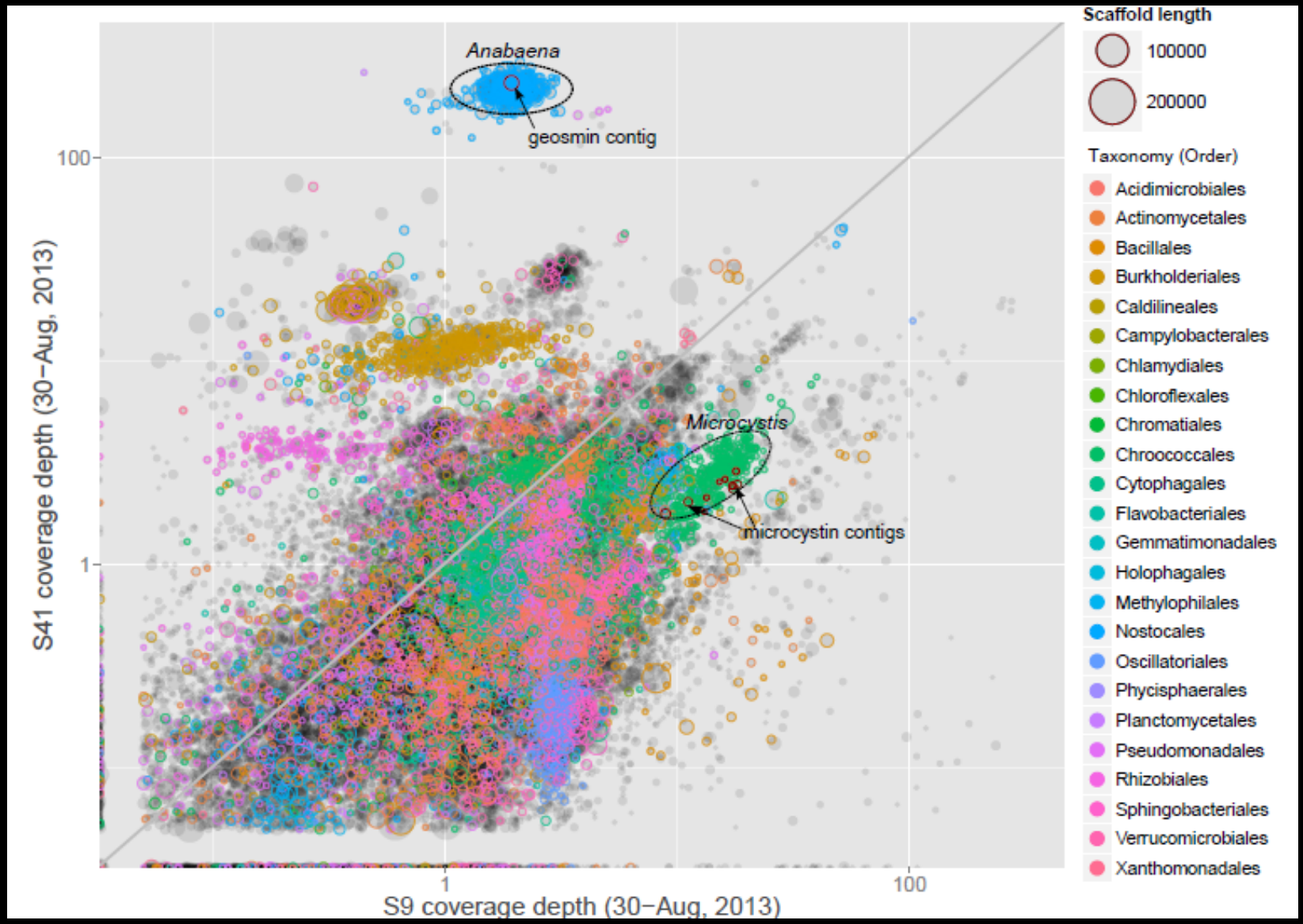
Otten, *in prep*.

Shotgun metagenomics to ID toxin and T&O producers



Otten et al., 2016. AEM 82:5210-5220.

