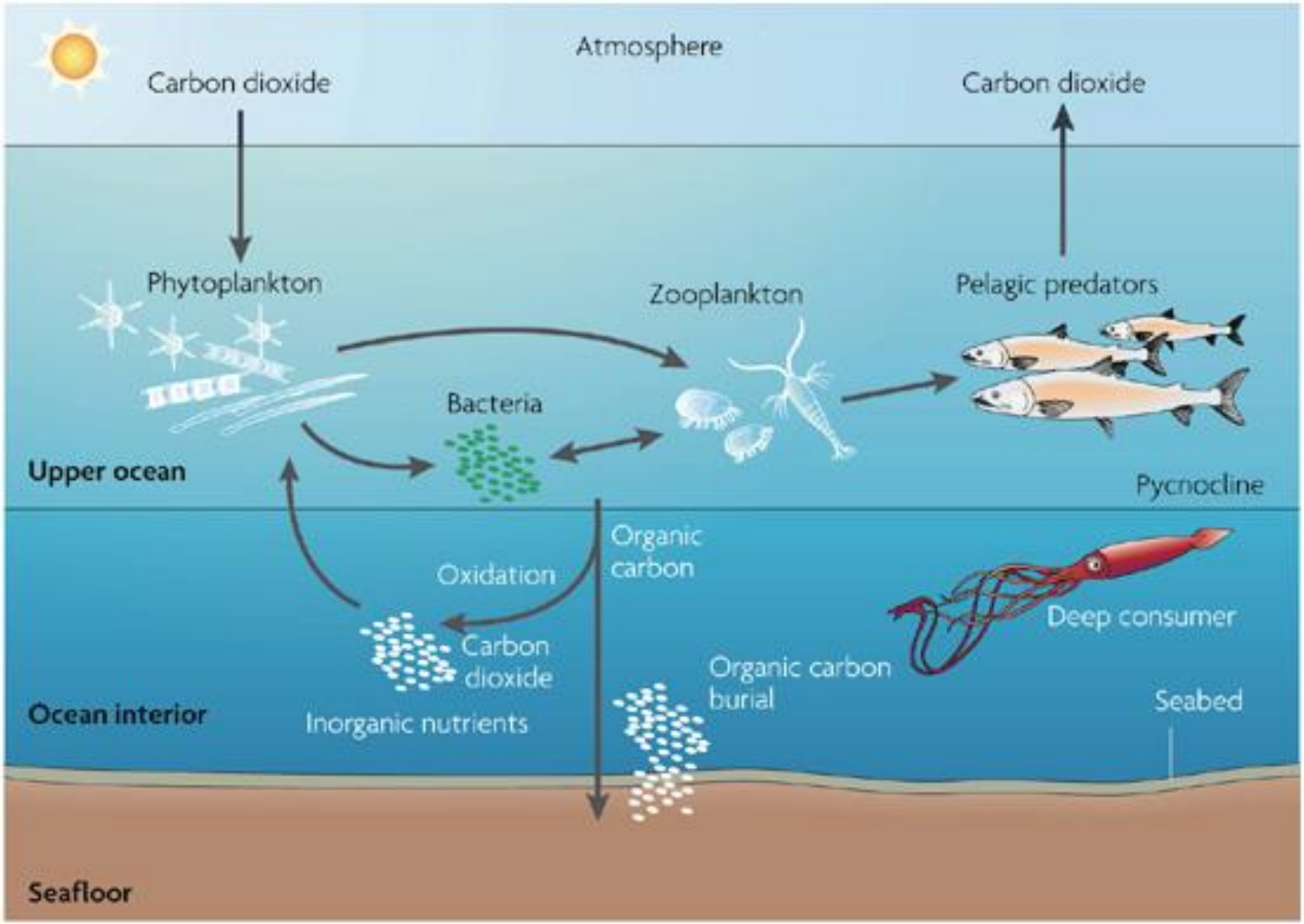
Evaluation of Zinc Pthalocyanine (ZnPc) as an Internal Standard for the Routine Chromatographic Analysis of Chlorophylls in Environmental Water Samples

