A Strategy for the Analysis of Cyanotoxins:

Grand Lake St. Marys and Beyond…..

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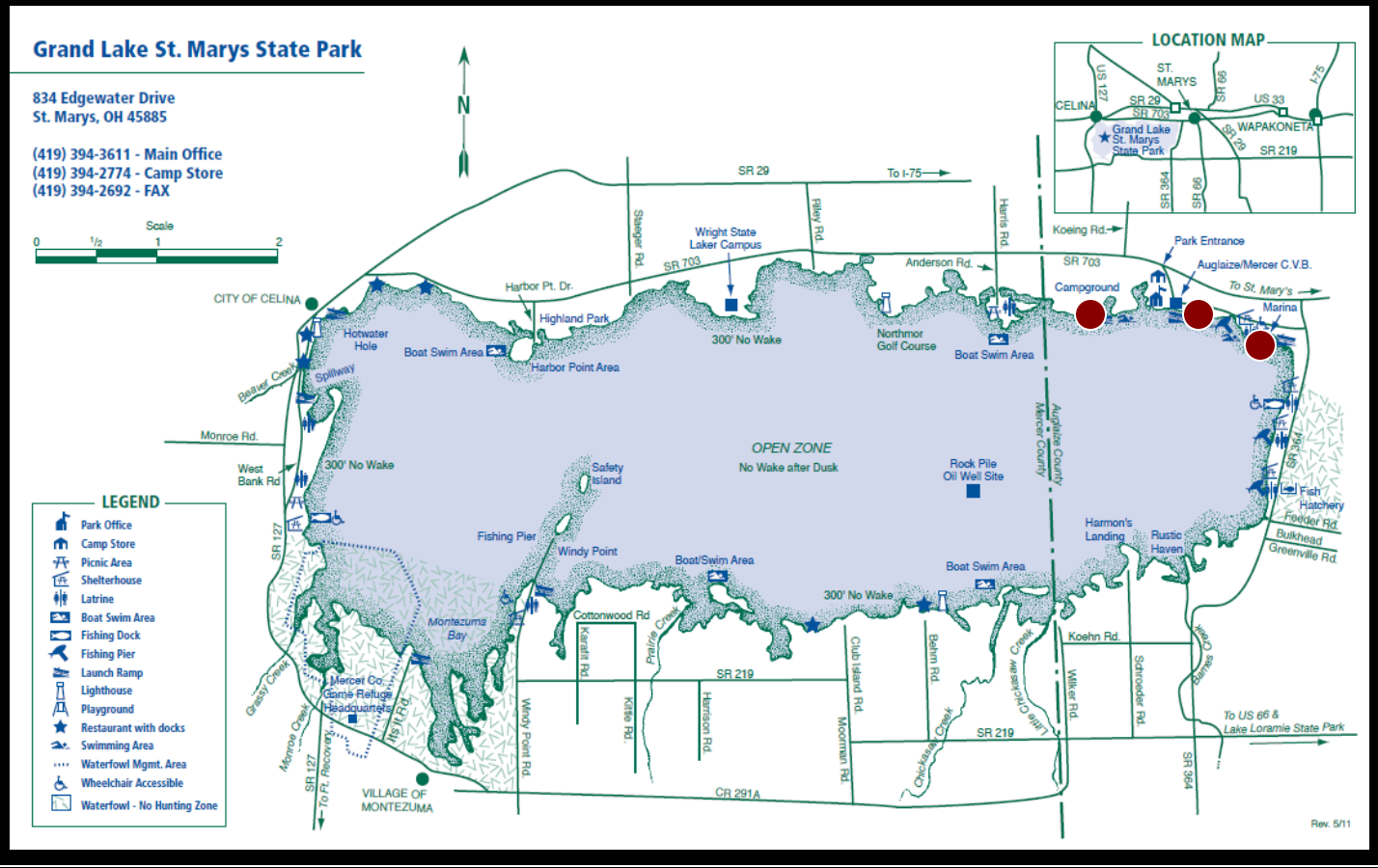
Timeline

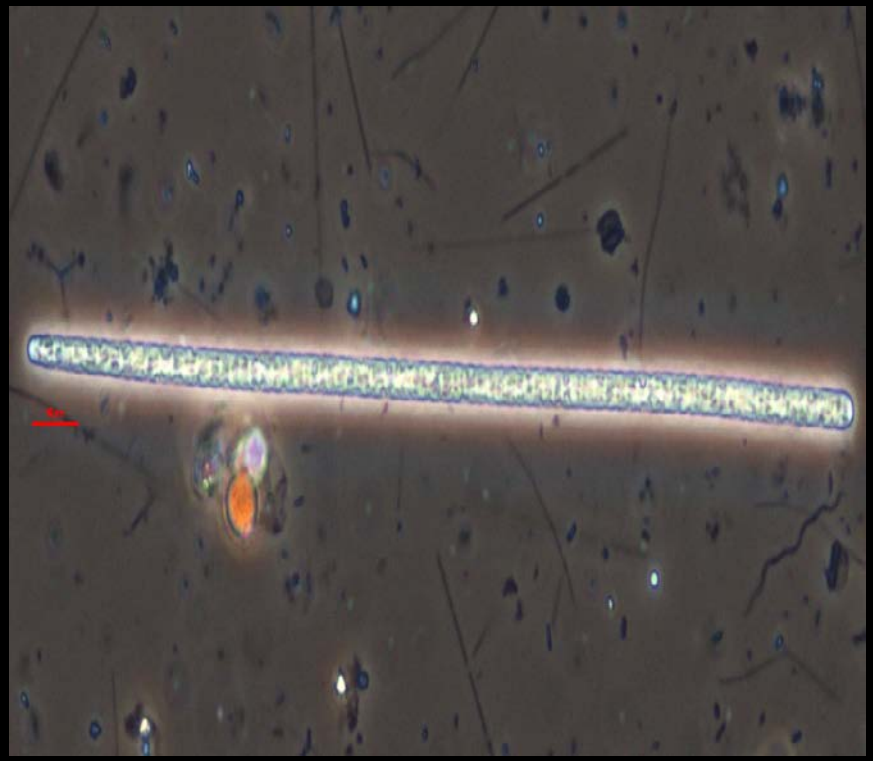
- Sept 2008
  - Ohio EPA inquired about cyanobacteria testing
- 2009 (May – Sept)
  - Microcystin analysis
- 2010 (June – Oct)
  - Anatoxin-a (ANTX) analysis
  - Cylindrospermopsin (CYN) analysis
  - Saxitoxin (STX) analysis

