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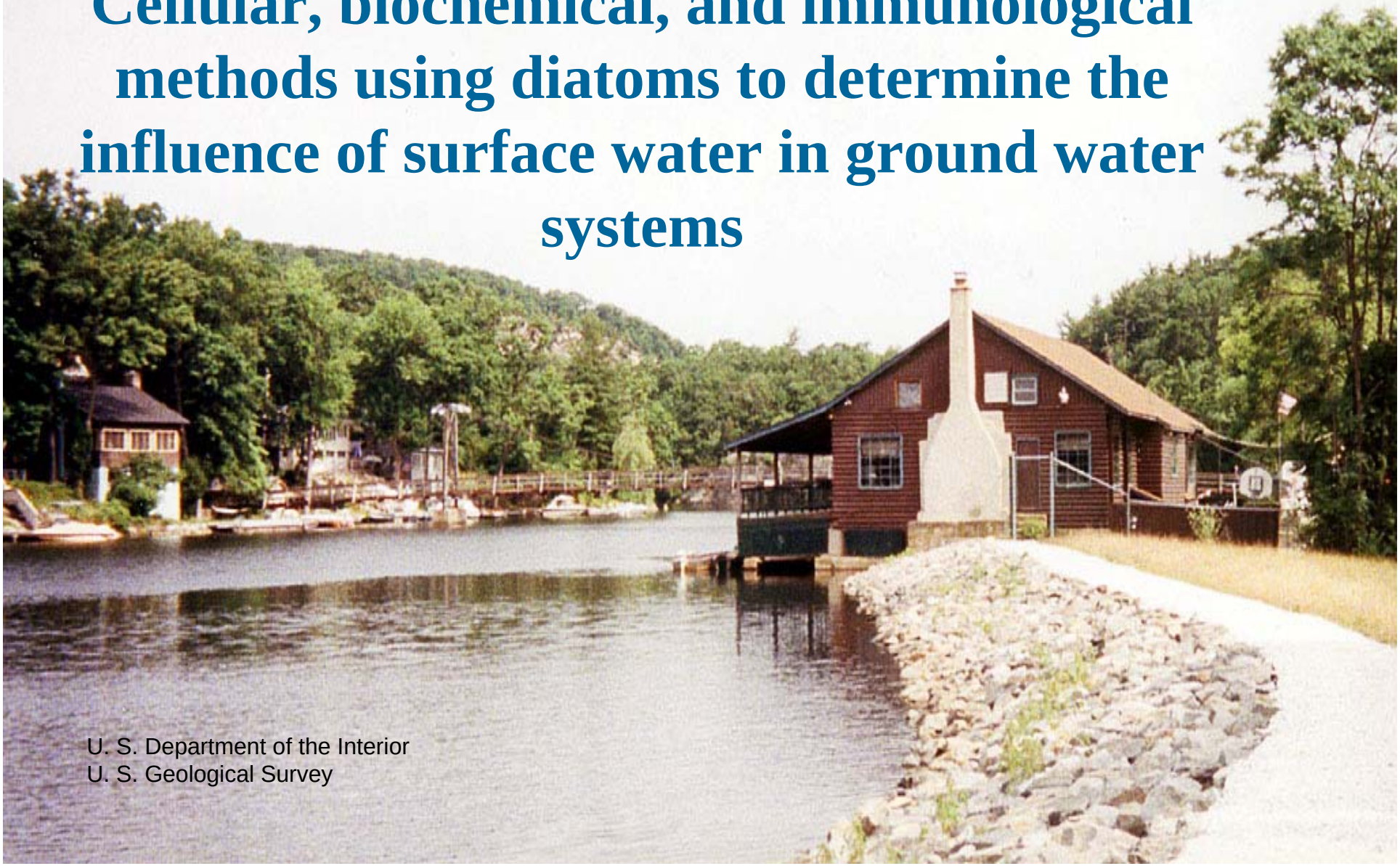
science for a changing world

Cellular, biochemical, and immunological methods using diatoms to determine the influence of surface water in ground water systems



U. S. Department of the Interior
U. S. Geological Survey

Interdisciplinary Team

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Overview

- Lakes & domestic wells in lakeside communities
- Ground water/surface water interaction
- Diatoms, biochemistry, immunology
- The future

