Detection of *Prymnesiun parvum* Toxins by LC-MS

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History of *Prymnesiun parvum*

- Known as a toxic golden algae since the 1930s (Carter, N. 1937)
- Japanese workers first isolated two ichthyotoxic and hemolytic compounds prymnesin 1 and 2 in 1994 (Igarashi, T.; Aritake, S.; Yasumoto, T.)
- This group determined the structure of the two compounds in 1996.

What is Golden Algae?

- **A naturally occurring microscopic flagellated algae that typically occurs in brackish waters**
- **Under certain environmental stresses, this alga can produce toxins which can cause massive fish kills**

Prymnesium parvum cell

Golden Algae in Texas

•Fish kills from the golden alga in inland waters in Texas since 1985

•The alga has caused fish kills in several river basins in Texas

•This algal species is found worldwide in estuarine waters (estuaries are mixing zones between water from rivers and seawater)

•Found in freshwater bodies that have relatively high salt content.

•High salt freshwater is where Texas fish kills have occurred

•Texas Parks Wildlife biologists were the first to report occurrence of this alga in freshwater bodies in the Western Hemisphere

•Other states have reported its occurrence or possible occurrence

•Fish kills caused by the alga can be significant resulting in both ecological and economic harm to the affected water bodies

Environmental Impacts

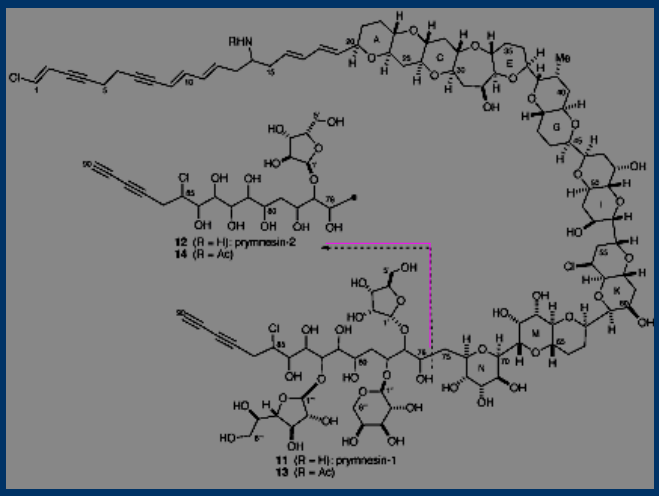
•**Fishery** - all species of fish are affected and can die from *Prymnesium parvum*, toxins. The numbers of fish killed depends on how long the bloom remains active and if toxins are present.

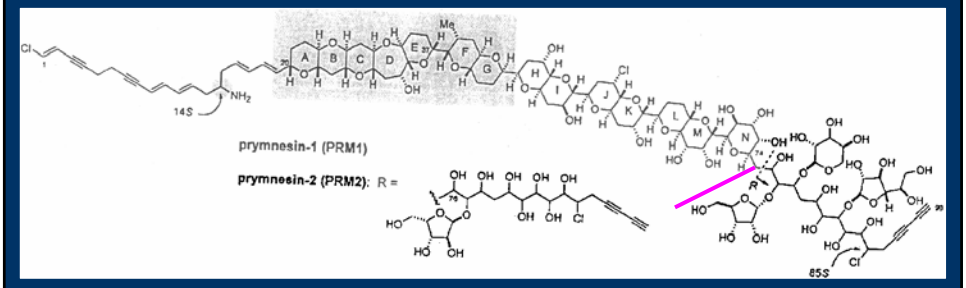
Appearance of gills of dying fish during bloom conditions

Aerial Photograph showing water color during Golden Algae bloom

P. parvum toxins prymnsin-1 and prymnesin-2 structures

Prym-1 (2263 average mass) Prym-2 (1969 average mass)





Sample collection and preparation:

