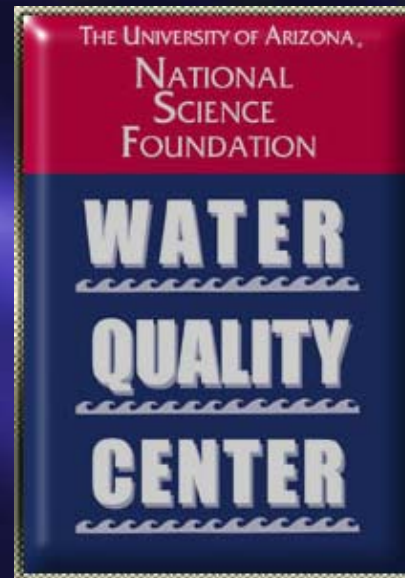


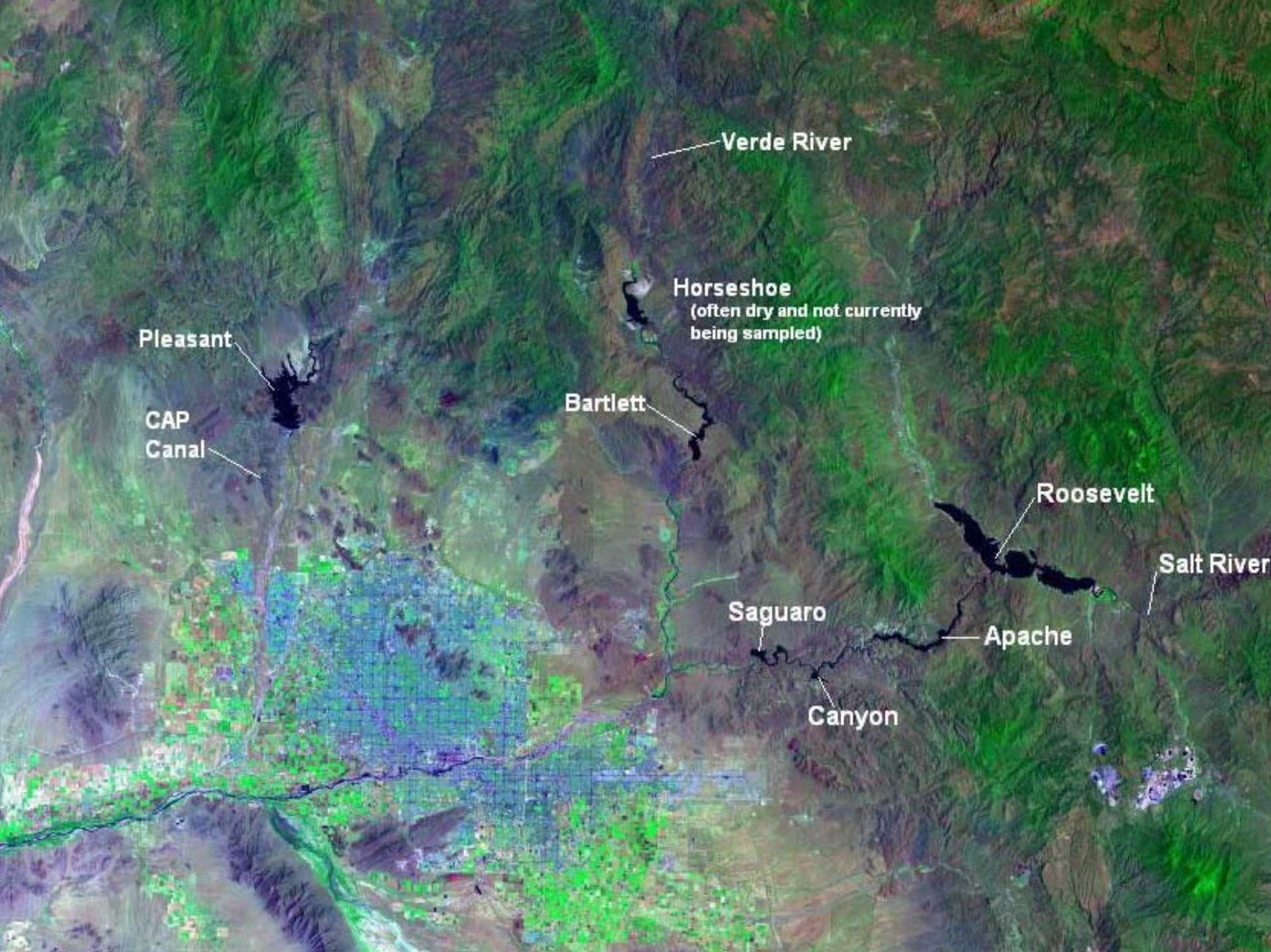
**COMPREHENSIVE WATERSHED MANAGEMENT FOR THE  
VALLEY OF THE SUN AND THE CENTRAL ARIZONA BASINS**