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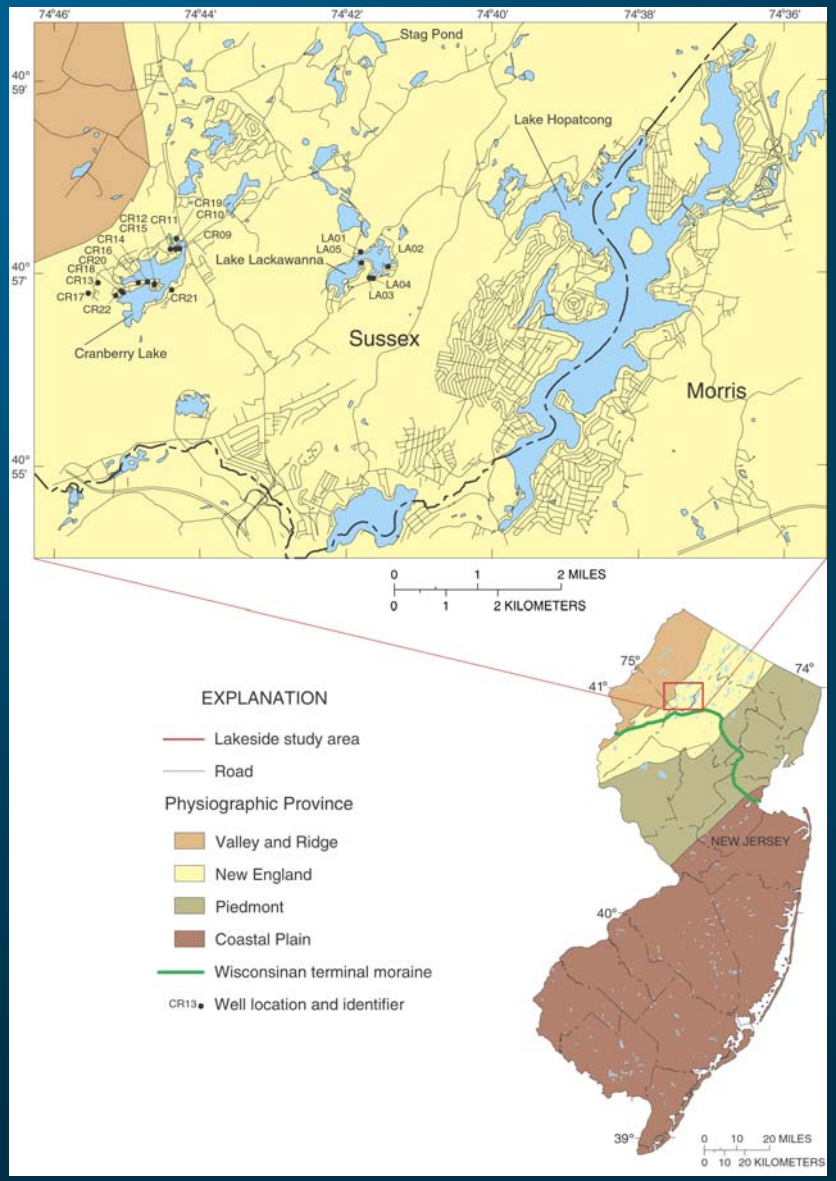
Study area



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Background

- Over 500 lakes in NJ - surface areas > 33 acres
- Most are located in the northern NJ fractured bedrock or glacial fill terrains
- Many are impounded
- Many are heavily used for recreational pursuits (including motorized boating = MTBE)

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Indicators of GW↔SW interaction

- Physical
  - Static and stressed ground water elevations versus lake surface elevation
- Chemical
  - MTBE
  - Herbicides/algaecides
- Biological
  - Fecal coliform/streptococcus
  - Diatoms

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Surface water flow from lakes to wells?

- Altitude of the lake compared to well head?
- Static or stressed ground water levels in well?

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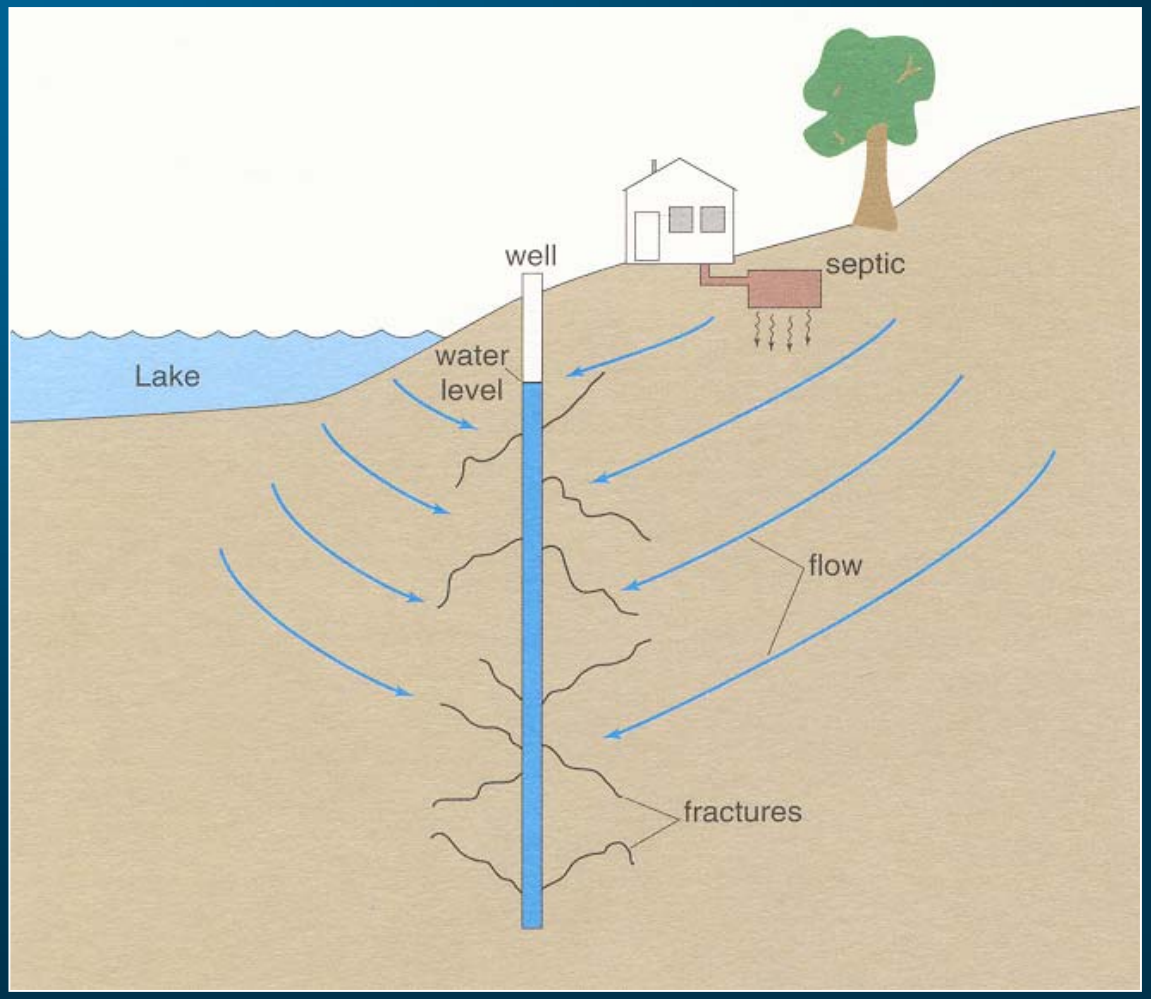
What would that look like?



