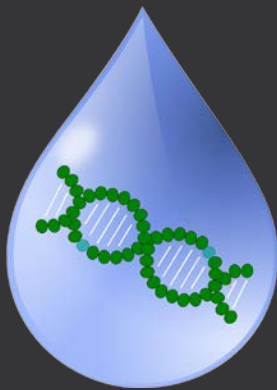


Effect of sample handling on anatoxin-a stability

January 25, 2018



**Bend
Genetics**

Gabie Gutierrez

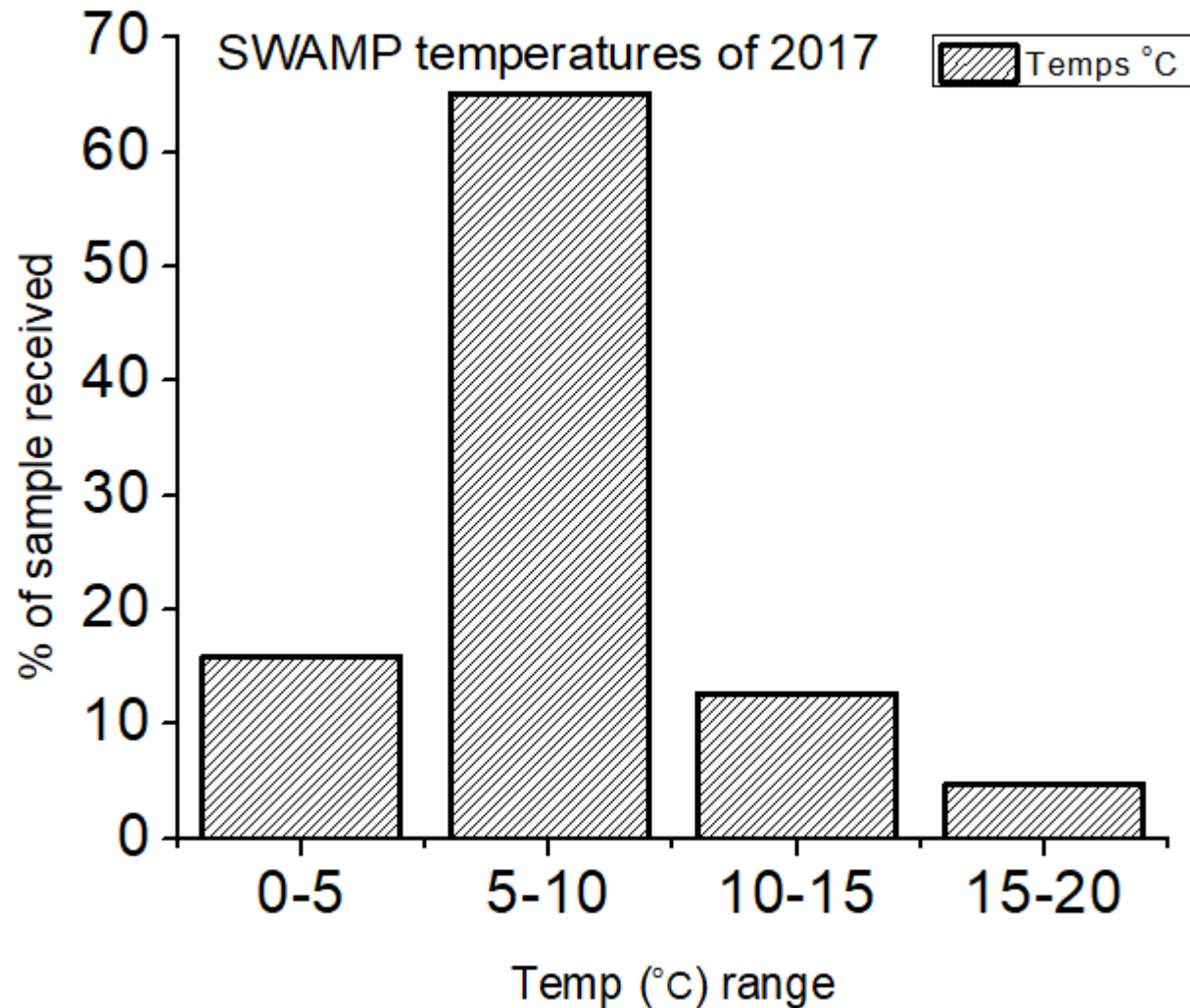
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Study Objectives