By Patrick Kohler

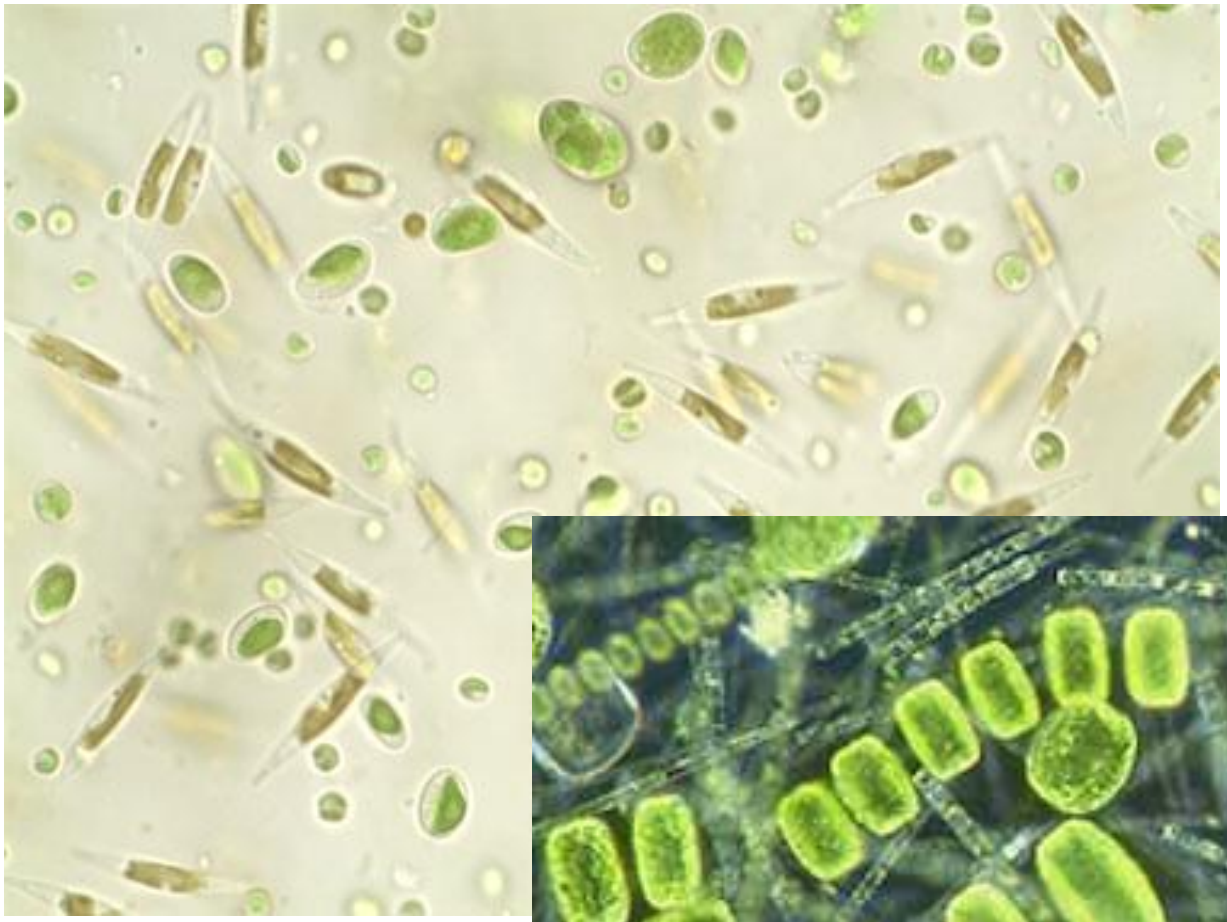
April 29, 2011

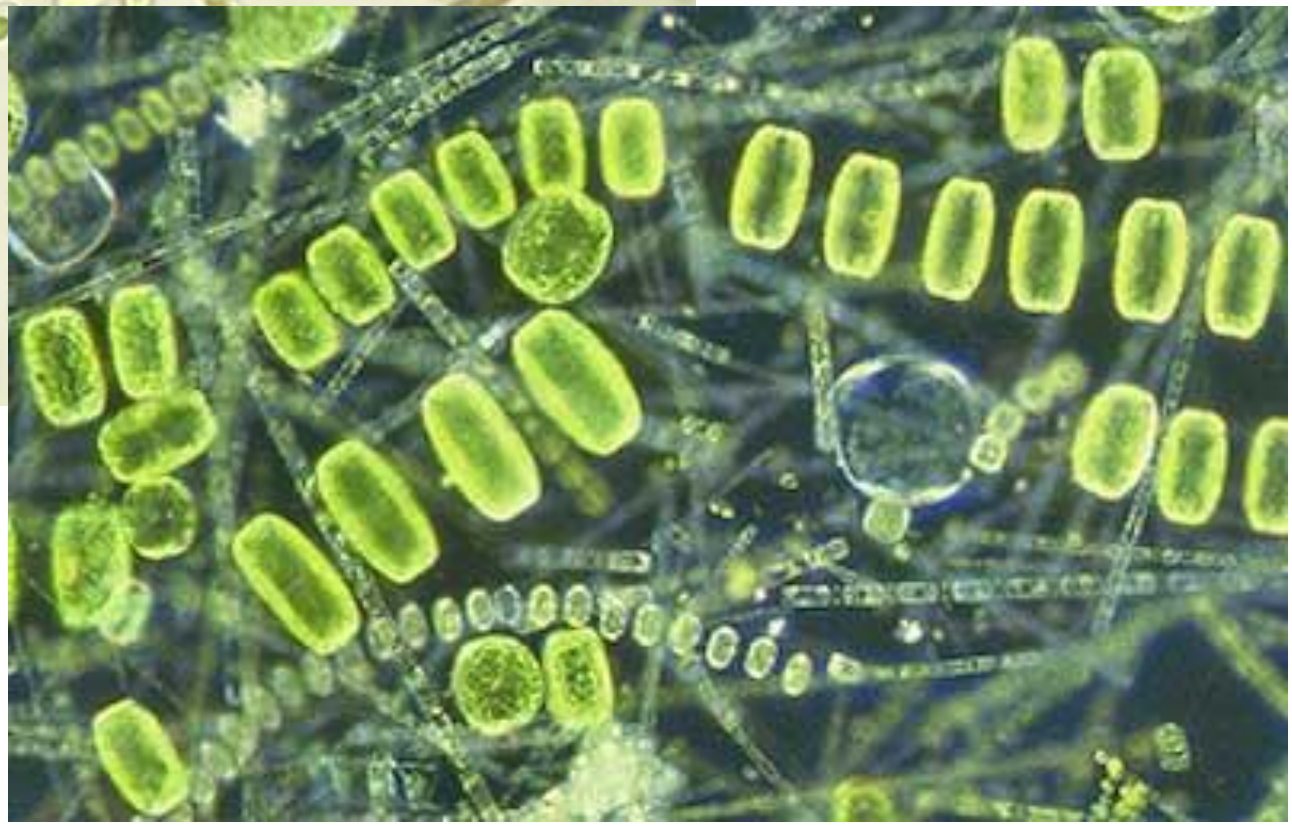
Phytoplankton (aka algae)

- Phyto = plant, plankton = wanderer
- Obtain energy from photosynthesis
- Base of the food chain
- Live near the surface of the water
- Produce much of the oxygen in our atmosphere
- Regulated by sunlight and nutrients in the water
- Contain chlorophylls and related pigments to capture light for photosynthesis