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Status of Study Area

- 9 of 13 static water levels and 9 of 10 stressed water levels lower in wells than Cranberry Lake's surface elevation
- Possibility exists for seepage of lake water into the local aquifer and domestic wells

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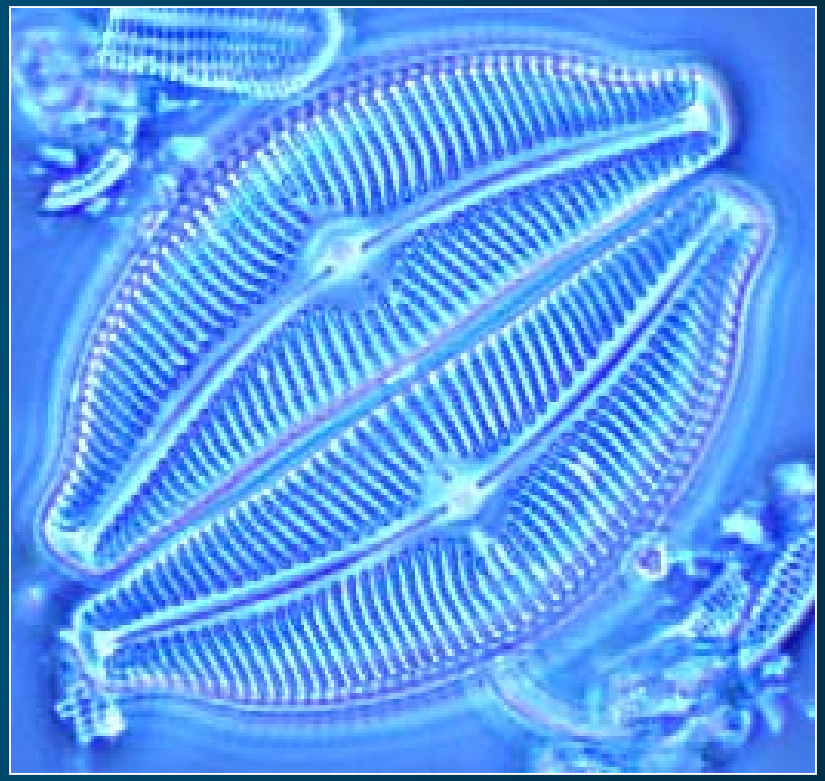
What is a diatom?

- Photosynthetic autotrophic protists

- Diverse ~10,000 living species



- Use silica to produce a rigid cell wall (frustule)

- Frustules can be a variety of shape and are used to identify species

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Presence of diatoms in ground water

- Raw water from the lake and wells
- Similar species found in lakes and wells
- MTBE and water level data suggest seepage from lake to local aquifer

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Implications

- **EPA's Ground Water Under the Direct Influence of Surface Water (GWUDISW)**
  - **"any water beneath the surface of the ground with significant occurrence of insects or other macroorganisms, algae, or large diameter pathogens such as *Giardia lambila* or (for systems serving at least 10,000 people only) *Cryptosporidium*, or significant and relatively rapid shifts in water characteristics such as turbidity, temperature, conductivity, or pH which closely correlate to climatological or surface water conditions" (40 CFR 141.2).**