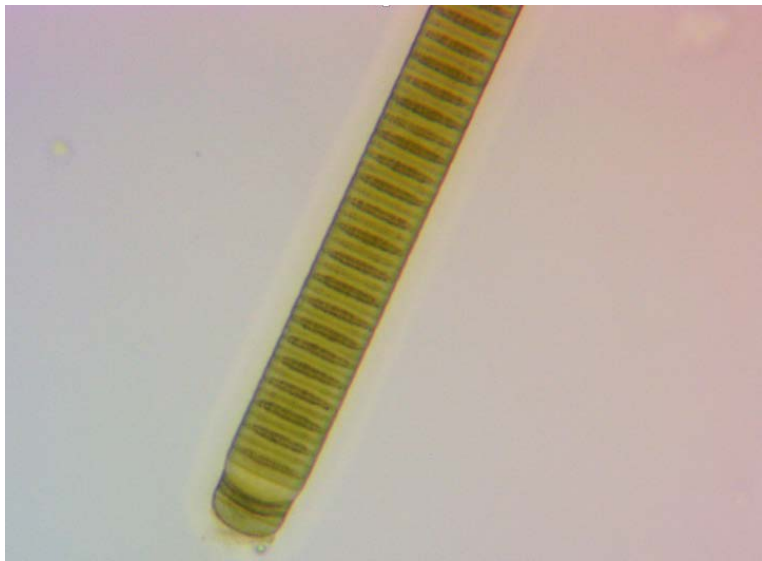
- Assess the effect of temperature and holding time on anatoxin-a degradation.
 - Evaluate risk of false negatives.
- Determine the effect of preservative on anatoxin-a stability



Background

- Potent neurotoxin
- Anatoxin-a can be produced by planktonic or benthic species of cyanobacteria
 - Common genera include:
Anabaena/Dolichospermum, Oscillatoria,
Phormidium
- Majority of anatoxin-a is stored intracellularly





Phormidium



Dolichospermum

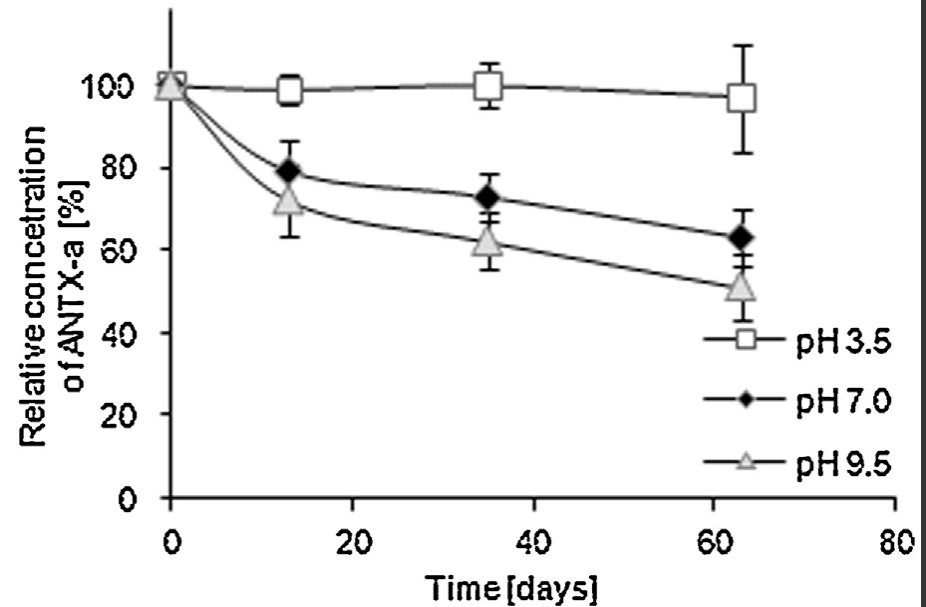
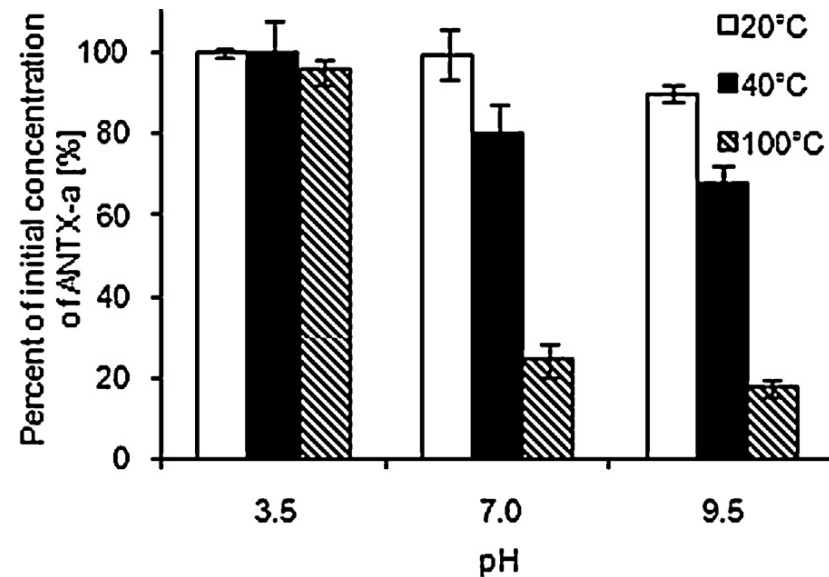