Nature Reviews | Microbiology





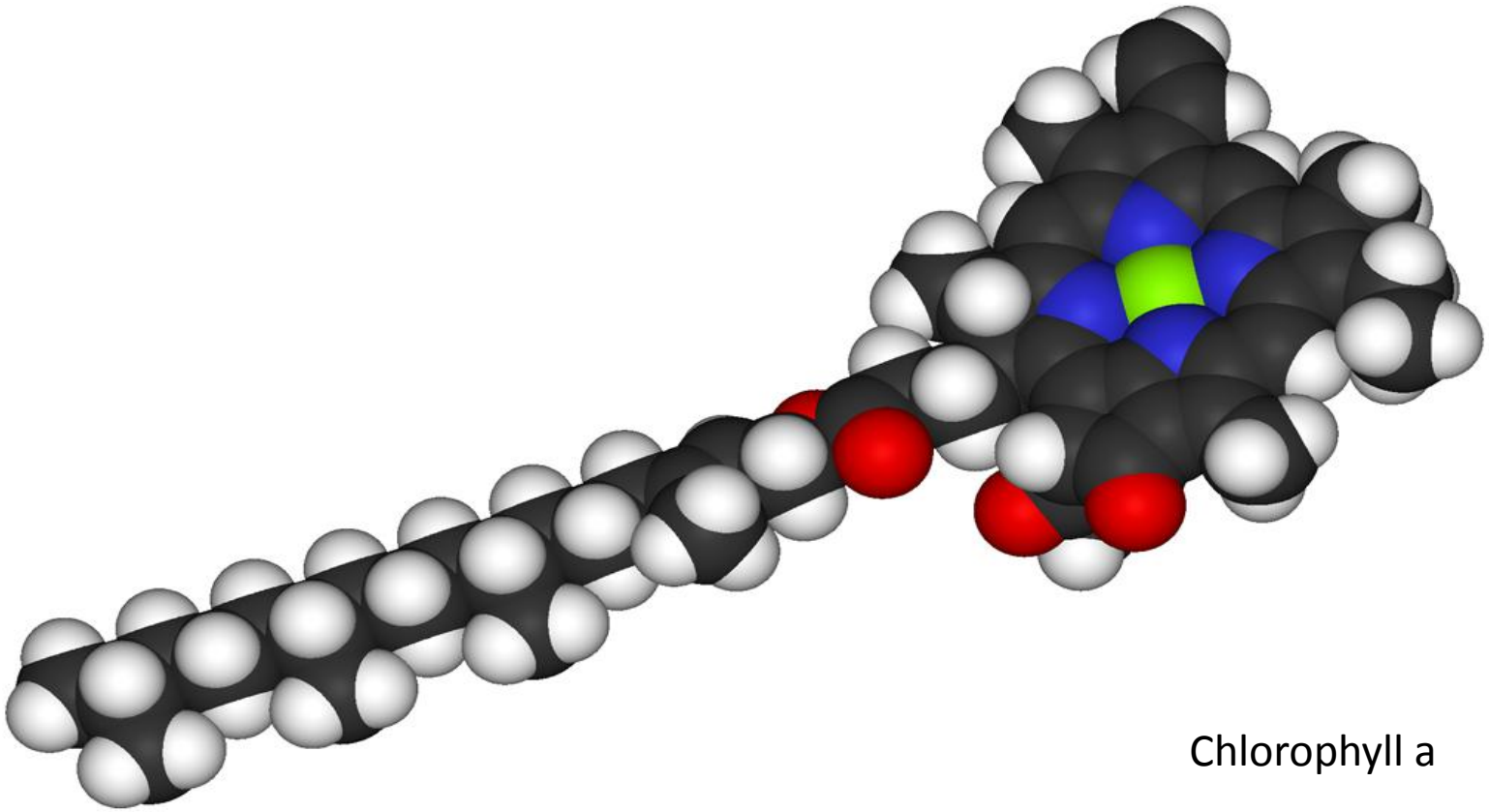
Examples of various phytoplankton found in water

Phytoplankton Biomass

- Lakes, rivers and canals need a proper amount of phytoplankton to be productive
- Too much phytoplankton results in so called “algae blooms”
- Measure the amount of phytoplankton by measuring the amount of chlorophyll

An over-abundance of phytoplankton creates an “algae bloom”