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Verde River

Horseshoe  
(often dry and not currently  
being sampled)

Pleasant

Bartlett

CAP  
Canal

Roosevelt

Salt River

Saguaro

Apache

Canyon

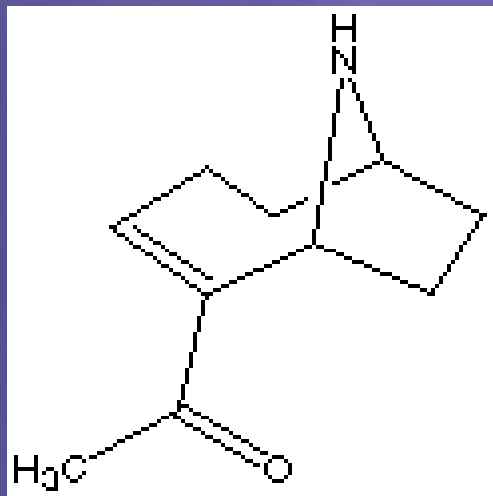
- All reservoirs routinely sampled for anatoxin-a, cylindrospermopsin, microcystin, and saxitoxin.
- Analyses are done by Dr. Greg Boyer at SUNY-CESF (Syracuse).

# Analytical Methods

- Anatoxin-a, Saxitoxin
  - HPLC after fluorescent derivatization.
- Microcystin
  - Protein phosphatase inhibition assay.
    - If greater than 0.5  $\mu\text{g/L}$ , confirmed by HPLC using a PDA detector.
- Cylindrospermopsin
  - HPLC using a photodiode array detector
- Detection limit for all assays is less than 0.1  $\mu\text{g/L}$

# Anatoxin-a (ethanone, 1-(1R,6R)-9-azabicyclo[4.2.1]non-2-en-2-yl-)

- It is one of the most potent agonists at the nicotinic acetylcholine receptor discovered to date.
- LD<sub>50</sub> of 250 µg/L (i.p. mouse).
- Small molecular size means rapid onset of symptoms (otherwise known as Very Fast Death Factor).



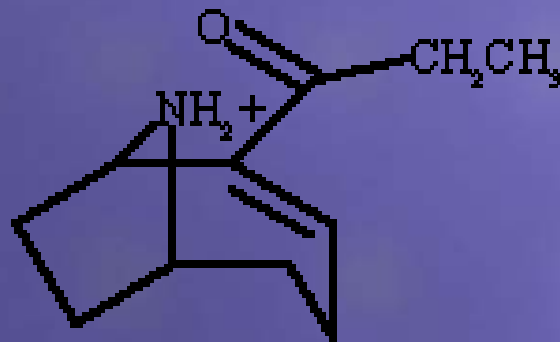
# Mode of Action

- Binds to the acetylcholine receptor and acts as an agonist.
- Not ejected by the nerve after binding so impulses do not fade.
- Additionally, it inhibits acetylcholinesterase used to inactivate the acetylcholine released by normal nerve impulses.

- The result is an overstimulation of chest muscles which may lead to limp paralysis, dyspnea, cyanosis, cardiac arrhythmia and death.
- Onset of symptoms is extremely rapid (< 5 min.).
- No antidote, supportive care only.

# Other Anatoxins

- Homoanatoxin
  - Homoanalogue of anatoxin-a.
  - Toxicity is the same as anatoxin-a.



HOMOANATOXIN-A



# Anatoxin-a(S)

- Much more potent than anatoxin-a or homoanatoxin.
- LD<sub>50</sub> of 20 µg/kg<sup>-1</sup> i.p. mouse.
- The only naturally occurring organophosphate known.

# History of Anatoxin Production in the Salt River Reservoirs

- Summer of 2001, called to investigate a large die-off of *Corbicula fluminae* in the upper reaches of Saguaro.





- During this event, we found 120-140  $\mu\text{g/L}$  of anatoxin-a.
- These were the highest levels ever recorded by the reporting lab and posed an imminent risk to public safety.
- Levels quickly diminished with distance toward Stewart Mtn. Dam.