Map of Grand Lake

- Largest inland lake in Ohio
- 13500 acres
- Depth: 3-5 ft
- Max length: 8.2 miles
- Max width: 2.8 miles



Grand Lake Cyanobacteria 2009



Planktothrix agardhii - 400x

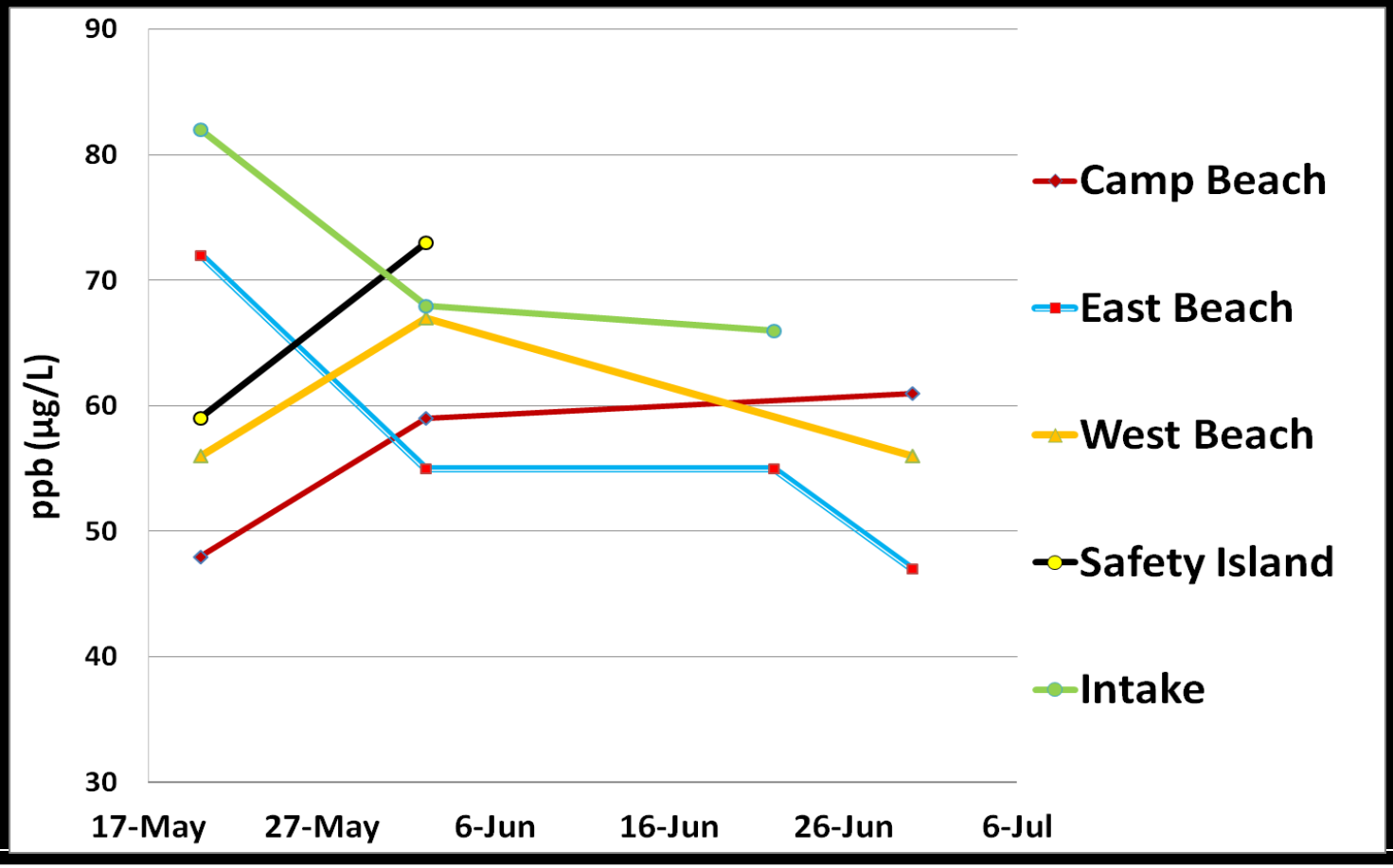
Toxins Associated with *Planktothrix agardhii*

- Microcystins
  - Briand et al. 2008
  - Yéprémian et al. 2007
  - Kurmayer et al. 2006
- Anatoxin-a
  - Suspected, but not confirmed in uni-algal culture
  - Closely related to *P. rubescens*, which is a producer
    - Viaggiu et al. 2004

2009 Analyses

- Microcystins
  - Abraxis Adda ELISA
- Anatoxin-a
  - Not tested---

2009 Microcystin Data



Grand Lake Algal Bloom
June 2010

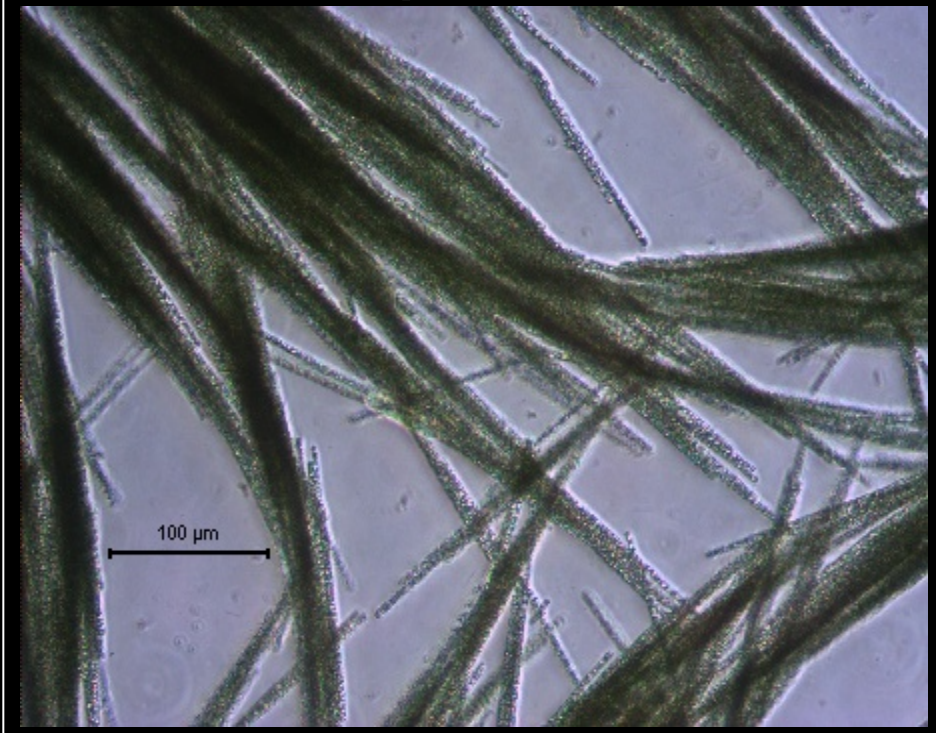
State Parks Monitored in 2010 (Ohio DNR)