Lake Apopka, Florida



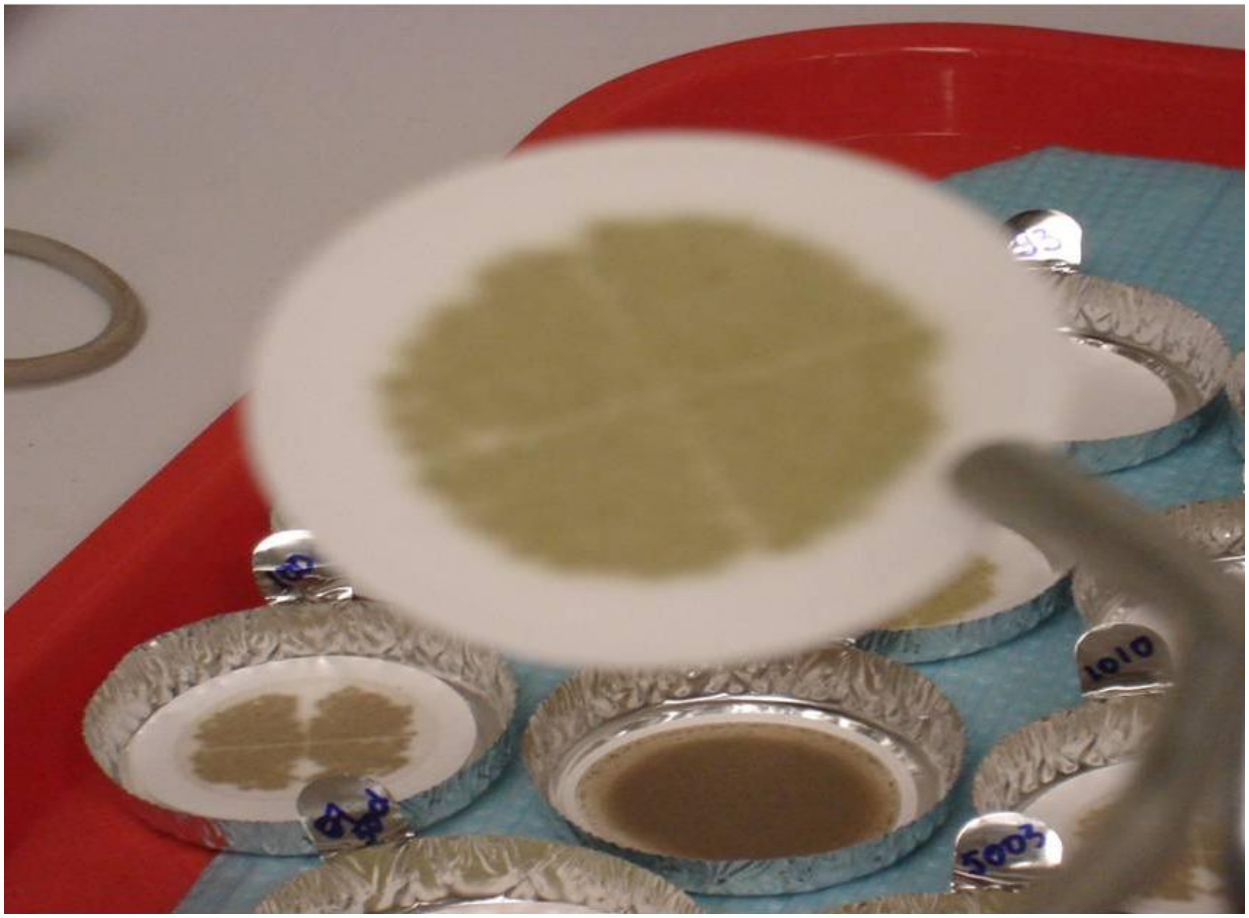
Chlorophyll a

$C_{55}H_{72}O_5N_4Mg$