# Suspect Organisms Capable of Anatoxin-a Production

- *Anabaenopsis circinalis*

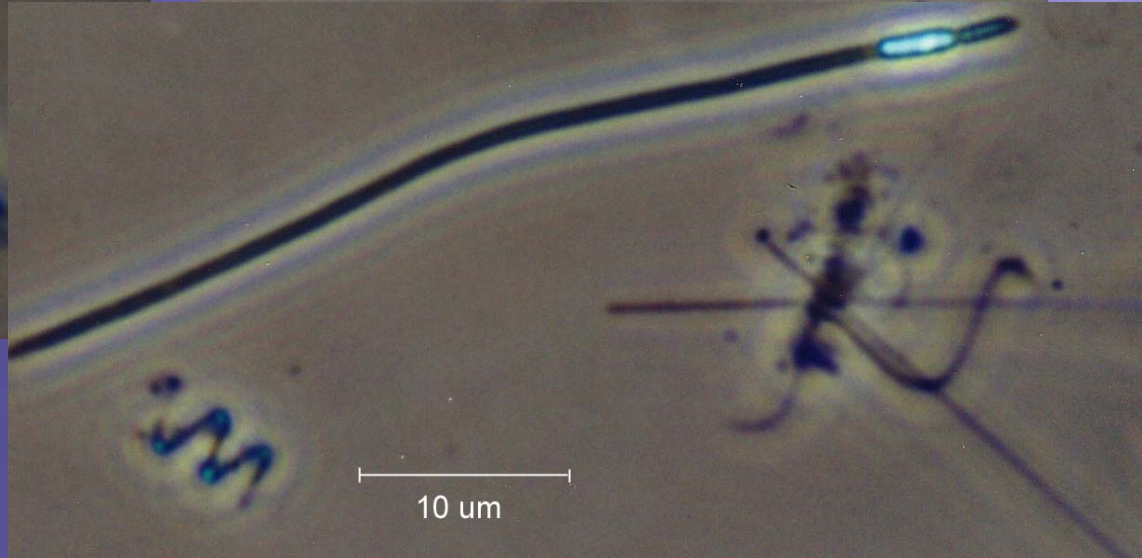
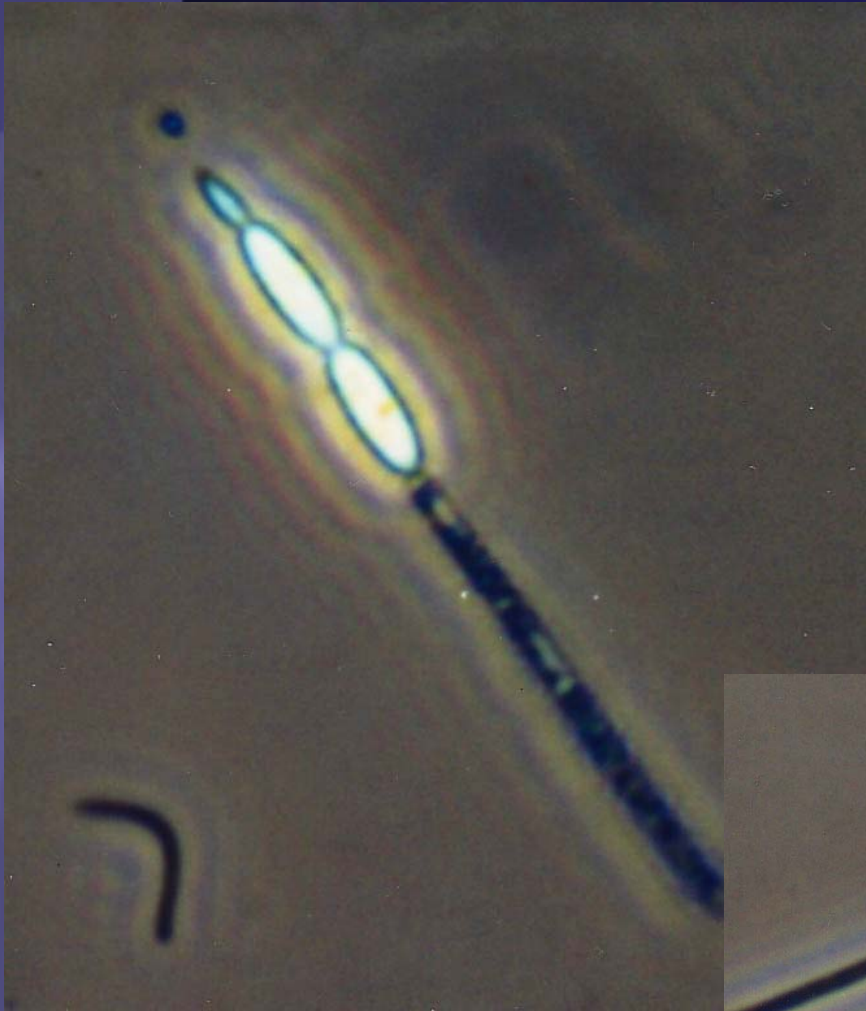
Division Cyanophyta  
Order Nostocales  
Family Nostocaceae  
Genus: *Anabaenopsis*