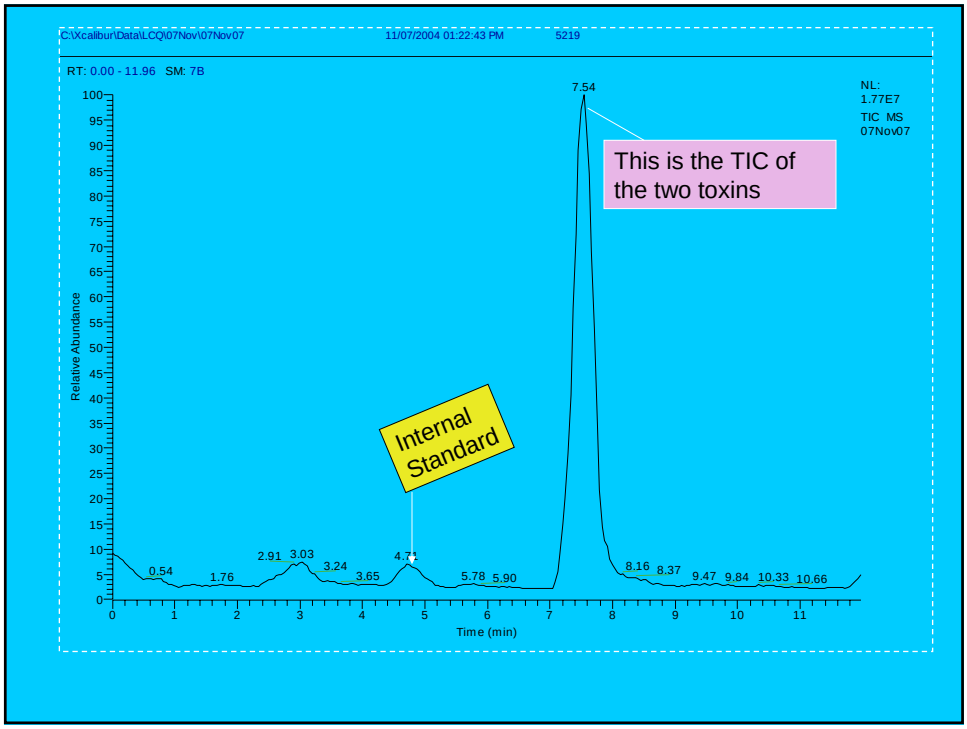
- Water samples were collected in amber glass vials , no head space, stored on ice or refrigerated.
- Water samples were filtered using a 0.2 micron filter
- Internal standard was added (reserpine) to 1 mL sample.
- 20 µL injection