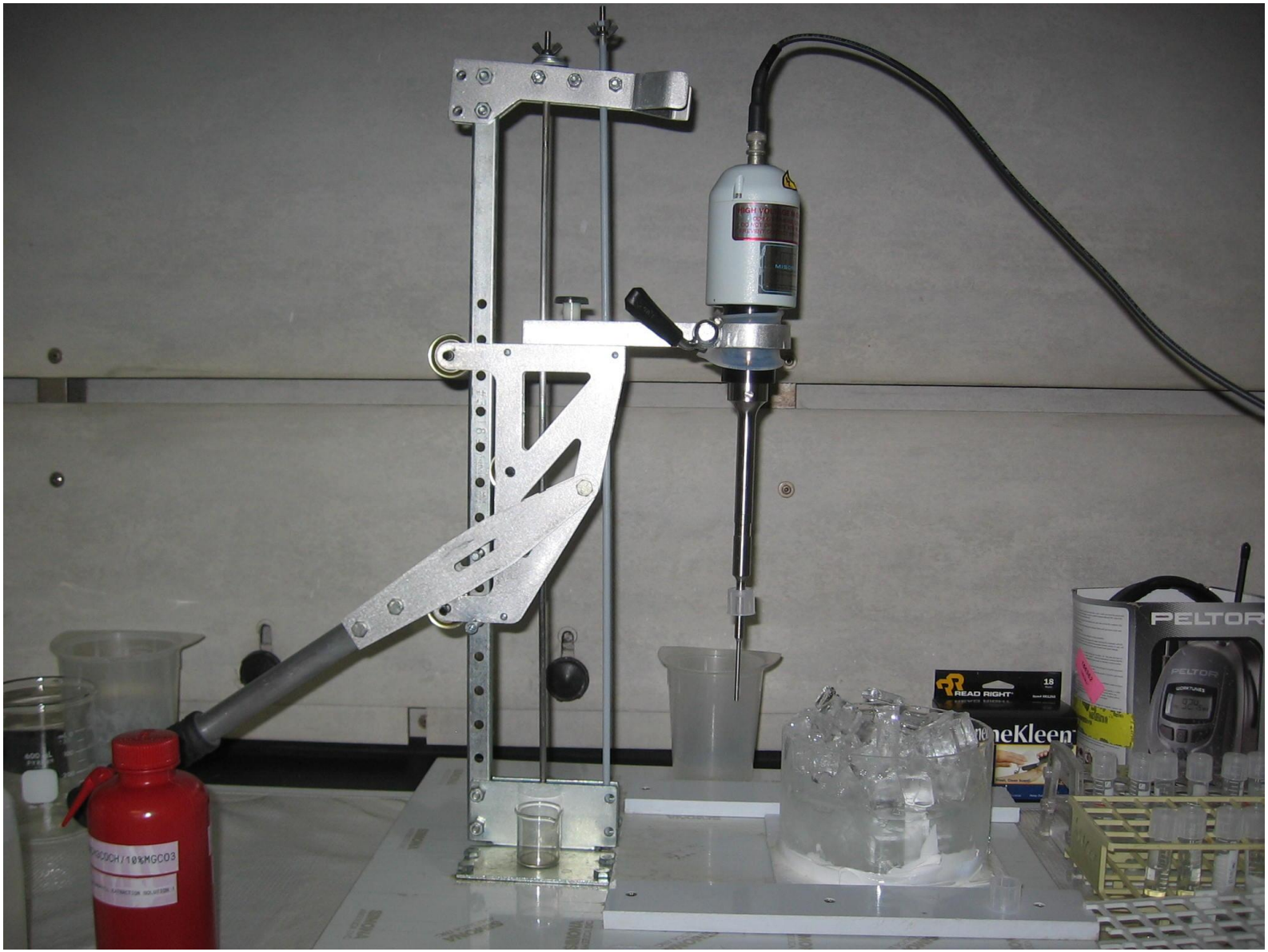
Chlorophyll a is natures light harvesting antenna