- 13 of 17 Ohio State Parks and associated water bodies monitored in 2010

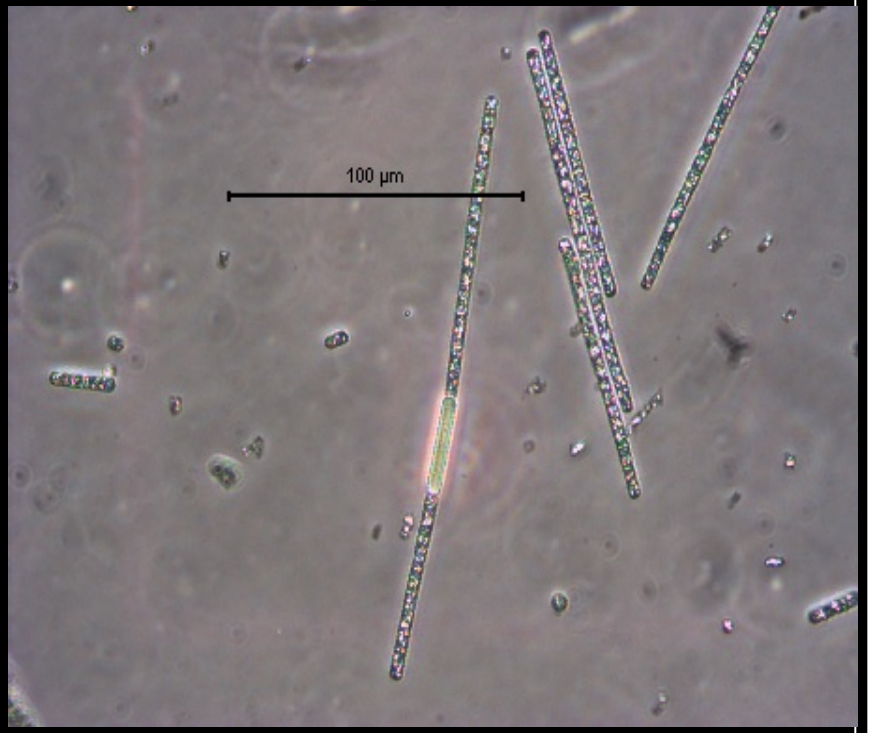
Warnings, Notices, Advisories, Postings, etc.

Grand Lake
Dominant cyanobacteria - 2010

Aphanizomenon cf. *flos-aquae*



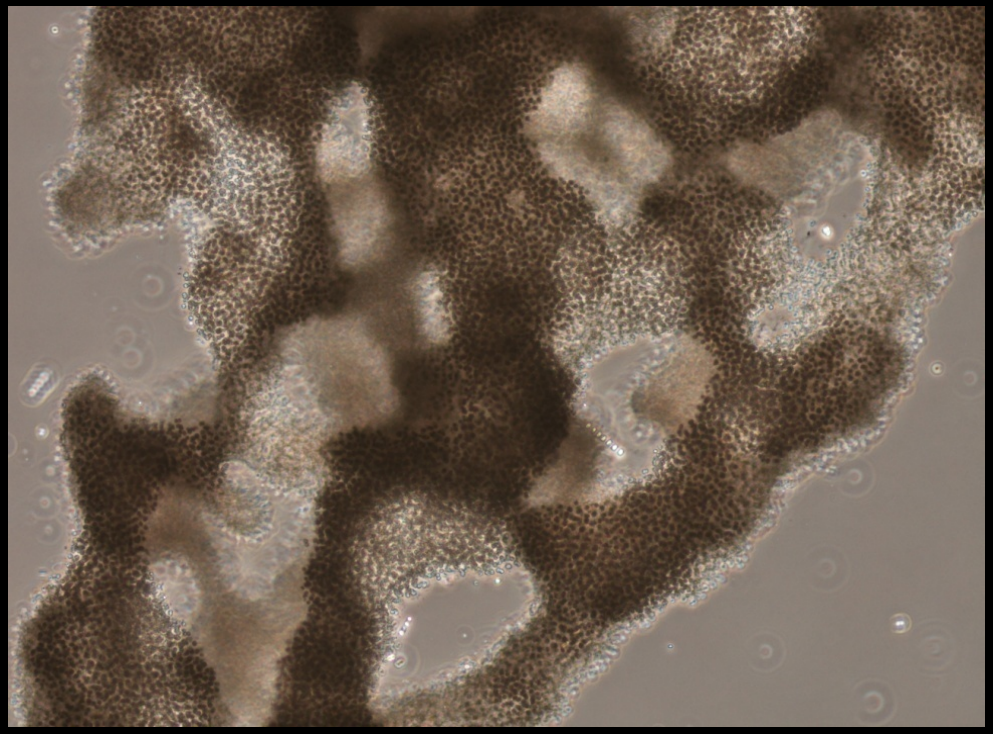
200*x*



400*x*

Grand Lake
Dominant cyanobacteria - 2010

Microcystis aeruginosa



Potential Toxins (2010)

- *Aphanizomenon flos-aquae*
  - Cylindrospermopsin
    - Preussel et al. (2009)
  - Anatoxin-a
    - Rapala et al. (1993)
  - PSTs (paralytic shellfish toxins/saxitoxin)
    - Species re-evaluated in literature
    - Now reclassified as *Cuspidothrix issatschenkoi*
- *Microcystis* spp.
  - Microcystins

Analyses conducted in 2010

- Celina WTP
  - Conducted microcystin analysis (ELISA)
- GreenWater Labs
  - Anatoxin-a (LC/MS/MS)
  - Cylindrospermopsin (ELISA & LC/MS/MS)
  - Paralytic Shellfish Toxins (ELISA)