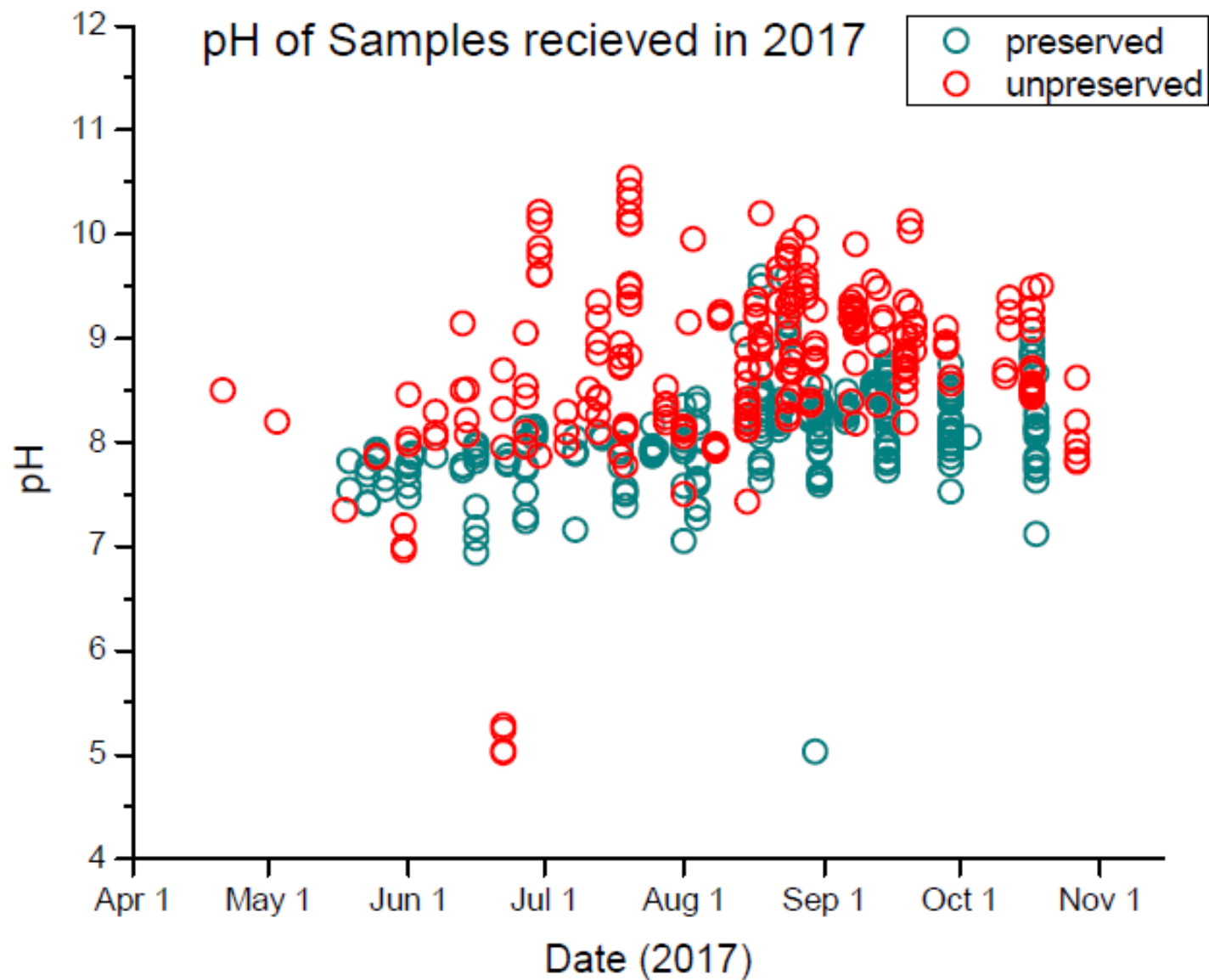
Cylandrospermum

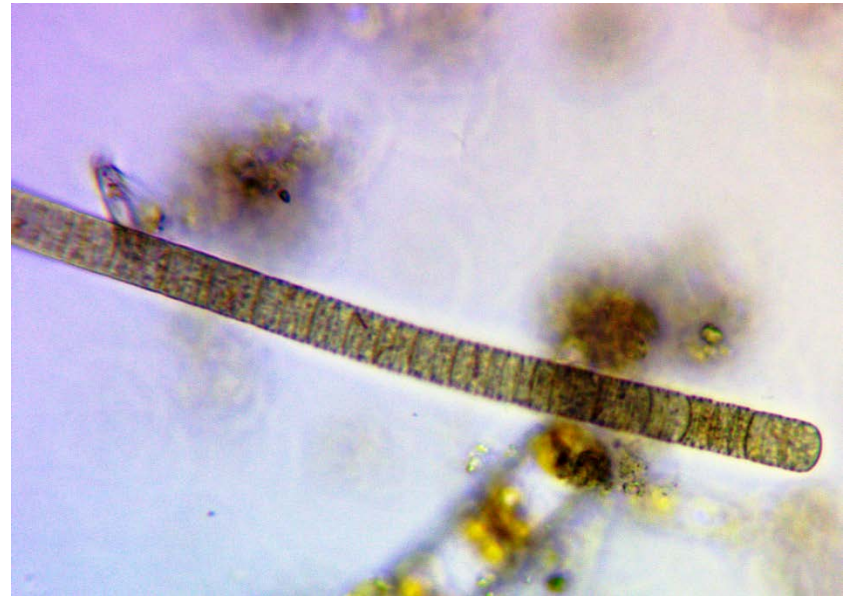