Chlorophyll Analysis

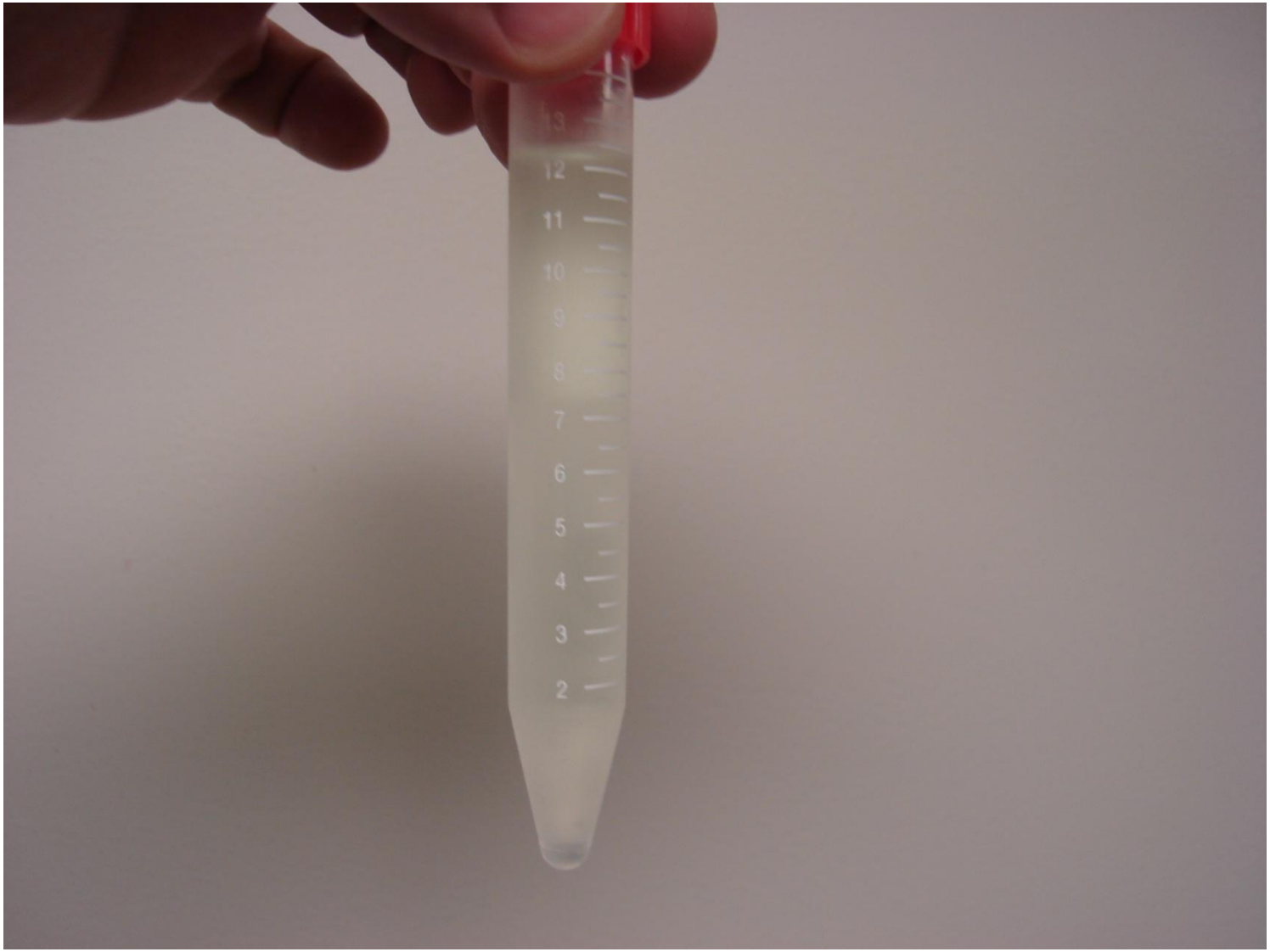
- Need to filter water sample to remove phytoplankton
- Must extract chlorophyll from the phytoplankton by rupturing cells and steeping in a solvent. The