# *Cylindrospermopsis raciborskii*

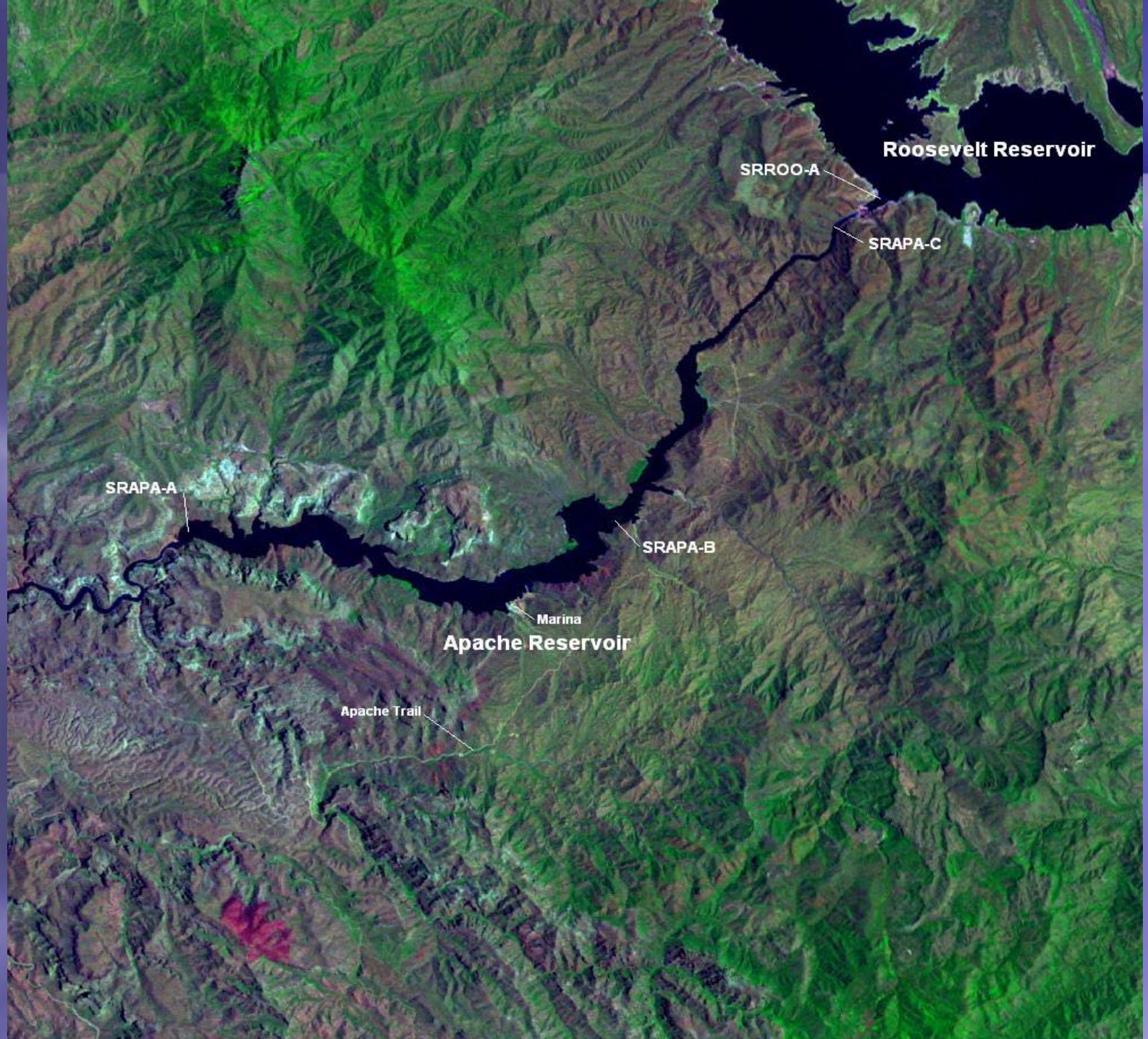
- Invasive exotic recently discovered during this project to be in all of the reservoirs surrounding the Valley.
- *C. raciborskii* can produce 3 distinct toxins:
  - Cylindrospermopsin (hepatotoxin)
  - Saxitoxin (hepatotoxin)
  - Anatoxin-a



- Most other planktonic forms of toxic cyanobacteria produce blooms on top of the water.
- *C. raciborskii*, however, is always found well-distributed throughout the water column and has the highest concentrations below the surface.
- If toxins are produced at depth near the dam of a reservoir, these could be released into the riverine portion of downstream reservoirs or into rivers/canals.

# Current Fish Kills

- Starting in May, a fish kill occurred in Apache from Roosevelt Dam down to Burnt Corral Campground.
- Anecdotal reports indicated thousands to tens of thousands of effected fish of varying species including threadfin shad, bass, bluegill, and carp



Roosevelt Reservoir

SRROO-A

SRAPA-C

SRAPA-A

SRAPA-B

Marina

Apache Reservoir

Apache Trail



- A few weeks later, a fish kill occurred in Canyon.
- This was followed by a fish kill in the upper reaches of Saguaro and recently, another fish kill in Apache.