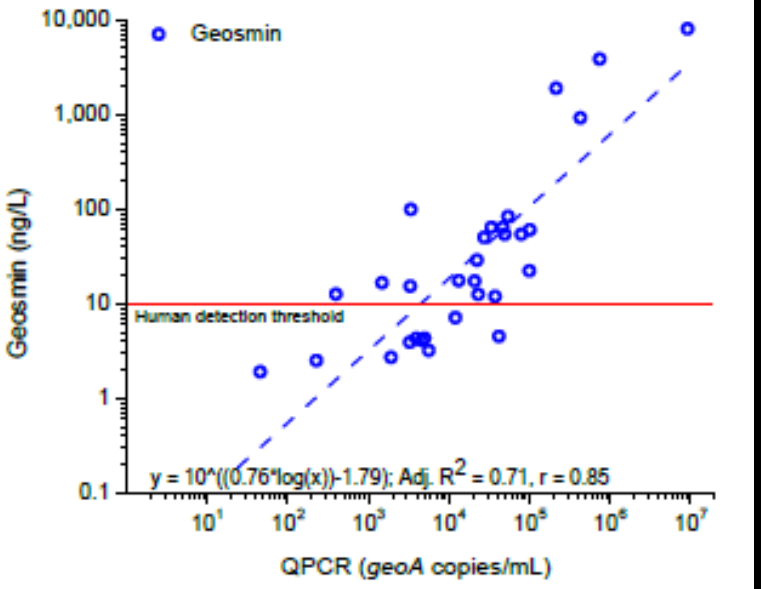
Differential binning of microbial genomes

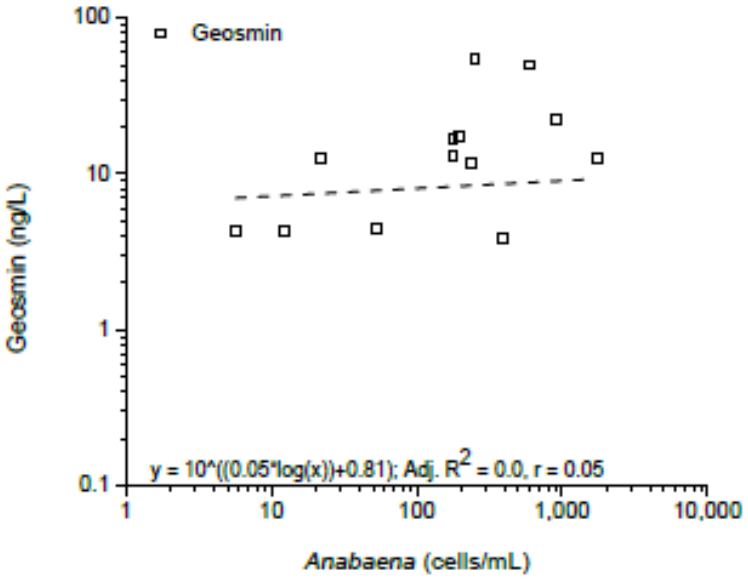


From 2 m depth-integrated samples

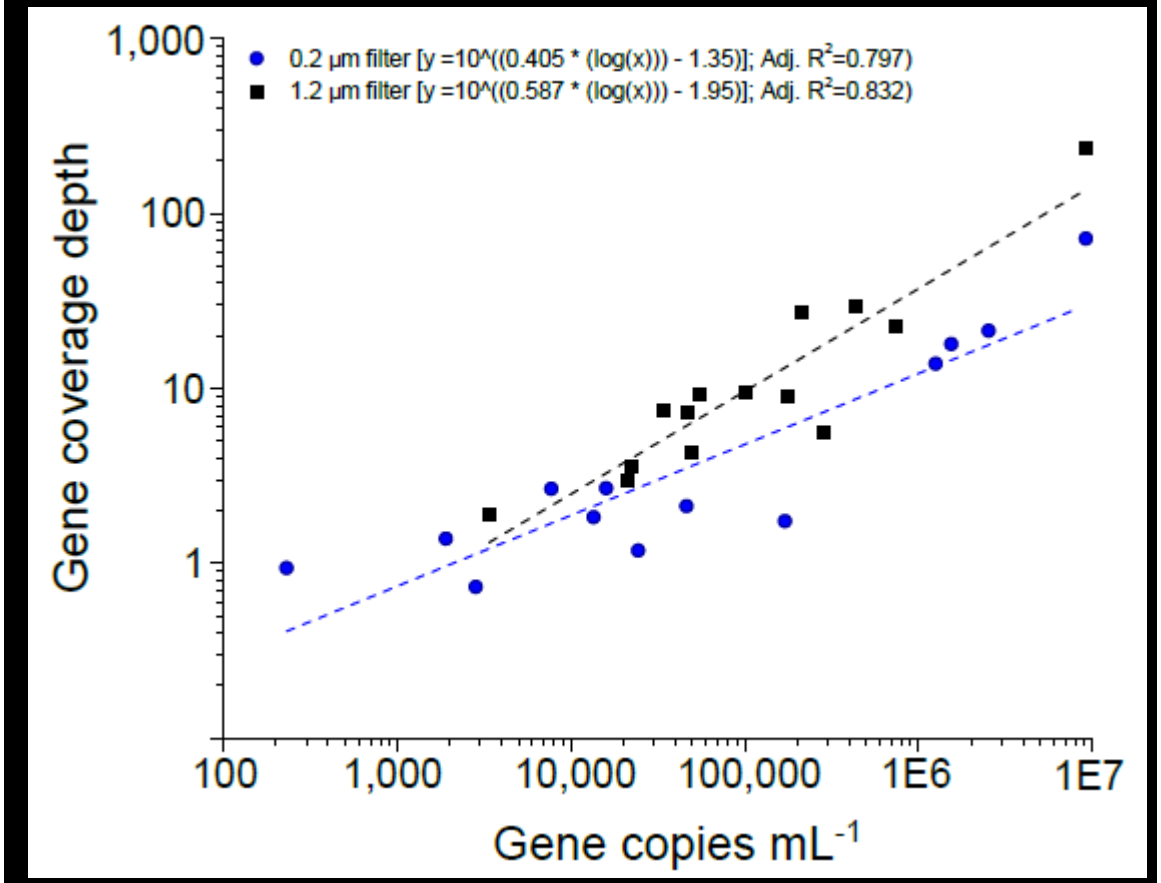
Otten et al., 2016. AEM 82:5210-5220.