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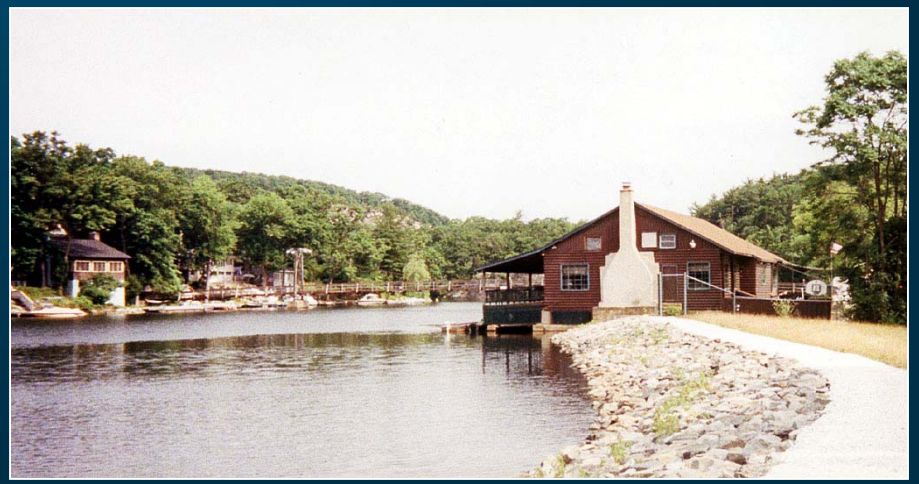
Real world implications

- ~500,000 people live within ¼ mile of a lake (>33 acres) within major Northeastern basins
- If a diatom can be transported, what about pathogens or hazardous chemicals?



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Modern biology methods and diatoms

- Stainable protein and plant fragments
- Research partners (and got funding)
- Applied modern immunological and biochemical methods to GW/SW interaction

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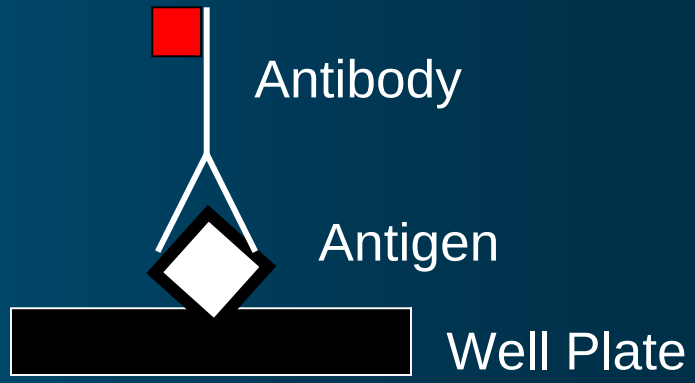
Definitions



- **Antigen**
  - **Any substance capable of inciting an immune response and reacting with the products of that response**
- **Antibody**
  - **A compound synthesized as part of the immune response to a specific antigen**

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More definitions

- **Polyclonal antibody (pAB)**
  - **A mixture of antibodies resulting from the immune response of an animal to an injected antigen**
- **Enzyme linked immunosorbant assay (ELISA)**
  - **A test using antibodies and an enzymatic reaction to detect antigens**

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Conventional approach

- Filter 500-1,000 gallons of water
- Microscopic Particle Analysis (EPA)
- Enumerate organisms associated with SW
- Labor intensive + well capacity is an issue

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Our approach

- Determine protein types providing best detection
- Develop antibodies from selected protein types
- Develop ELISA for detection of diatoms in GW
- Field truth methods

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Mass Cultures

- Collect diatom samples – field & lab cultures
- Isolate target species
- Grow purified cultures
- Extract protein for antibody production

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Protein types for antibody production

- Diatom cell walls
  - Comprised of many proteins
  - Less specific
- Frustulins
  - Family of proteins
  - More specific

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Development of pABs

- Inject antigen (diatom compound = cell wall components or proteins) into lab animal
- Wait 8-12 weeks
- Extract antibody

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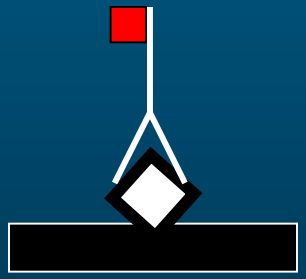
Direct ELISA design