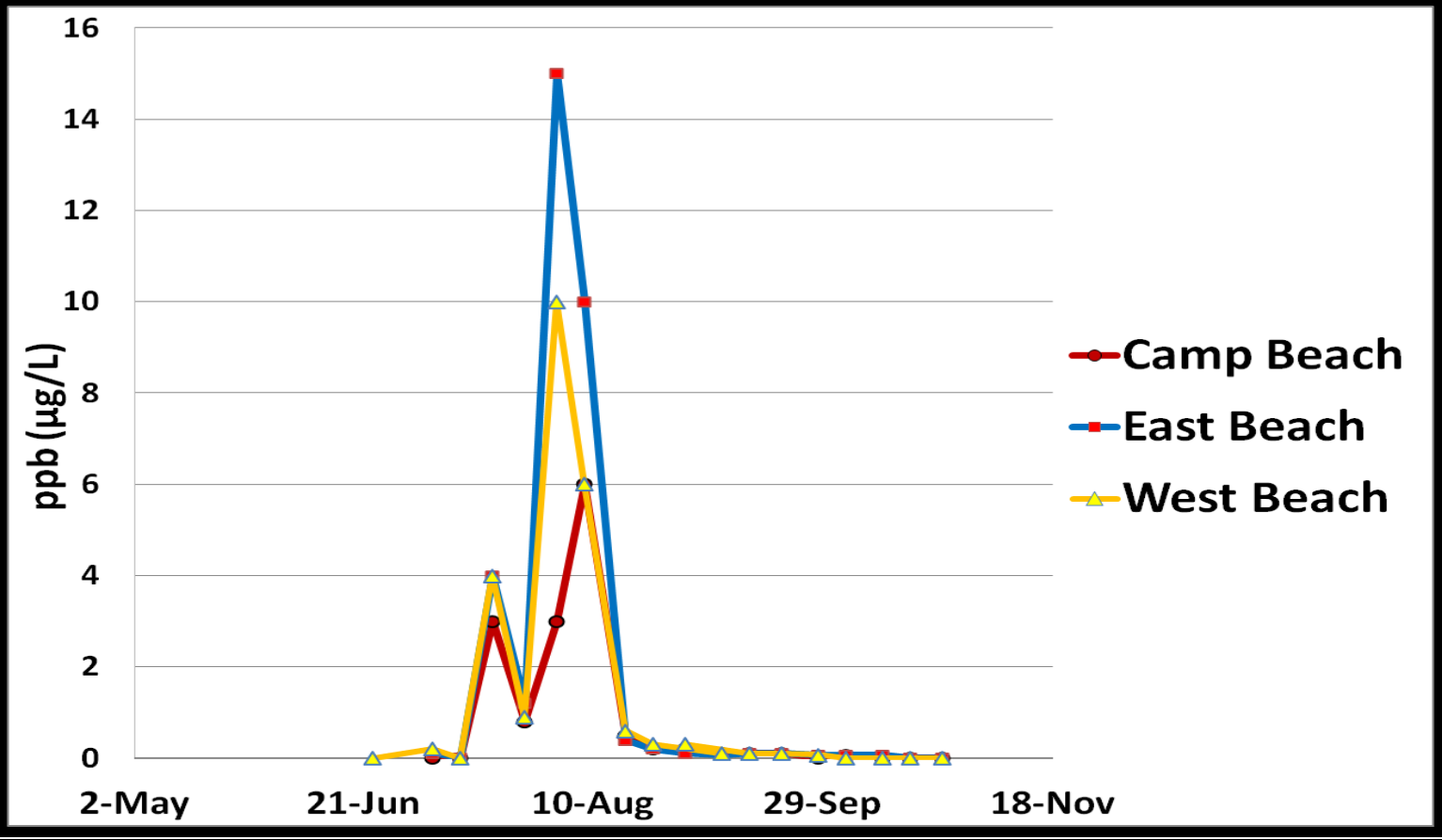
Anatoxin-a Analysis

- Currently no ELISA available

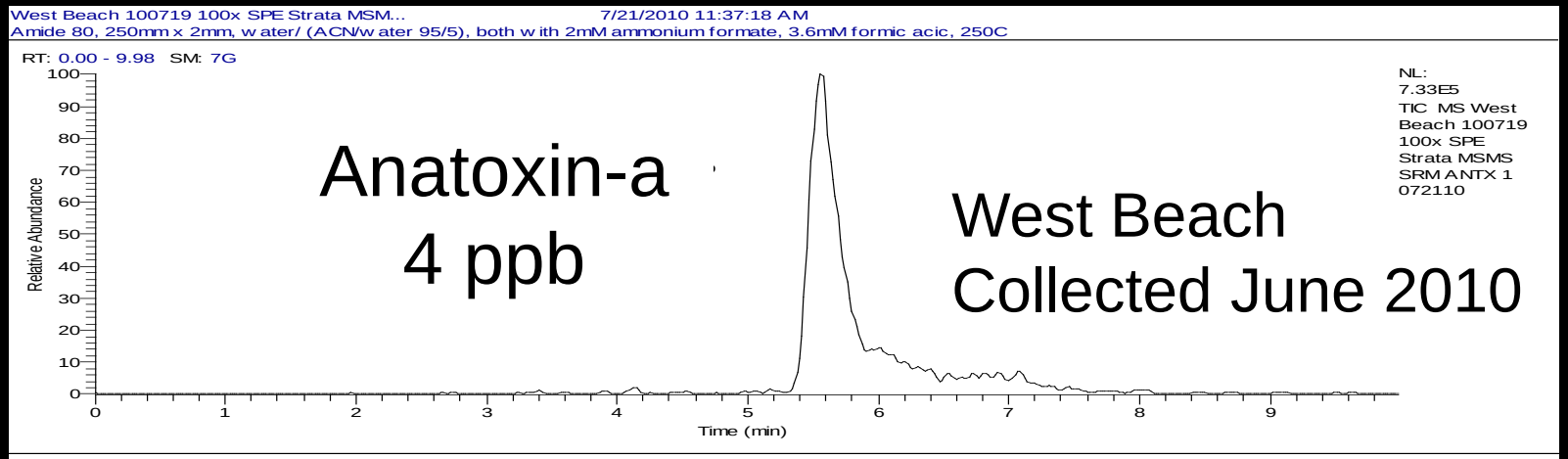
- LC-MS/MS
  - $[M+H]^+$ ion (*m/z* 166) fragmented and product ions (*m/z* 149, 131, 107, and 91) monitored.
  - (LOD) = 0.05 µg/L (@ 100x preconcentration)
  - (LOQ) = 0.1 µg/L