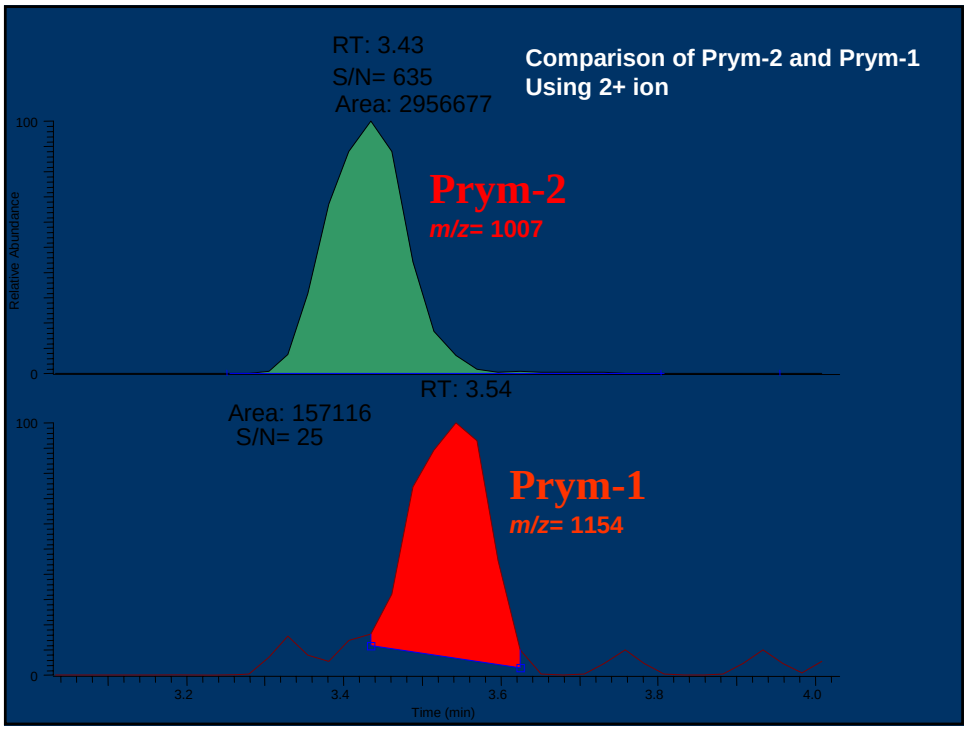
Instrument method

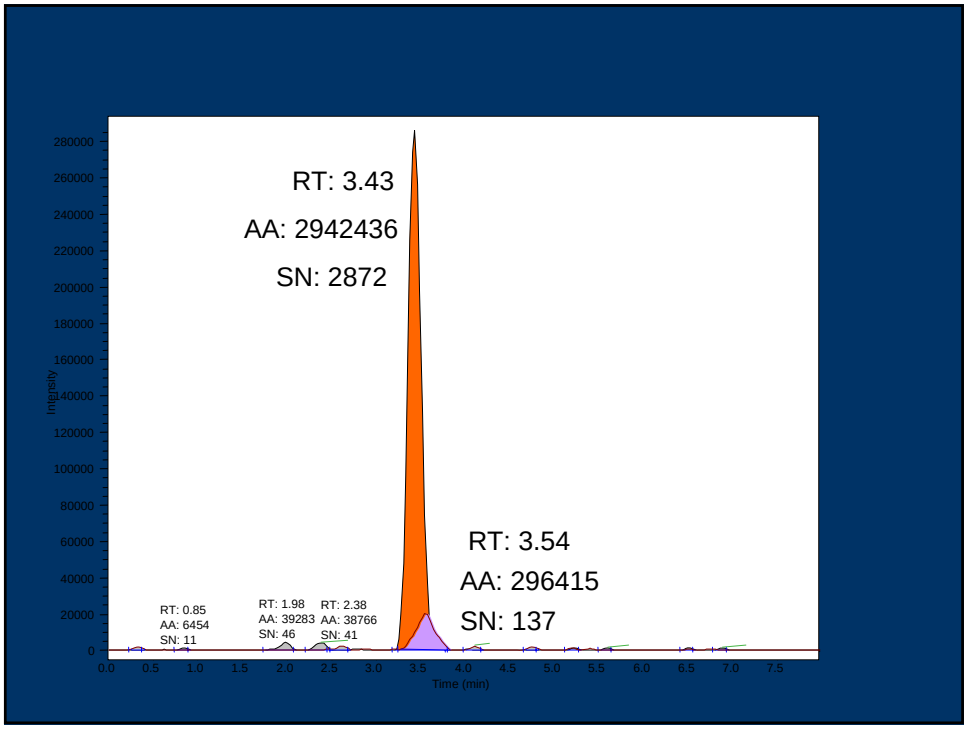
- LCQ Advantage (Thermo-whoever)
- C18 100 mm X 4.6 mm particle size 5 um, pore 100 A
- Isocratic: 20%H2O (0.5%amonium acetate): 80% acetonitrile
- LC flow of 400µL/minute
- Run time 10 minutes
- ESI positive ionization