most common solvent used is acetone.
- Measure amount of chlorophylls in extracted solution by chromatography



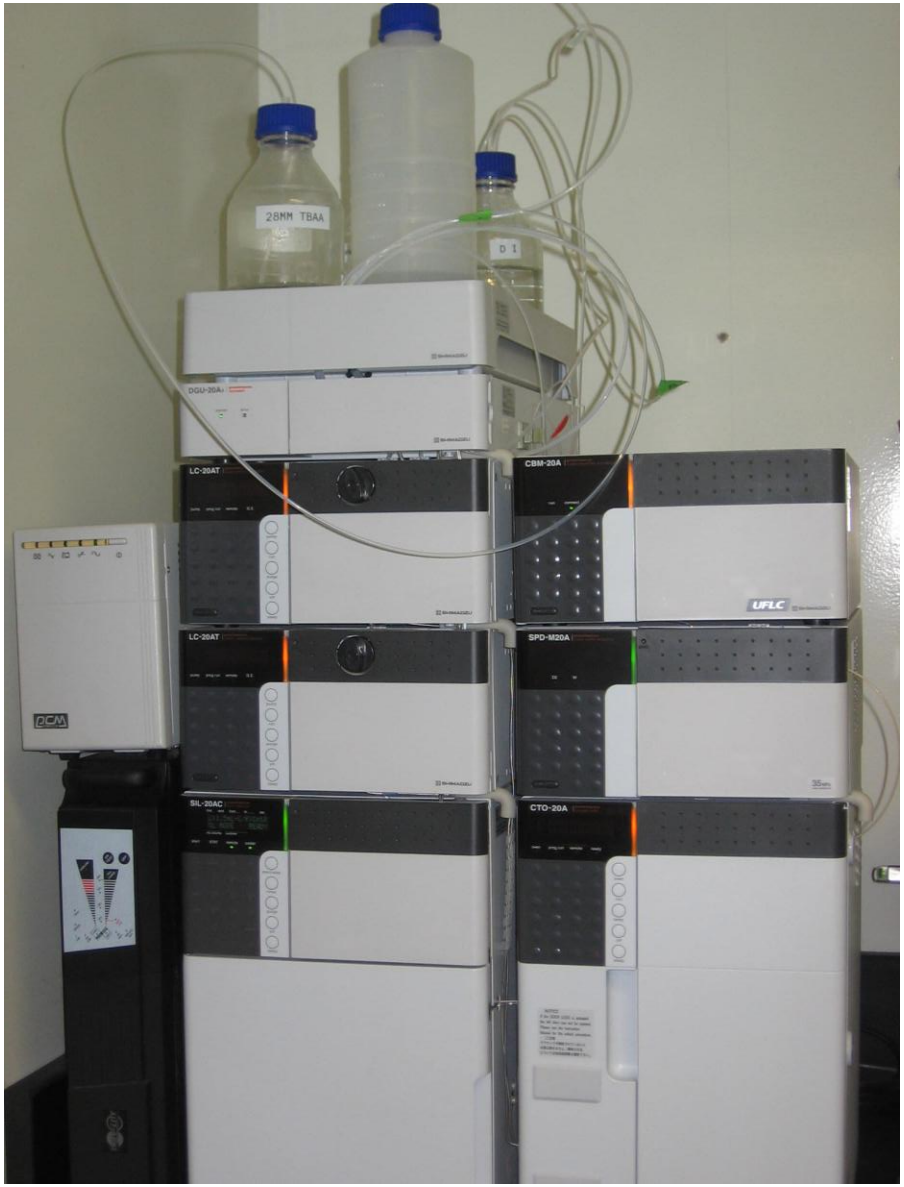
Phytoplankton containing chlorophylls trapped on the filter