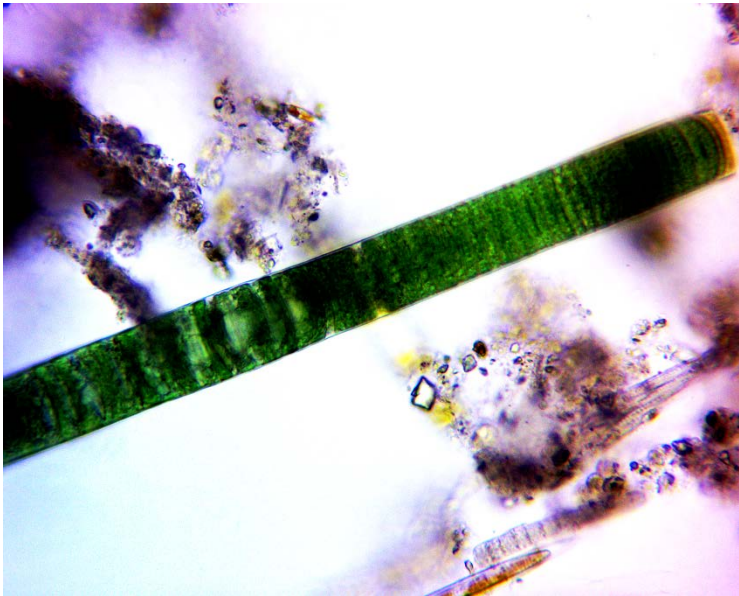
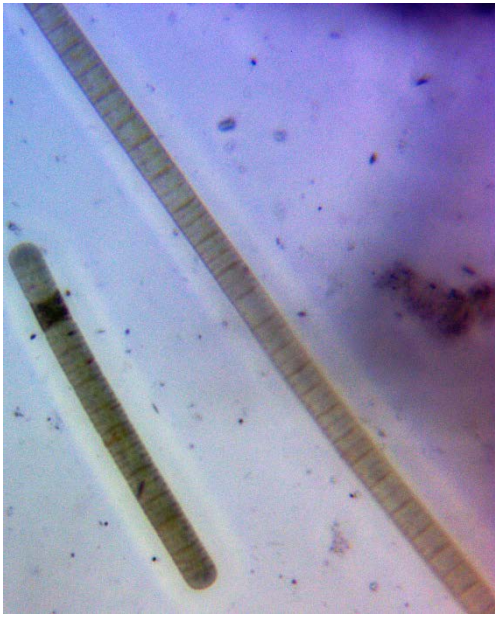