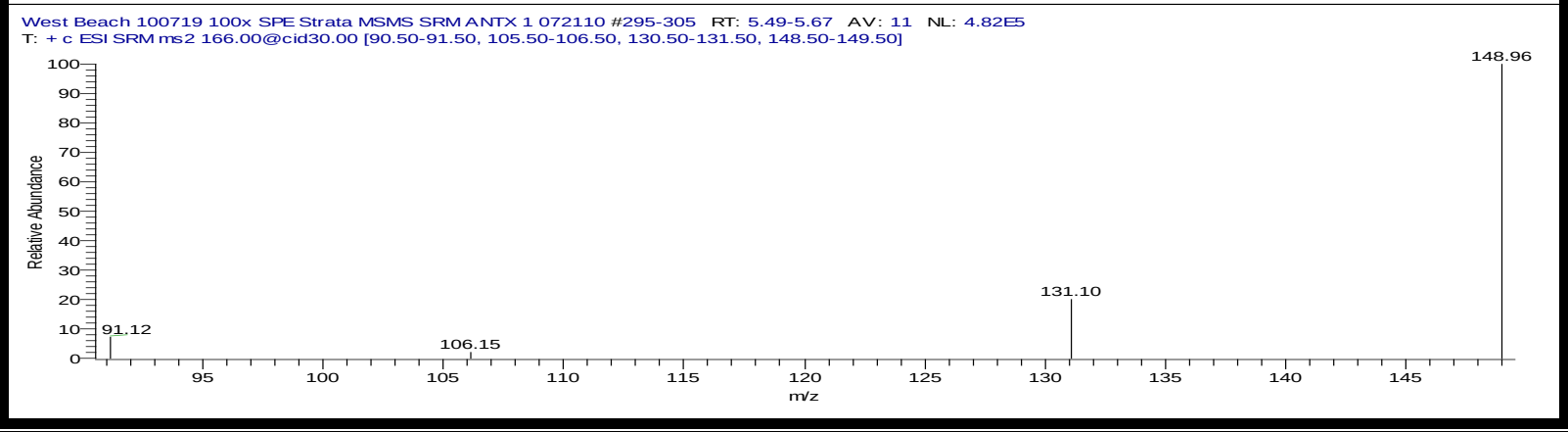
  - Phenylalanine (*m/z* 166)

Grand Lake 2010
Anatoxin-a Data (LC-MS/MS)