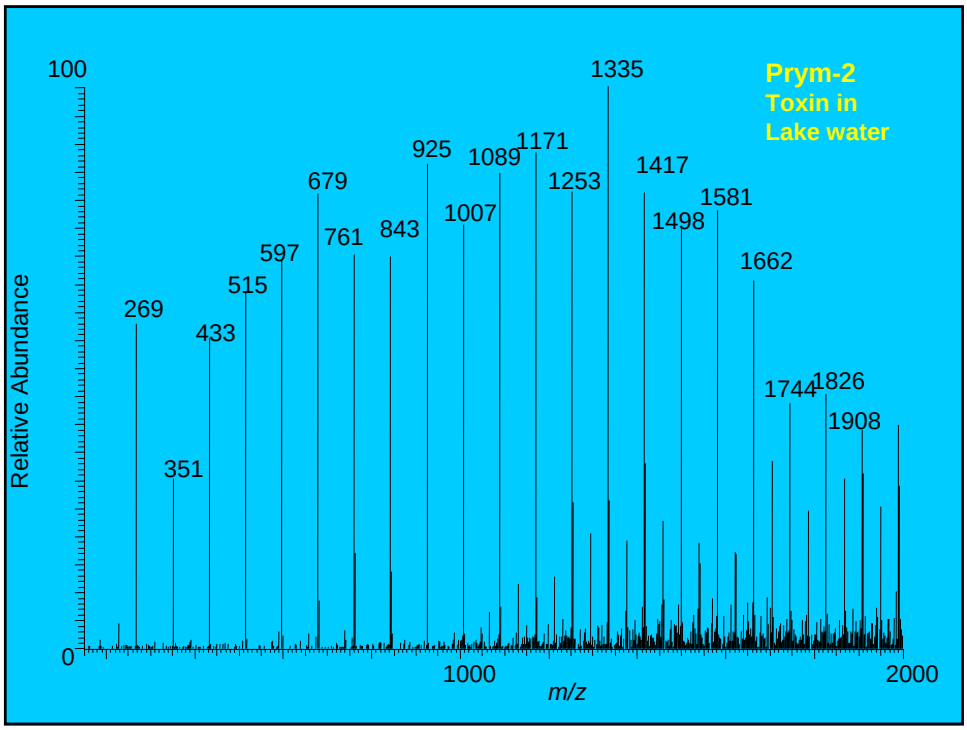
- Scan 150 to 2000 *m/z*

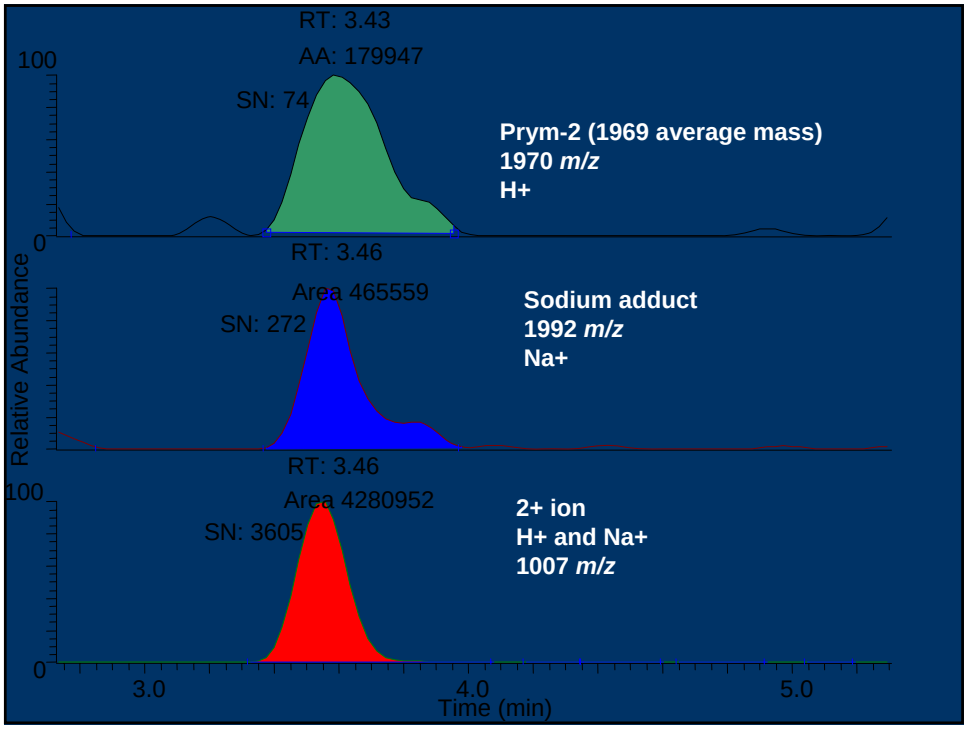
Capillary Temp (C):	250.00
Source Voltage (kV):	4.50
Source Current (µA):	80.00
Capillary Voltage (V):	46.00
Tube Lens Offset (V):	55.00
Multipole RF Amplifier (Vp-p):	450.00
Multipole 1 Offset (V):	-1.70
Multipole 2 Offset (V):	-5.50
InterMultipole Lens Voltage (V):	-54.00
Trap DC Offset Voltage (V):	-10.00