Anabaena

Abiotic factors influencing ATX degradation

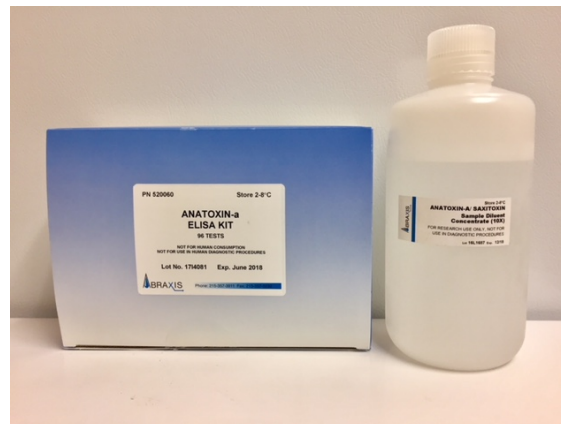






Experimental design

- Abraxis anatoxin-a ELISA kit (PN520060)
- Used American River water to dilute a highly concentrated ATX sample \rightarrow $\sim 15 \mu\text{g/L}$
- +/- Anatoxin-a preservative
- Factorial design (triplicates)
- pH measured at start and finish.



Experiment set up

Preserved

| Day - 0 | Day - 1 | Day - 2 | Day - 3 | Day - 5 | Day - 14 | |
|---------|---------|---------|---------|---------|----------|-------|
| 3 | 3 | 3 | 3 | 3 | 3 | 4° C |
| | 3 | 3 | 3 | 3 | 3 | 25° C |