Grand Lake 2010
Anatoxin-a



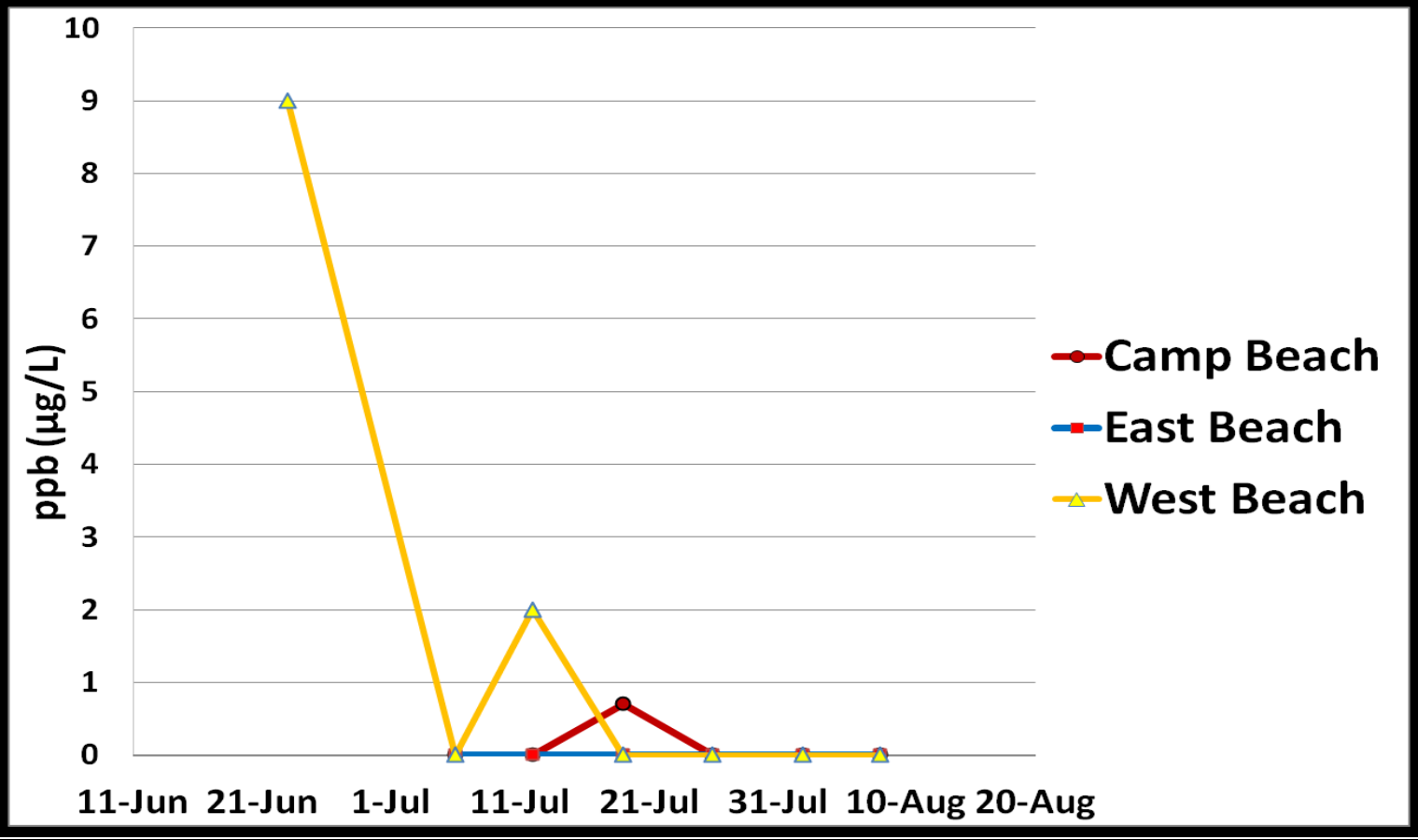


Cylindrospermopsin Analysis

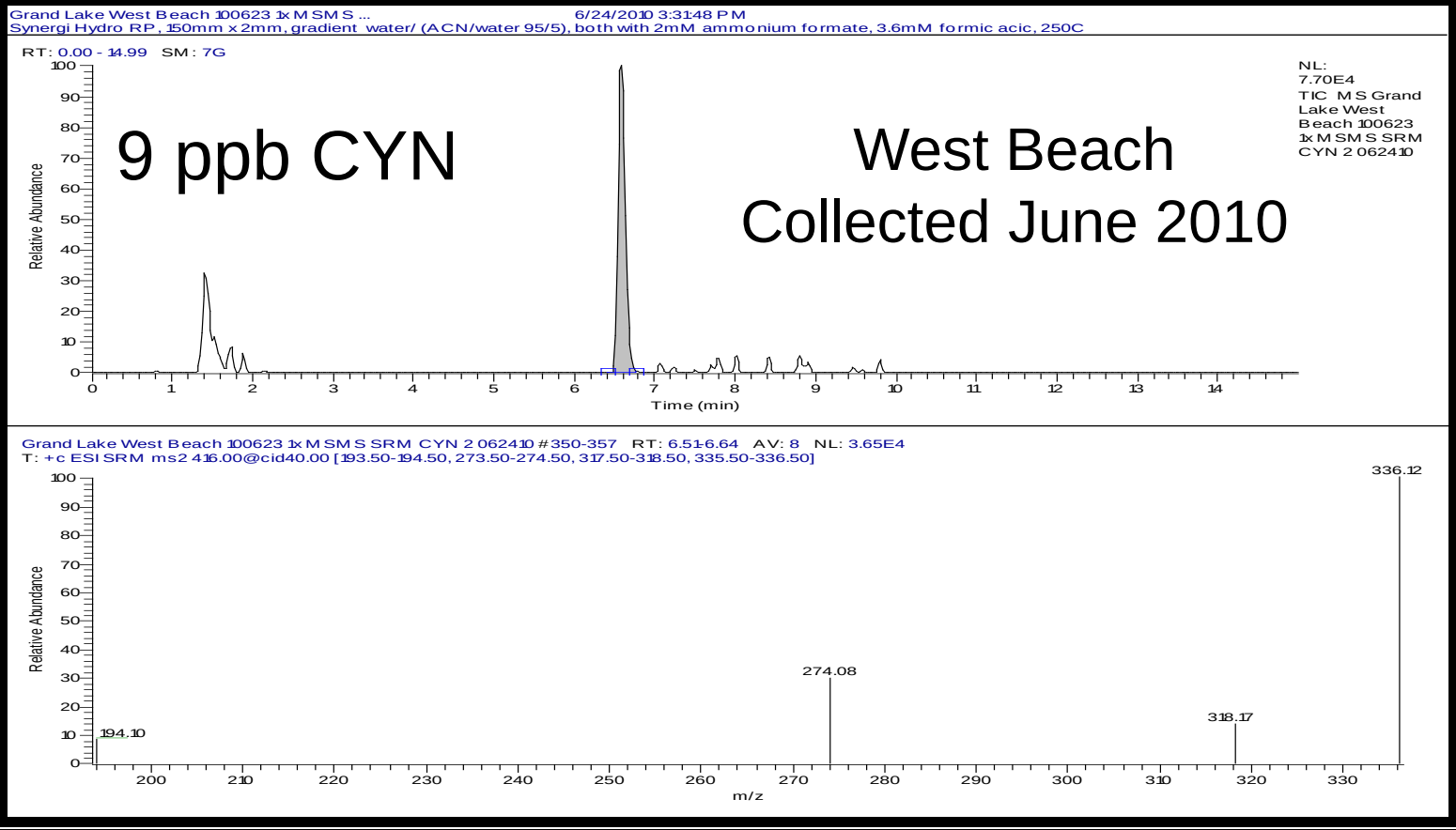
- ELISA
  - Subject to interference and false positives
- LC-MS/MS
  - $[M+H]^+$ ion (*m/z* 416) fragmented product ions (*m/z* 336, 318, 274, and 194) monitored.
  - LOD = 0.05 µg/L (@ 100x preconcentration via SPE)
  - LOQ = 0.1 µg/L

Example: ELISA detected >200 µg/L in West Beach (7/12/10) sample
LC-MS/MS determined < 2 µg/L

Grand Lake 2010 Cylindrospermopsin Data (ELISA)