Development of *Anabaena*-specific QPCR for assaying geosmin genes





Obtained coverage depth from 30 million (100 bp) reads/sample relative to target abundance



Otten et al., 2016. AEM 82:5210-5220.

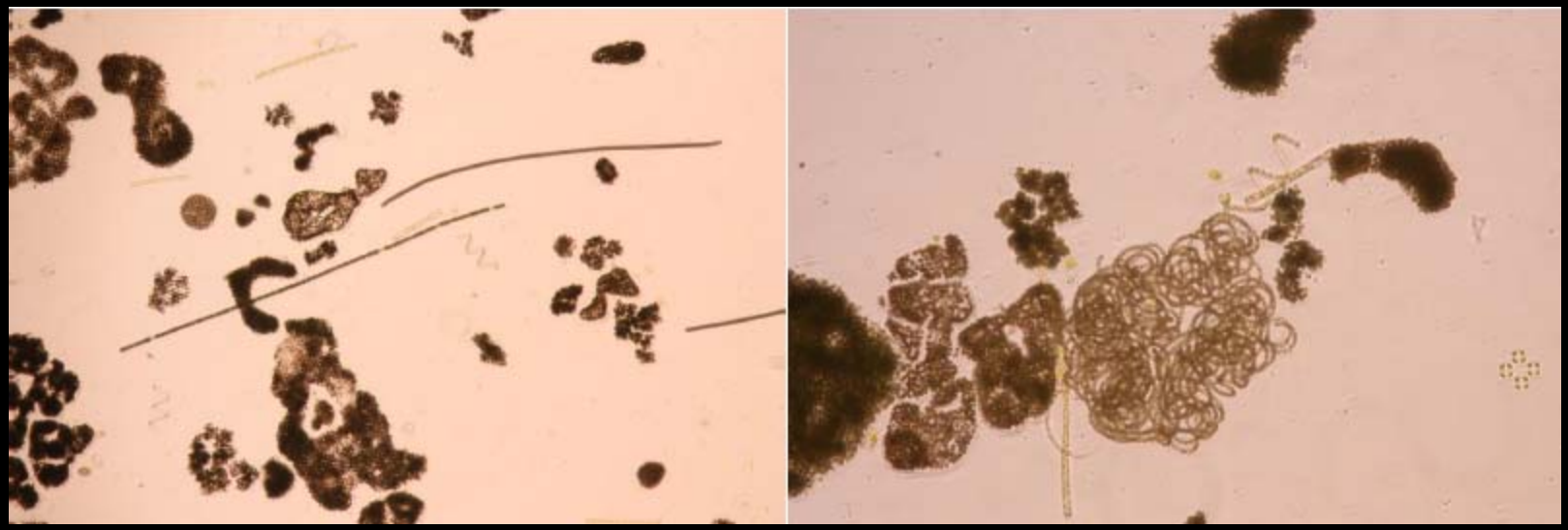
Sample collection & archival

- 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 → cells lyse=DNA lost
- Record volume filtered, required for QPCR quantification
- Store filters in microcentrifuge tubes at -20ºC; stable for years
  - Samples can be archived and batch processed

Conclusions

- QPCR is a useful proxy for cyanotoxin risks that is best used in a tiered monitoring framework
  - Assays are selected based on taxa present, samples with elevated toxin or T&O gene concentrations are candidates for toxin testing.
- Because DNA is amplified, toxigenic cells can be detected sooner than other methods
- QPCR can also be used for source tracking or benthic surveys
- Amplicon sequencing can be used to generate relative abundances of microbial taxa and/or phytoplankton when data are not time-sensitive
- Shotgun metagenomics allows for culture-free identification of problematic taxa
  - Data can be used to improve existing QPCR assays or develop novel ones

Thanks for your attention!



Please feel free to contact me with any questions.

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