Unpreserved

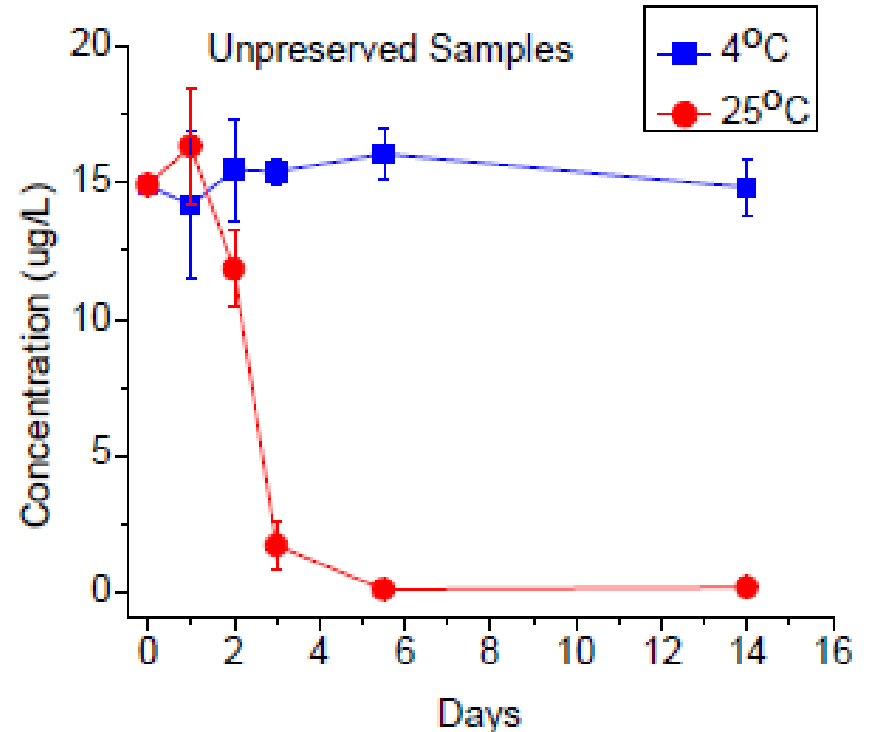
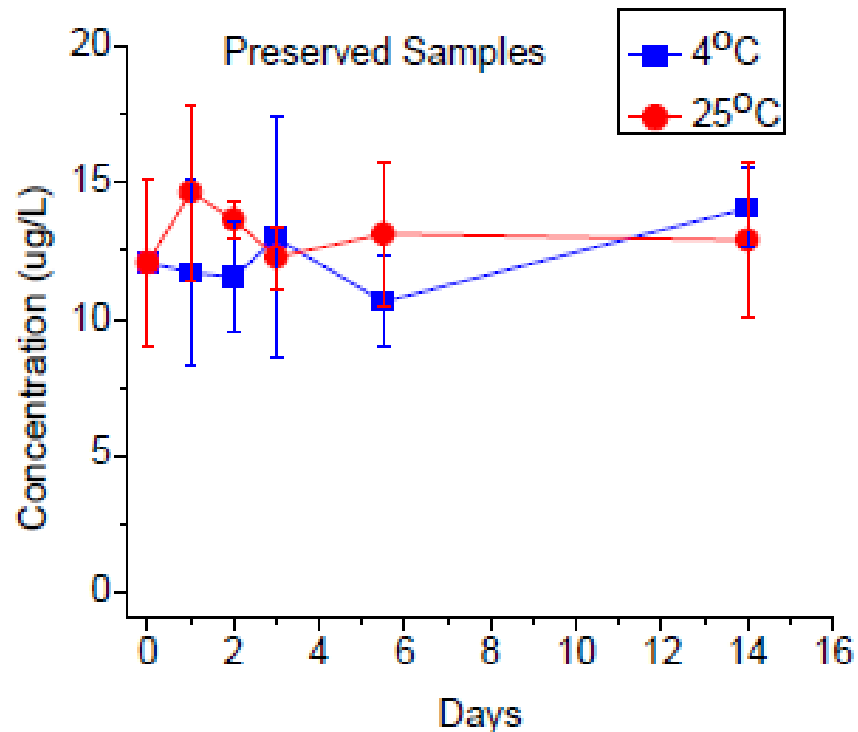
| Day - 0 | Day - 1 | Day - 2 | Day - 3 | Day - 5 | Day - 14 | |
|---------|---------|---------|---------|---------|----------|-------|
| 3 | 3 | 3 | 3 | 3 | 3 | 4° C |
| | 3 | 3 | 3 | 3 | 3 | 25° C |

Results

Preserved cold/warm = no change

Unpreserved cold = no change

Unpreserved warm = rapid loss of anatoxin-a



Conclusions

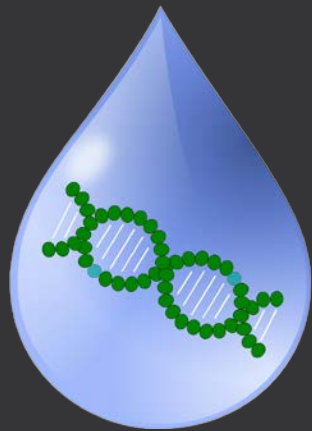
- The preservative kept anatoxin-a stable under normally encountered temps for two weeks
- Unpreserved samples kept cold showed no degradation over the same time period
- Unpreserved samples held at 25°C, exhibited a 28% reduction in two days and 99% by 5 days.
- The temp where ATX degradation begins to occur for unpreserved samples remains to be precisely determined (i.e., $4^{\circ}\text{C} < X < 25^{\circ}\text{C}$).
- Samples can be analyzed after holding time if kept cold or are preserved
- Samples can be analyzed if received above 10°C if they were preserved.

Questions?

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