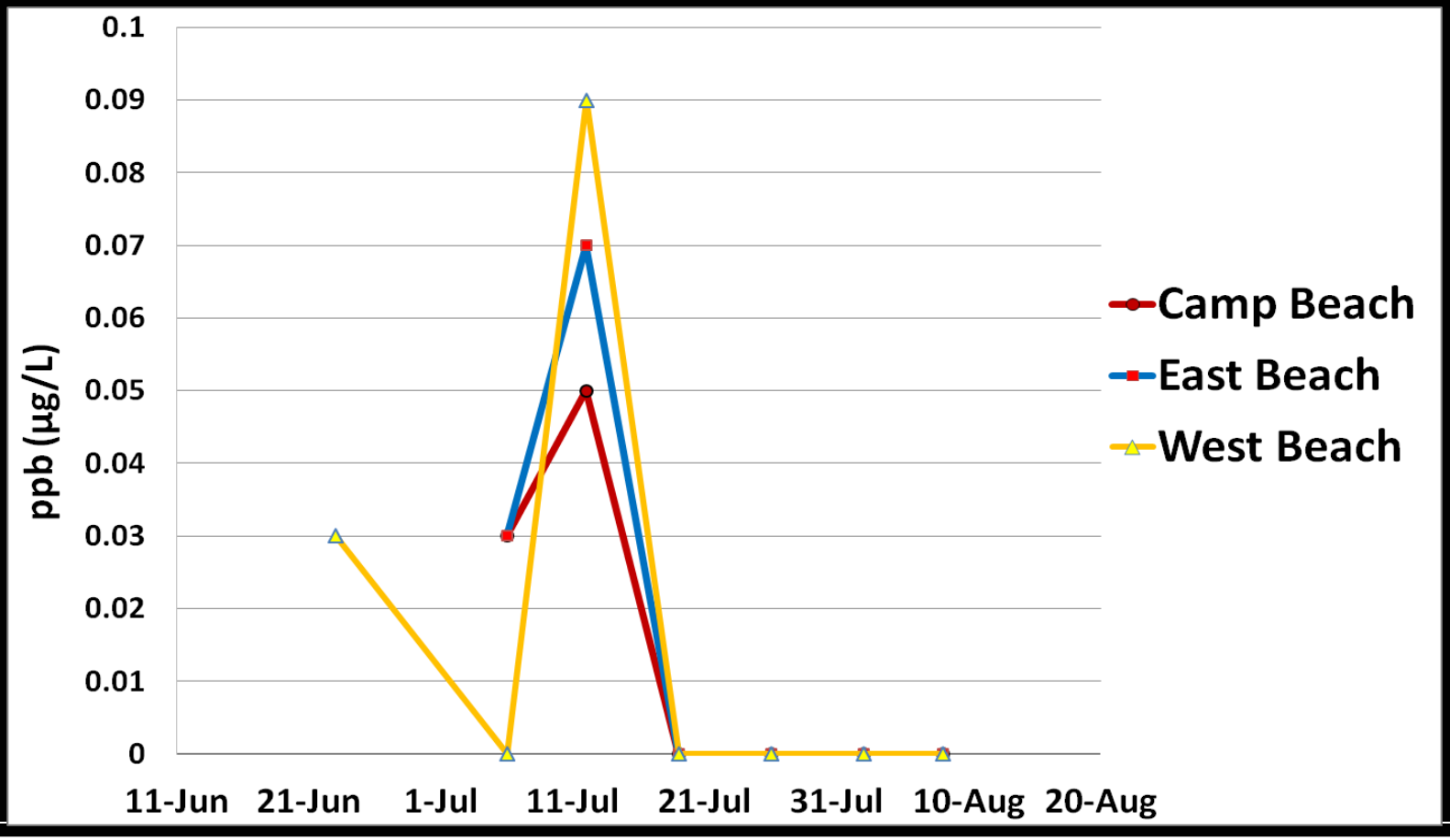
Grand Lake Cylindrospermopsin



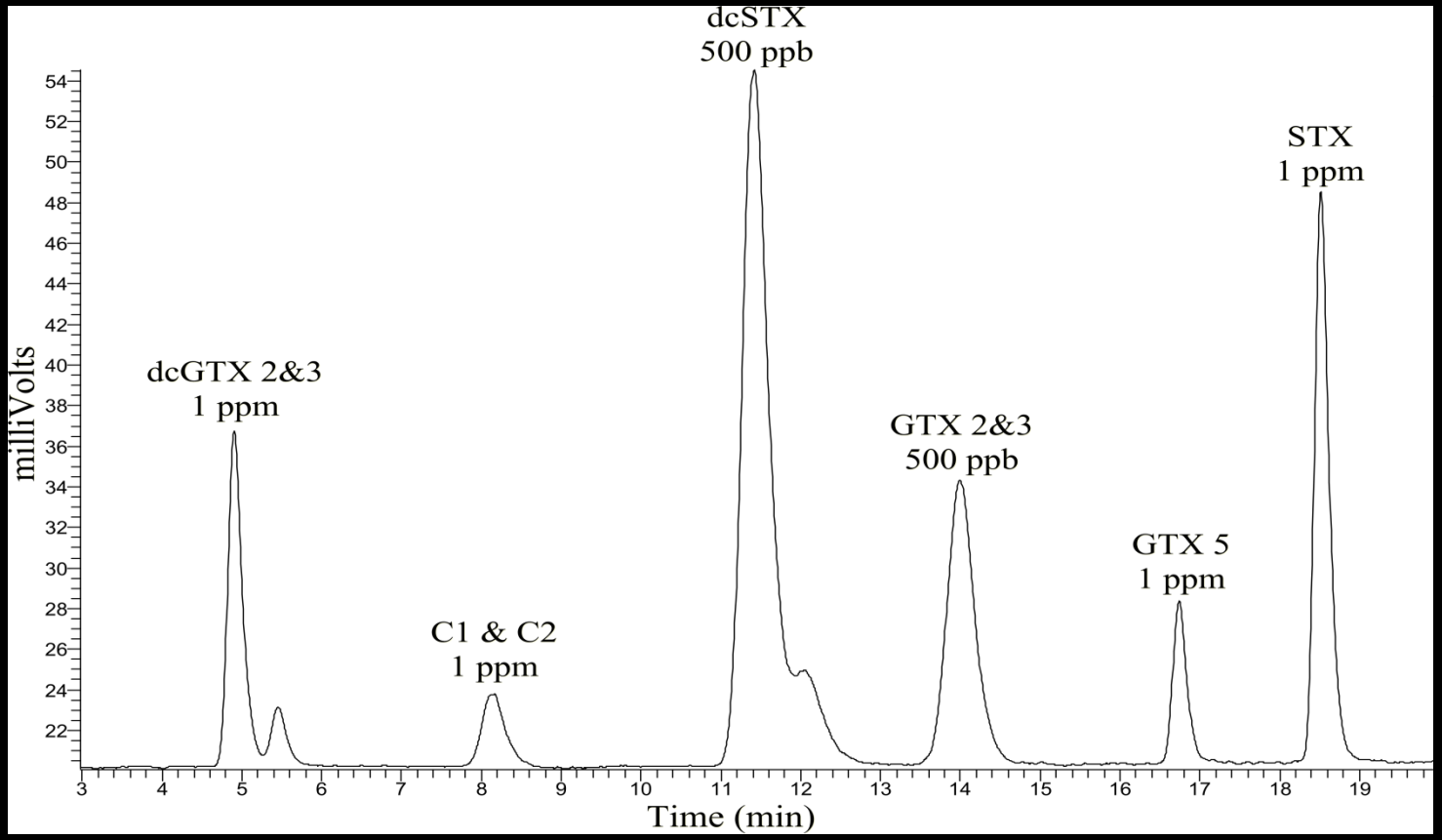
Paralytic shellfish Toxin Analysis (saxitoxins)

- ELISA (Enzyme Linked Immuno-absorbant Assay)
  - Limited sensitivity to variants other than STX
- LC-Fluorescence (AOAC First Action Methods)
  - LODs range from 0.01-0.5 ppb (100x pre-concentration)
  - Peroxide oxidation
    - dcGTX2&3, C1C2, dcSTX, GTX2&3, GTX5, STX, dcNEO
  - Periodate oxidation
    - GTX1,4, NeoSTX
- LC-MS^n
  - Provides confirmation
  - Less sensitive than LC-FL

Grand Lake 2010
Saxitoxin Data (ELISA)