- Samples were taken for 133 VOC's, 16 carbamates, 11 organophosphates, and 9 chlorinated pesticides in each reservoir.
- All came back as non-detects.







- No abnormal pathological findings upon gross necropsy.
- Algae identification at all sites revealed the presence of both *C. raciborskii* and *Anabaenopsis*.
- Thus far, all samples taken for anatoxin-a, saxitoxin, microcystin, and cylindrospermopsin have come back as non-detectable.

## *However,*

- Cylindrospermopsin, microcystin, and saxitoxin are extremely environmentally stable compounds.
- The non-linearity of the fish kills indicate that this was not a spill or an environmentally stable compound.

- If hepatotoxicity was involved, there would be gross pathological evidence.
- This pathological evidence would be lacking if neurotoxicity is involved.
- All physico-chemical parameters are within “normal” for the time of the year in question.

- We investigated the kills days after they occurred.
- The half life of anatoxin-a under laboratory conditions is 5 days.
- It is inactivated by both sunlight and highly alkaline conditions.

- *Under current field conditions in reservoirs on the Salt River, the half life of anatoxin-a probably drops to hours or even minutes.*
- *Difficult to quantify an extremely fast-acting acetylcholinesterase inhibitor that kills aquatic organisms leaving no traces in its wake.*

# Data Gaps/Research Needs

- Increased monitoring efforts on all reservoirs for potentially toxic organisms and the toxins themselves.
- Monitoring of rivers and canals downstream of reservoirs.





- Genetic isolation of different strains of potentially toxic species.
- Once these strains have been identified, we need a better understanding of environmental conditions leading to toxin production.

- Biological indicators as early warning of xenobiotic exposure.
- Specifically, *in vivo* measurements to quantify acetylcholinesterase inhibition in fish, zooplankton, and/or bivalves.

- Can be done either colorimetrically or through development of an *in vivo* enzyme inhibition assay based upon esterase activity.
- Controls/baseline values for individual species could easily be performed in the lab.
- Once developed, could be performed in the field in less than one hour.

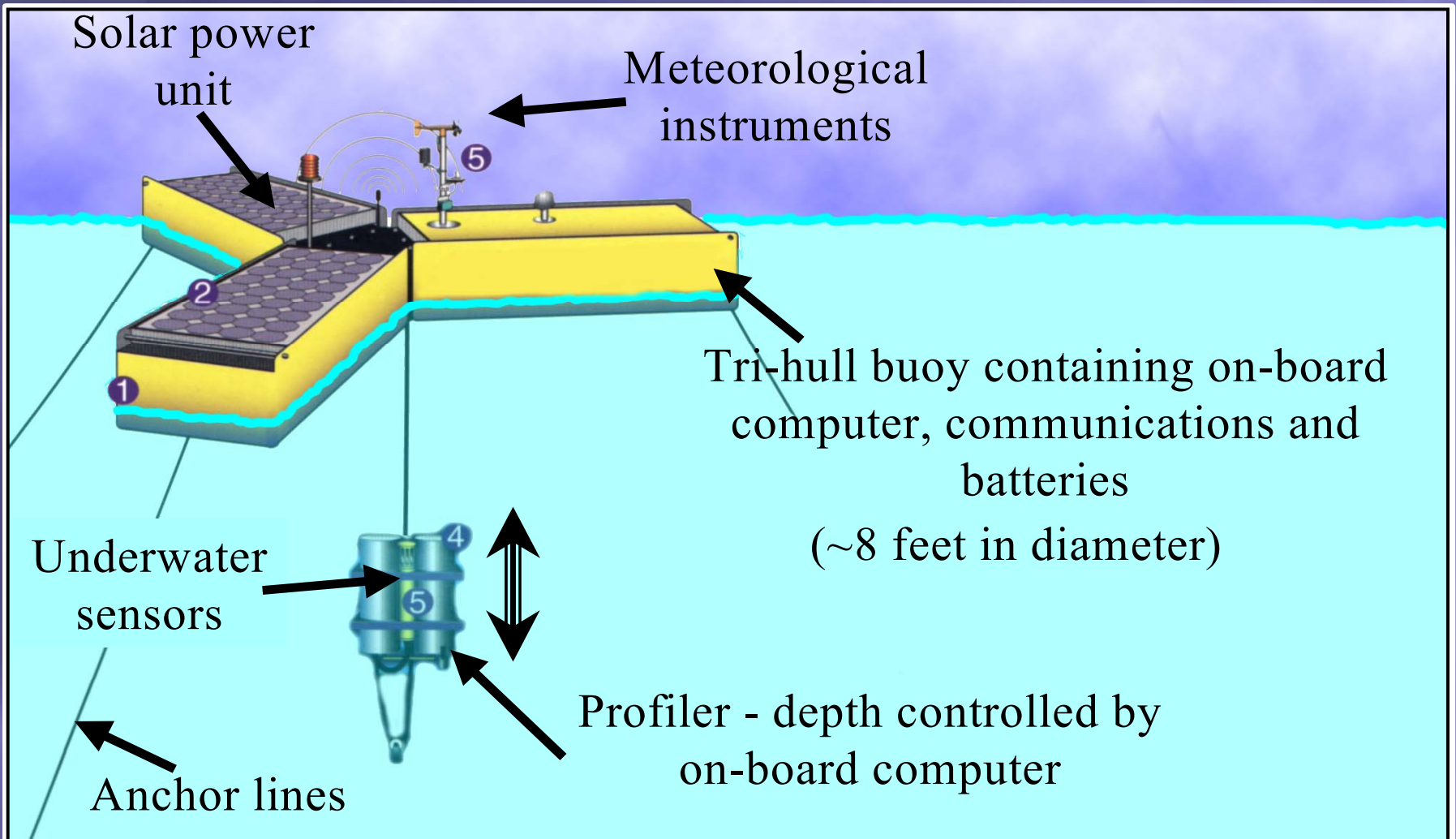
- Once toxin-producing strains have been genetically identified, modeling of production, fate, transport, and degradation rates of individual toxins would give some insight into what could be done, from a management standpoint, to alleviate the problem.