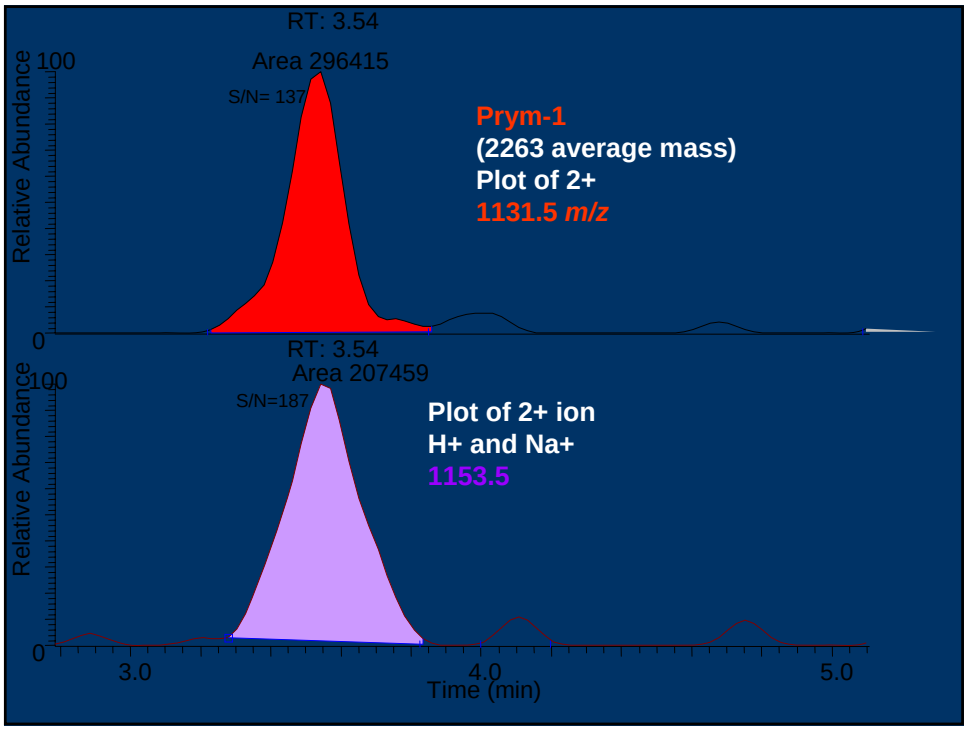

C:\Xcalibur\Data\LCQ\07Nov\07Nov07
11/07/2004 01:22:43 PM
5219
RT: 0.00 - 11.96 SM: 7B
NL:
1.77E7
TIC MS
07Nov07
This is the TIC of the two toxins
Internal Standard
Relative Abundance
Time (min)
7.54
0.54
1.76
2.91
3.03
3.24
3.65
4.71
5.78
5.90
8.16
8.37
9.47
9.84
10.33
10.66


RT: 3.43
S/N= 635
Area: 2956677
Comparison of Prym-2 and Prym-1
Using 2+ ion
Prym-2
m/z= 1007
Relative Abundance
RT: 3.54
Area: 157116
S/N= 25
Prym-1
m/z= 1154
Time (min)


RT: 3.43
AA: 2942436
SN: 2872
RT: 3.54
AA: 296415
SN: 137
RT: 0.85
AA: 6454
SN: 11
RT: 1.98
AA: 39283
SN: 46
RT: 2.38
AA: 38766
SN: 41
Intensity
Time (min)


Prym-2
Toxin in
Lake water
Relative Abundance
m/z
100
0
1000
2000
269
351
433
515
597
679
761
843
925
1007
1089
1171
1253
1335
1417
1498
1581
1662
1744
1826
1908


RT: 3.43
AA: 179947
100
SN: 74
Prym-2 (1969 average mass)
1970 m/z
H+
0
RT: 3.46
Area 465559
SN: 272
Sodium adduct
1992 m/z
Na+
Relative Abundance
RT: 3.46
100
Area 4280952
SN: 3605
2+ ion
H+ and Na+
1007 m/z
0
3.0
4.0
5.0
Time (min)