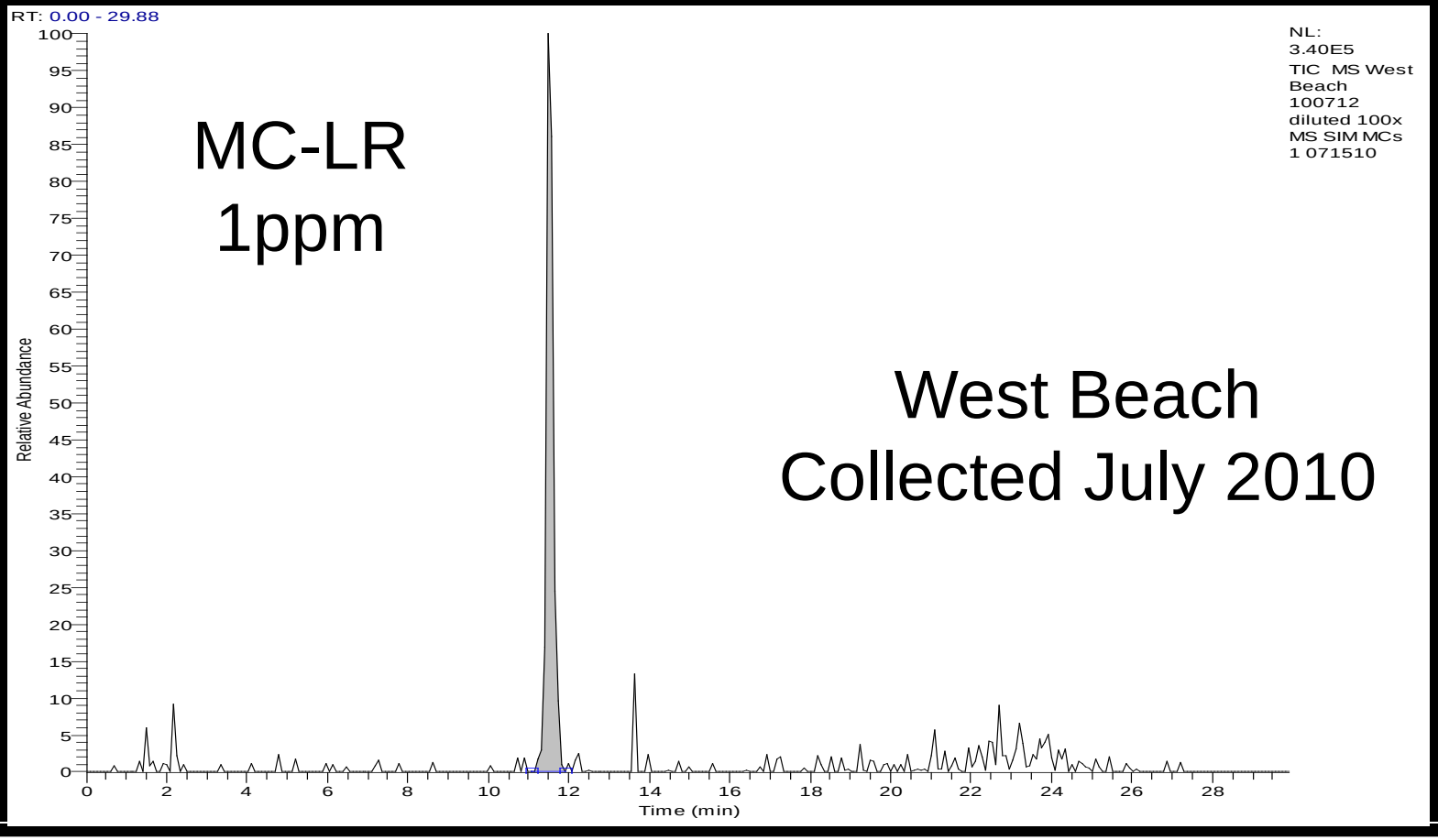
Pre-Column Peroxide Oxidation



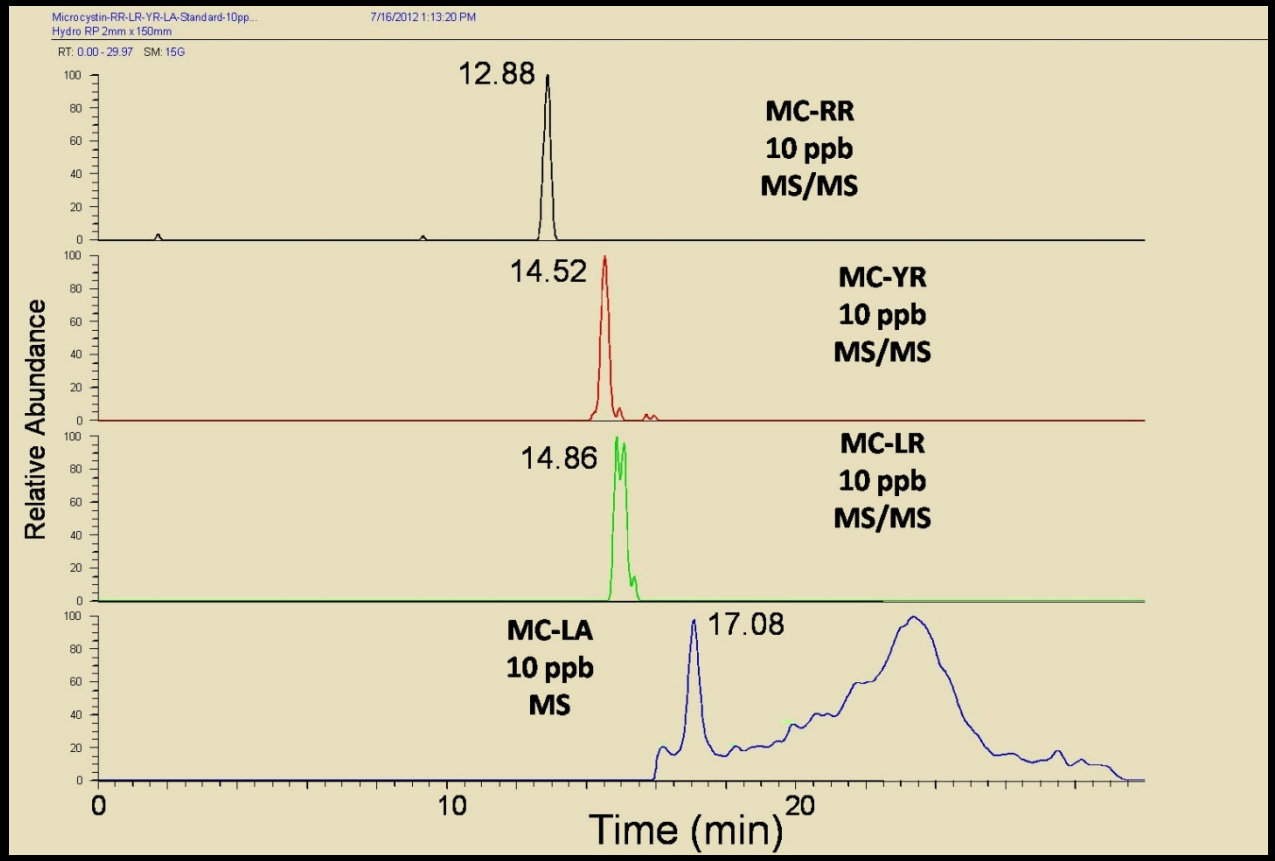
Microcystin Analysis

- ELISA (Adda) from Abraxis
  - Equally sensitive to all microcystin variants
  - Subject to matrix interference
- LC-MS/(MS)
  - -RR, -LR, -dmLR, -YR (LC-MS-MS)
  - - LA, LF, LW (LC-MS)
  - LOD/LOQ = 0.01 – 0.1

Grand Lake 2010
Microcystin –RR, YR, LR, LA (SIM)



Microcystin –RR, -YR, -LR, -LA 10 µg/L



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