Add water sample to well plate or tube and rinse

Add antibody (conjugated) and rinse



Measure color change, compare to standard curves, calculate concentration

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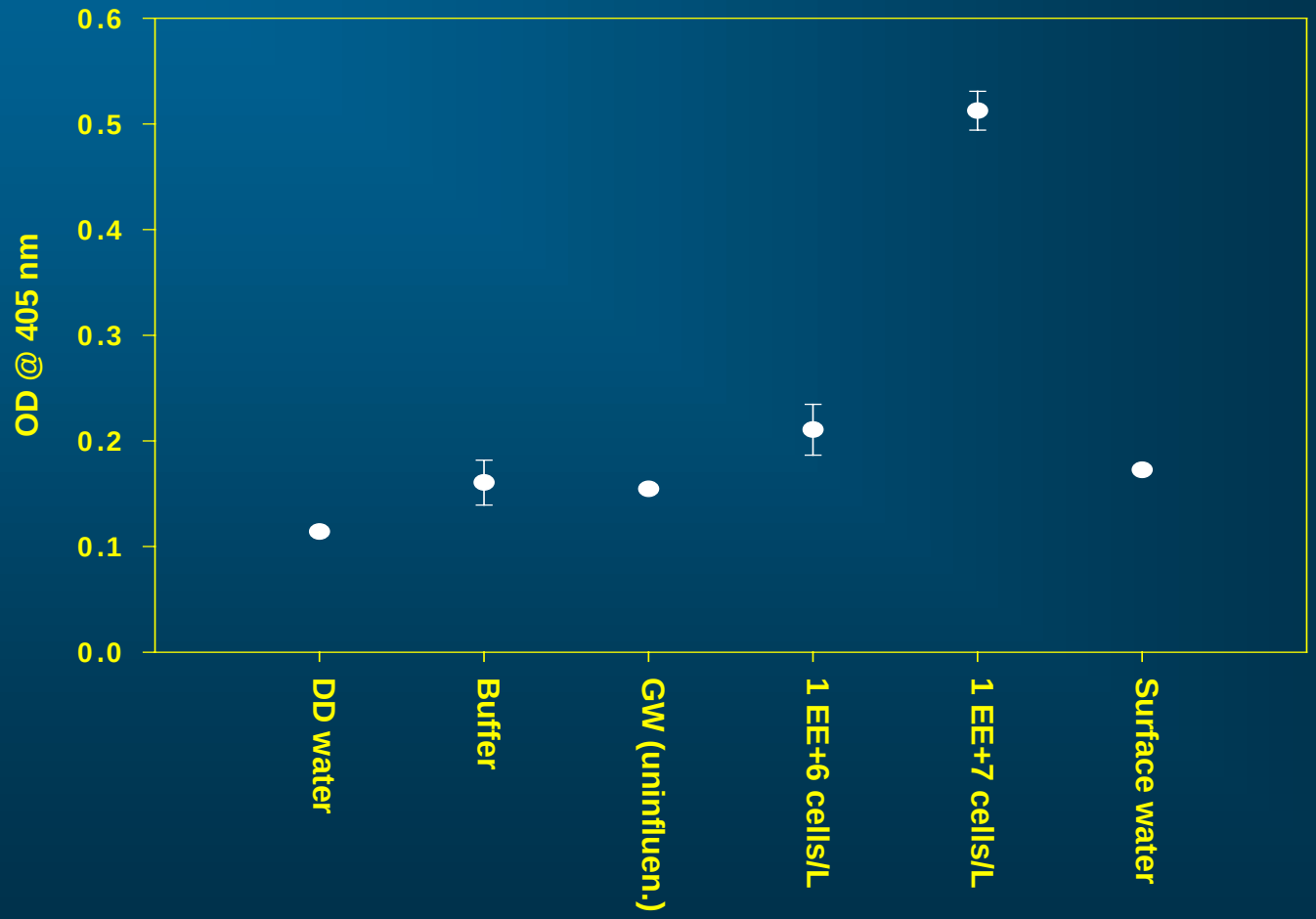
Results