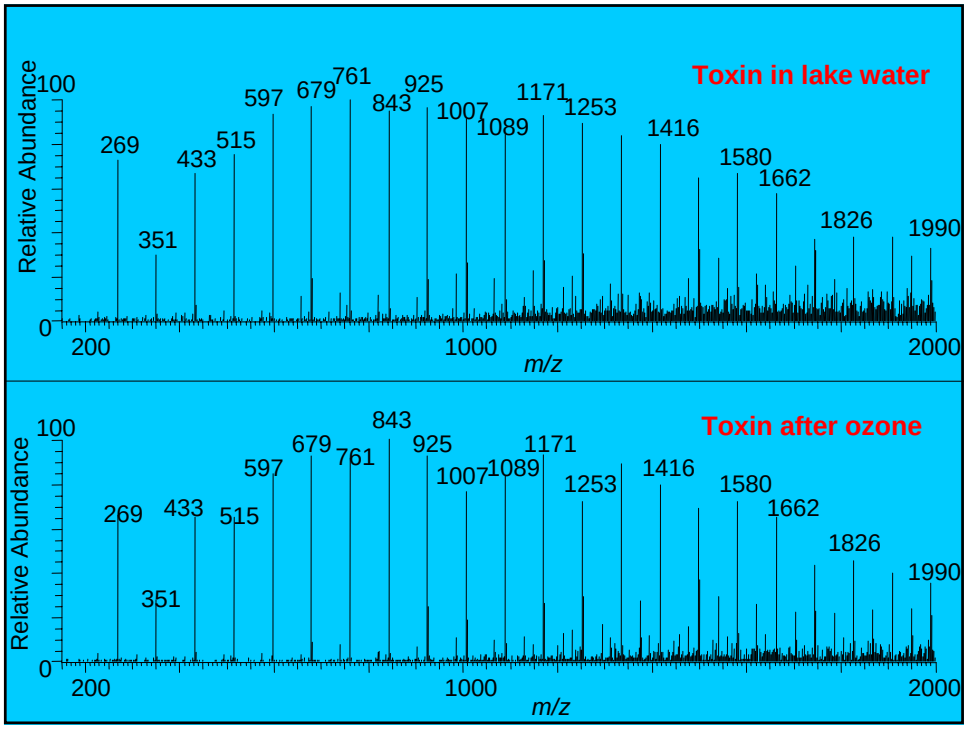

RT: 3.54
Area 296415
100
S/N= 137
Prym-1
(2263 average mass)
Plot of 2+
1131.5 m/z
Relative Abundance
0
RT: 3.54
Area 207459
100
S/N=187
Plot of 2+ ion
H+ and Na+
1153.5
Relative Abundance
0
3.0
4.0
5.0
Time (min)