# Development of Real-Time Monitoring Buoys

- Greg Boyer at SUNY already working on.
- Fully deployable systems are still 1-2 years away.
- The single best way to ensure public safety on the reservoirs surrounding the Valley.

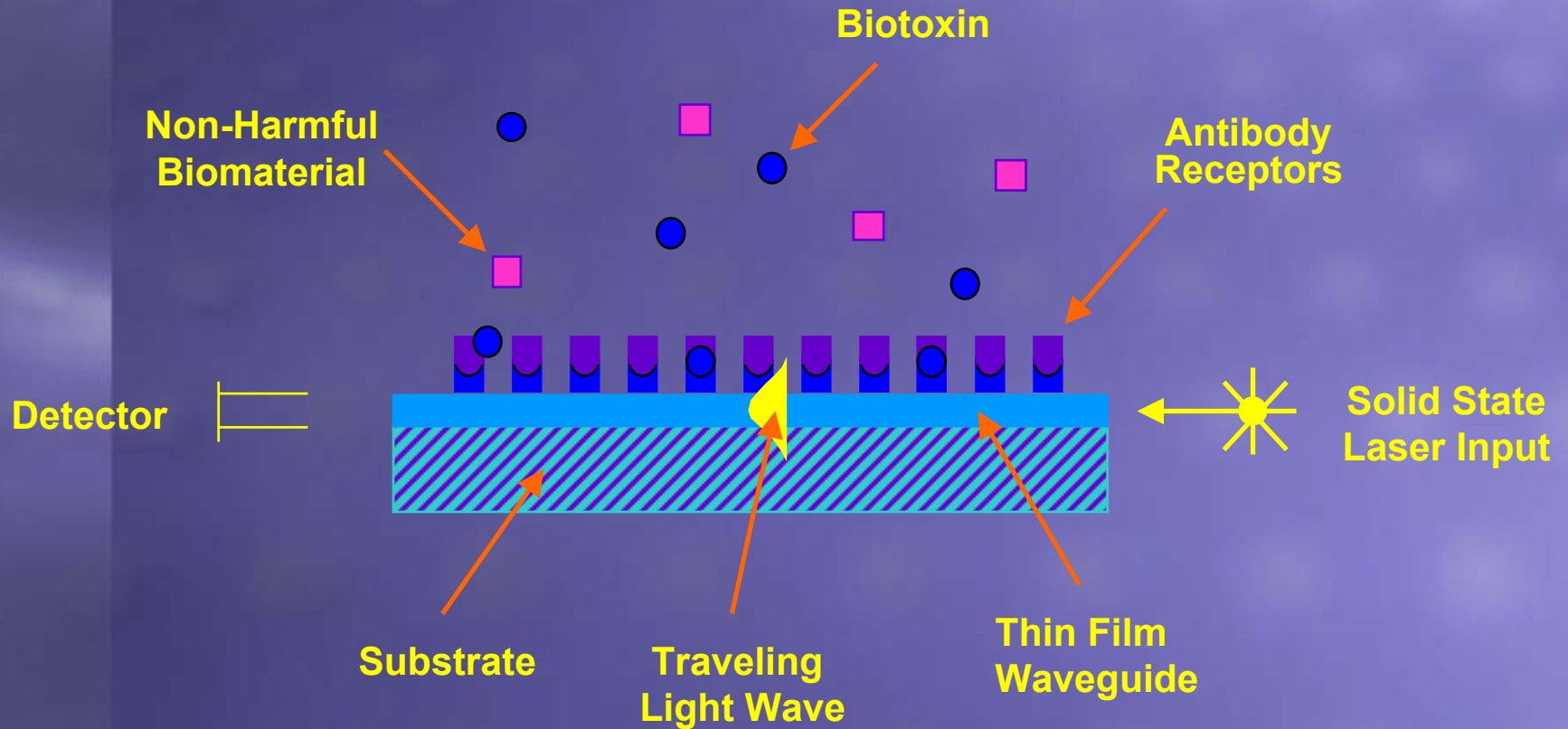
# Robotic Monitoring Platform Buoy

Slide courtesy of Dr. Greg Boyer, SUNY-CESF