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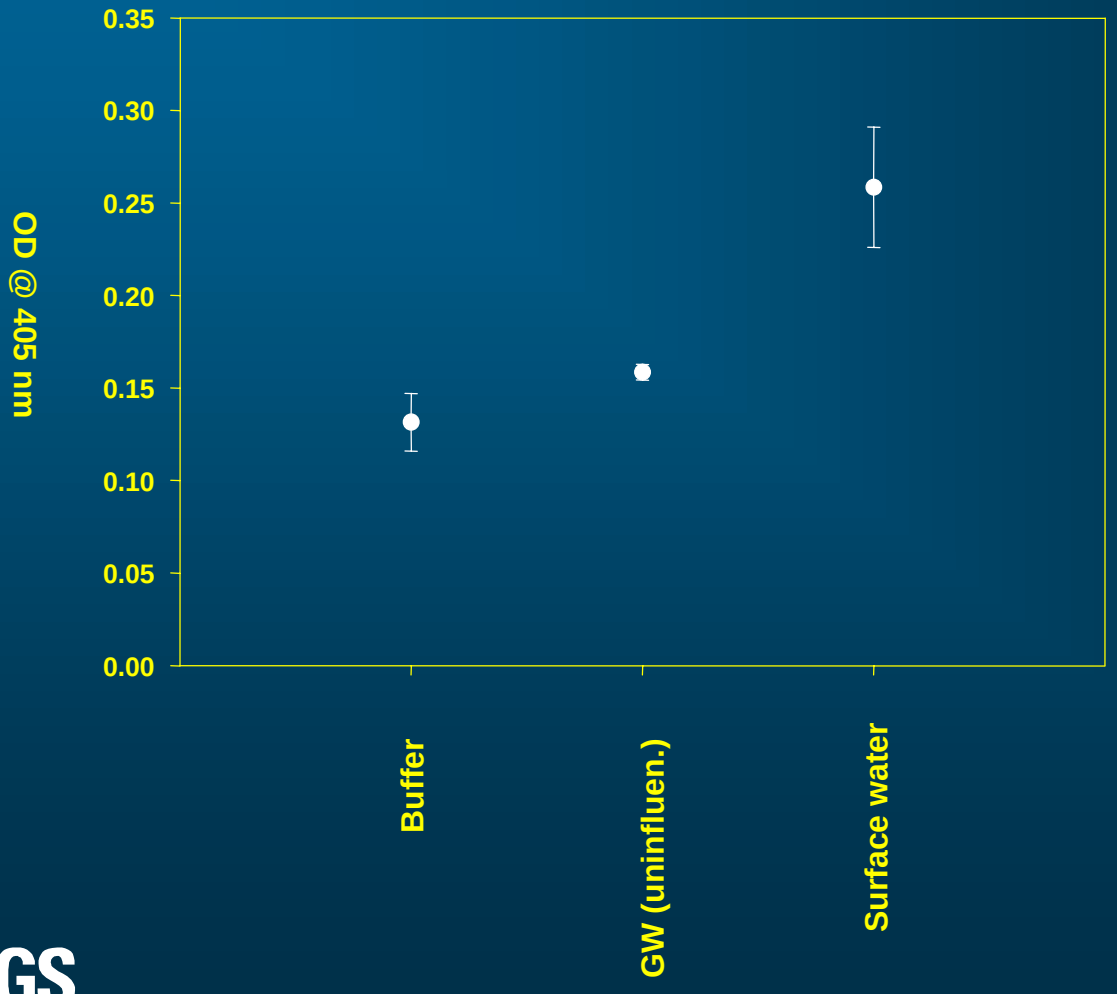
ELISA trial (Lake Water conc. 8x)



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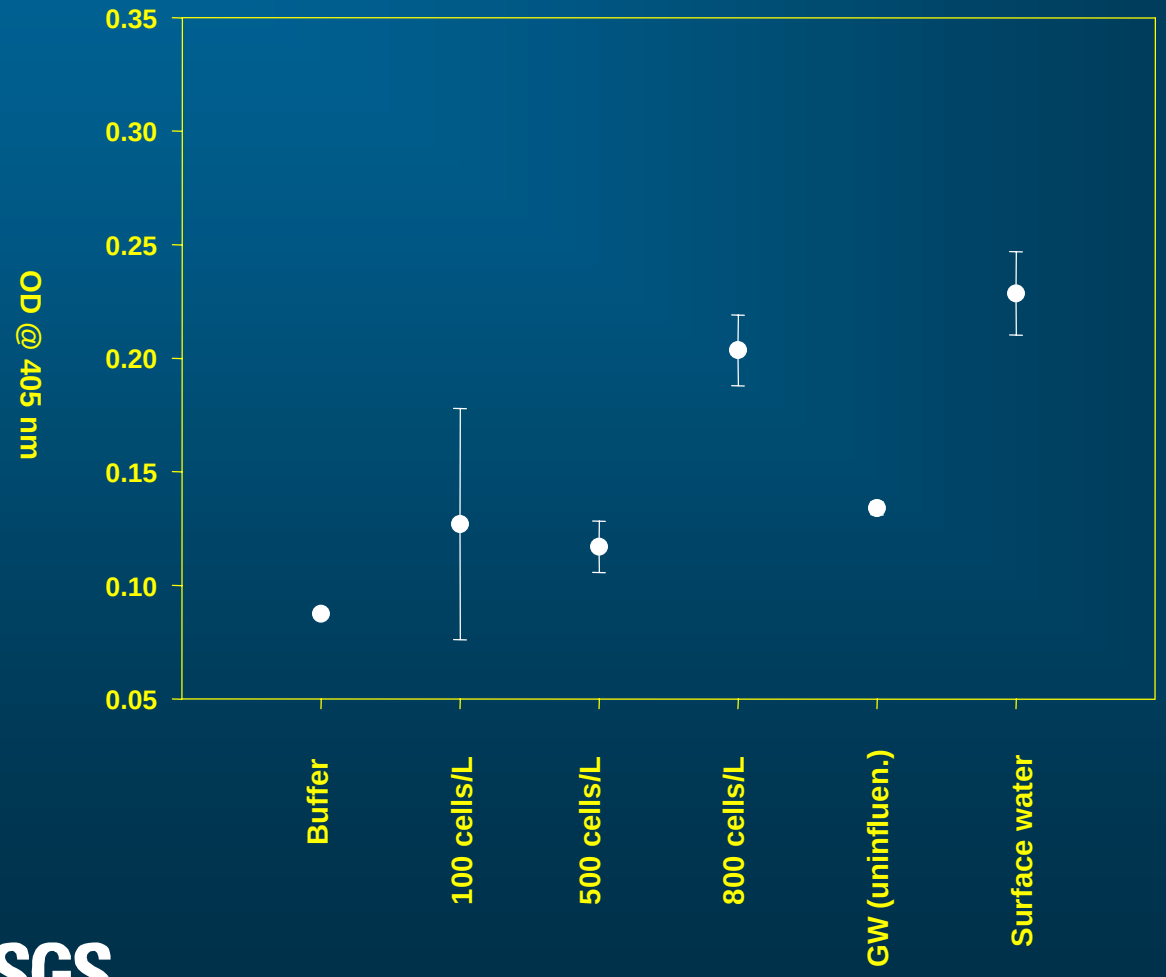
ELISA trial (test cultures conc. 10^4 x)