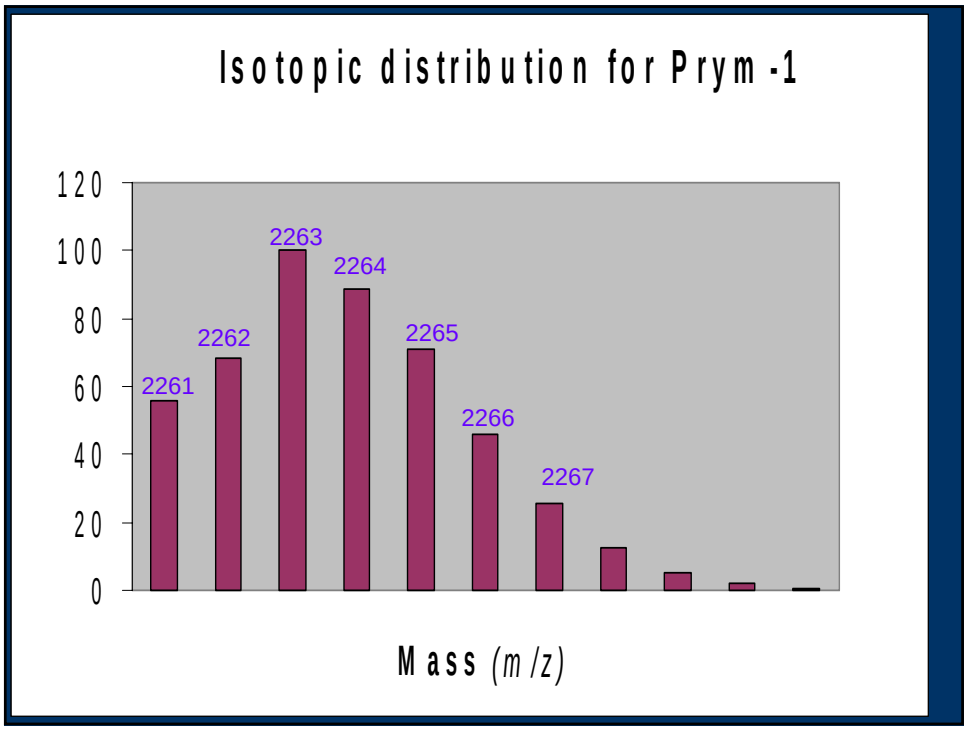
Treatment of Toxic Water

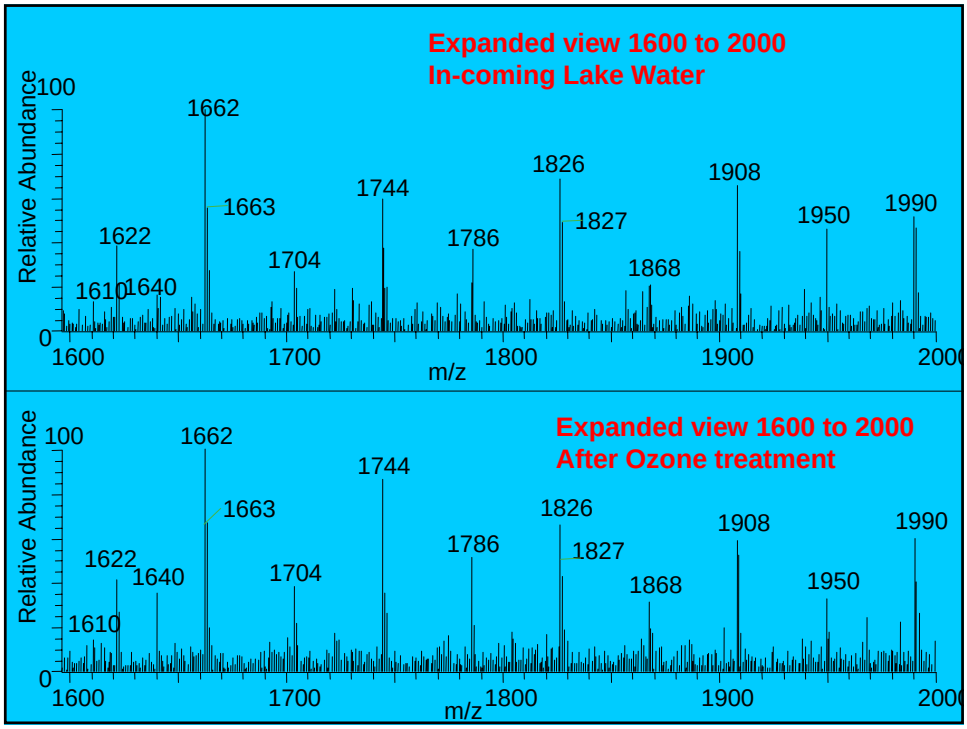
- pH adjustment
- Addition of ammonia
- Oxidative treatment
  - Oxidative Algicides
  - $KMnO_4$
  - O_3

And the winner is…..

OZONE!


Toxin in lake water
Relative Abundance
100
0
200
1000
2000
m/z
269
351
433
515
597
679
761
843
925
1007
1089
1171
1253
1416
1580
1662
1826
1990
Toxin after ozone
Relative Abundance
100
0
200
1000
2000
m/z
269
351
433
515
597
679
761
843
925
1007
1089
1171
1253
1416
1580
1662
1826
1990


Isotopic distribution for Prym-1
120
100
80
60
40
20
0
2261
2262
2263
2264
2265
2266
2267
Mass (m/z)



Acknowledgements

- USEPA National Coastal Assessment
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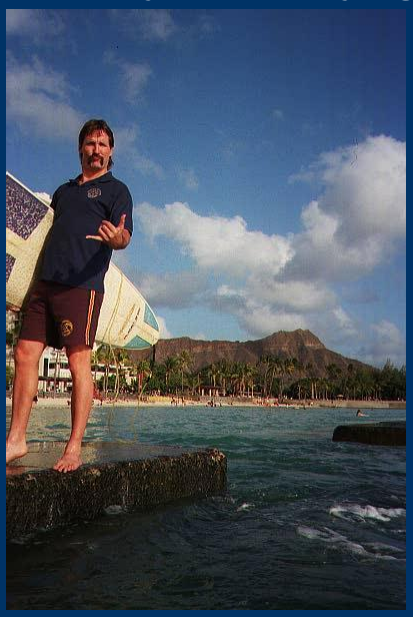
- Texas Golden Algae Working Group
- Texas State University Edwards Aquifer Research and Data Center

Thanks y’all for staying!



Now can we go surfing?