# Optical Biochip Sensor Technology

Slide courtesy of Dr. Greg Boyer, SUNY-CESF



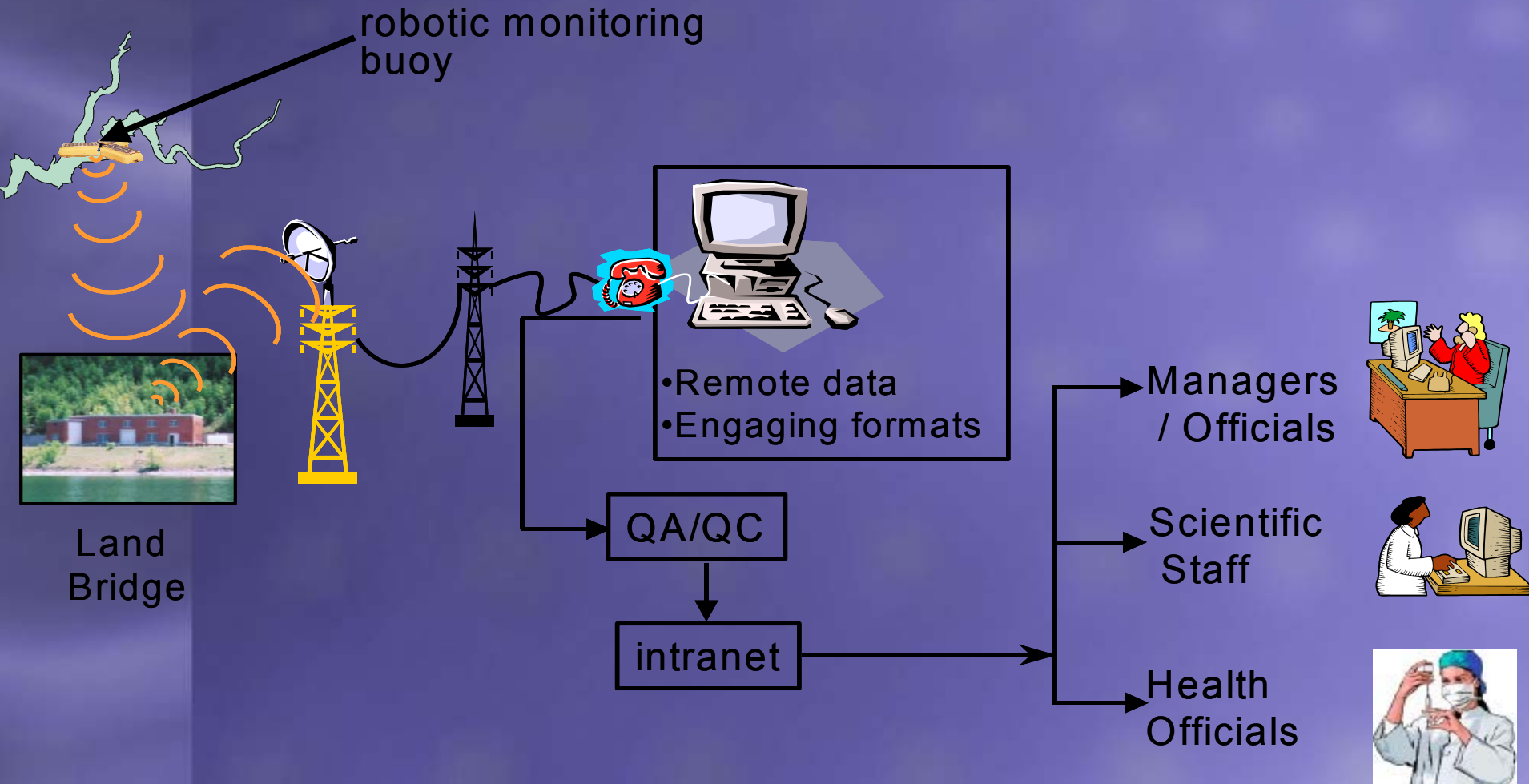
- ❖ Light is piped through an optical wave-guide whose index of refraction is dependent on surface conditions
- ❖ Antibodies can be used to specifically trap biotoxins on surface with high specificity
- ❖ The change in index of refraction creates a highly sensitive detector