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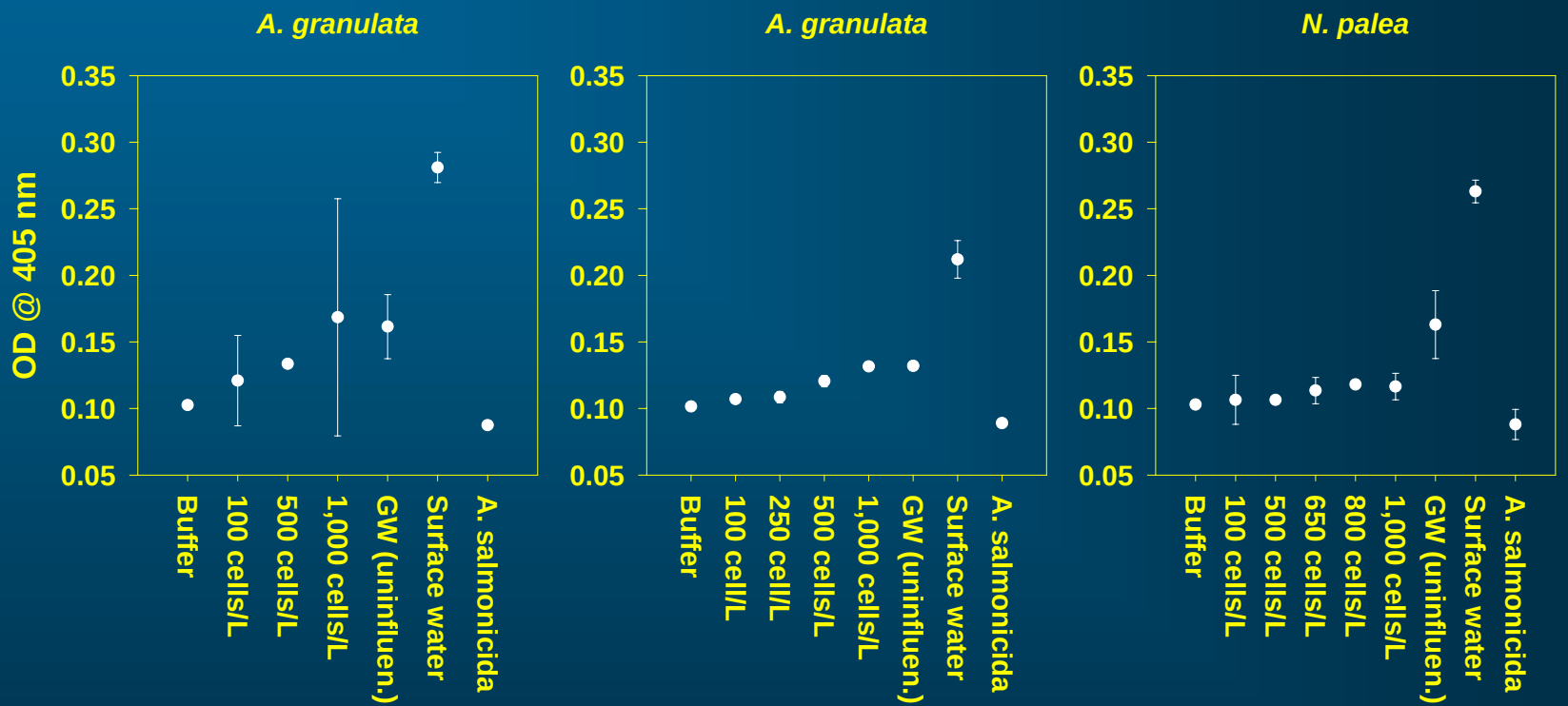
ELISA (reactivity)



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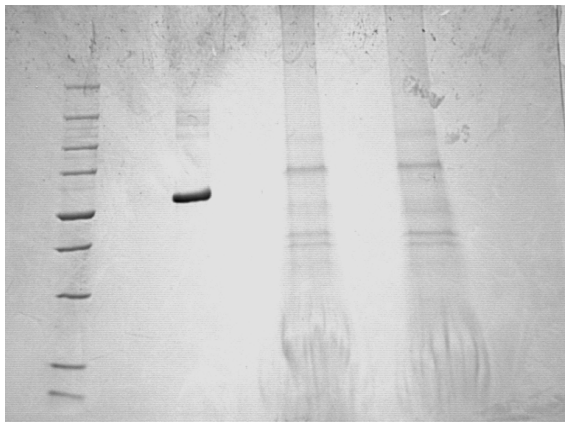
Light deprivation experiments

- **Objective:** Simulate movement from surface to ground water
- **Theory:**
  - Photosynthetic compounds – increase then degrade in absence of light
  - Documented in marine depth studies
- **Experiment:**
  - Controlled light deprivation experiments
  - Protein profiles at timed intervals



Electrophoresis gels used to characterize proteins

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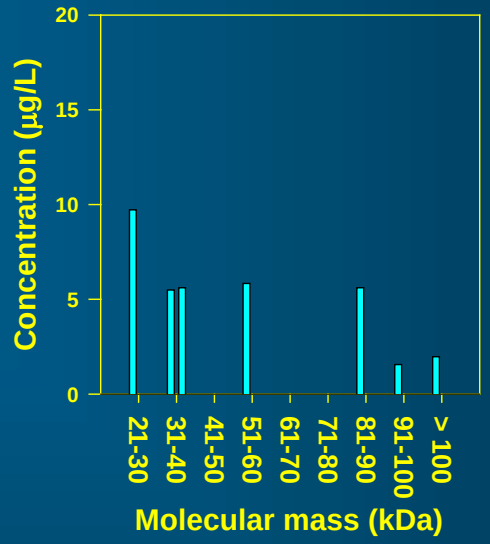
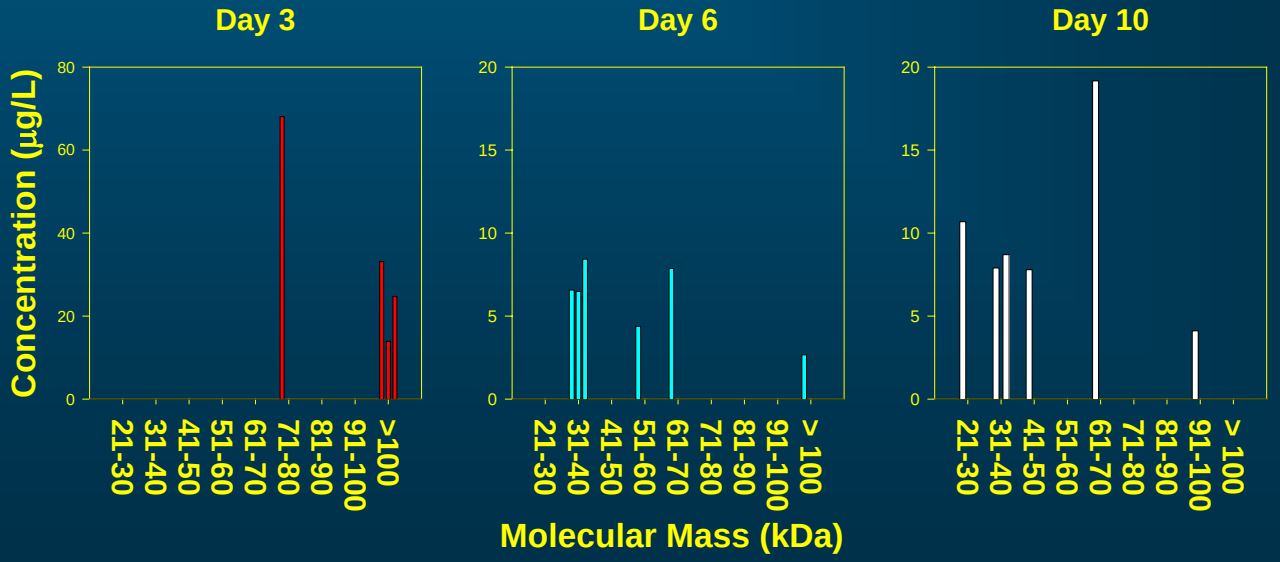
Compare sample to standard

Sample



Protein Profile Standard



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Conventional method vs. ELISA

Microscopic Particle Analysis (MPA)

- EPA approved method to determine GWUDISW
- 8- to 24-hour sampling period during which 1,890 to 3,785 L of ground water are filtered
- Filtered, examined, all particles counted, IDed
- Impractical for domestic well sampling
- Both lab and field intensive

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ELISA vs. conventional method

New ELISA

- Requires 1 L
- Relatively short (<1 hour) sampling period
- Does not require microscopic examination
- Capacity of well not an issue
- Substantially reduced field and lab cost

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Practical field application

- Obtain 1 L sample
- Ship to lab
- Concentrate by centrifugation
- Run ELISA on raw or lysed sample to determine antigen concentration

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The future?

- Stakeholder funded regional sampling and methods development support
- Parallel PCR-based detection method
  - Potentially more sensitive and selective than ELISA-based detection systems
  - Costly method development, problematic due to large number of potential environmental interferences
- Application of diatom ELISA methodology to other problems
  - Detection of salt-water intrusion in high chloride environments
  - Detection of invasive species

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