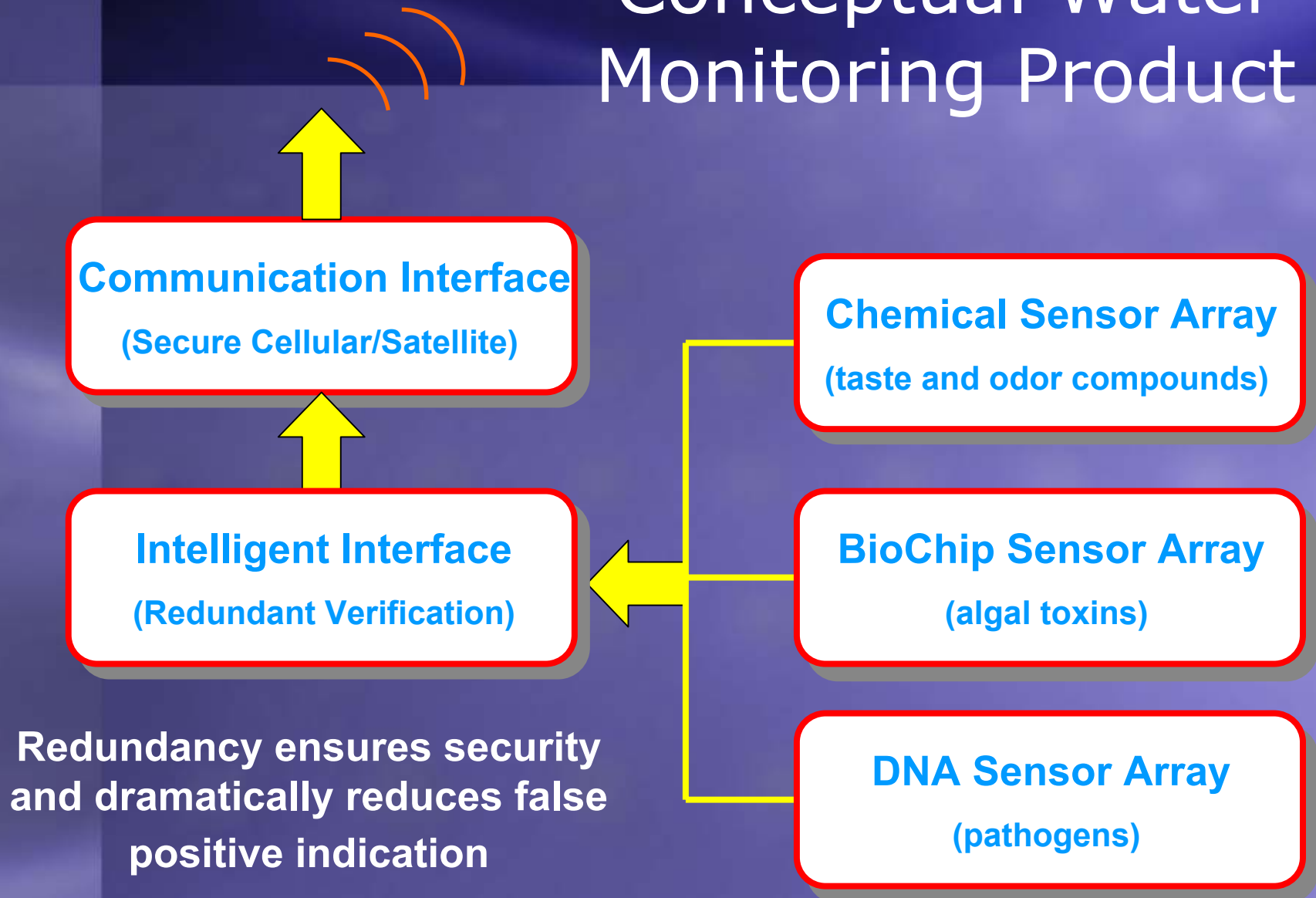
# Near Real Time Data Delivery

Slide courtesy of Dr. Greg Boyer, SUNY-CESF

## Delivering Remote Data



# Conceptual Water Monitoring Product



# Summary

- With the amount of nutrient in-loading into the Salt River reservoirs due to the Rodeo-Chedeski fire, eutrophication has occurred.
- Numbers of toxic cyanobacteria are, as of today, still relatively low.
- If these species continue to increase in number, potential toxicity to wildlife, and people, also increases.

- *During the summer of 2002, the first death in the U.S. directly attributable to anatoxin-a poisoning occurred in Wisconsin after teenage boys were swimming in a golf course pond.*



Questions?