Sonication station used to rupture the cells on the filter. Filters are placed in tubes (seen on right), immersed in solvent and then sonicated with the probe while the tube is placed in an ice bath to prevent degradation of chlorophylls



Sample extract containing chlorophylls extracted from cells on filter. Notice the pale green color of the solution.



Chromatographic system for measuring chlorophylls extracted from phytoplankton.

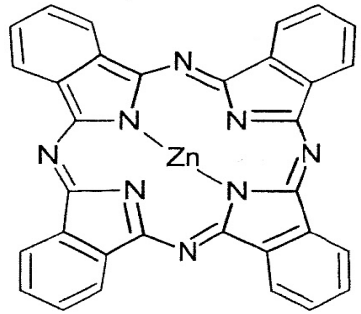
2 Big Problems with Analysis

- During extraction process chlorophyll may be lost due to light, temperature, sonication and other forms of degradation
- During extraction process solvent may also evaporate from the extract solution giving results that are falsely higher in concentration

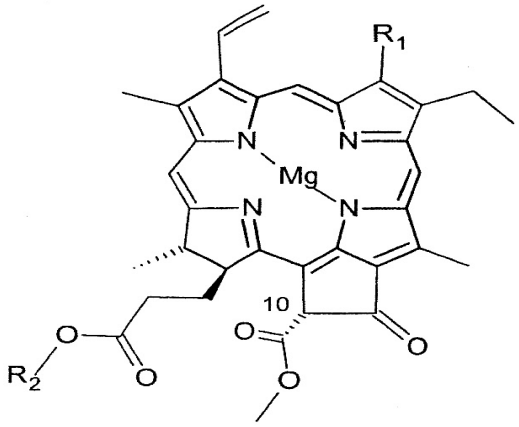
Solving these Problems with an Internal Standard

- In order to compensate for the amount of chlorophyll and/or solvent lost during extraction a known amount of an internal standard can be added prior to extraction
- To be a good internal standard it must be readily available, low in cost, similar in structure to chlorophyll, must not be naturally present in the sample, must degrade similarly to chlorophyll and must be well resolved from chlorophylls during chromatographic separation
- While some good internal standards exist for this method, they are expensive and not readily available. The purpose of my internship was to work on a team evaluating a potential new low cost, readily available, internal standard.

Potential Internal Standard Zinc-pthalocyanine (1) compared to chlorophylls a and b (2)



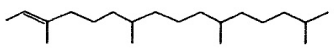
1

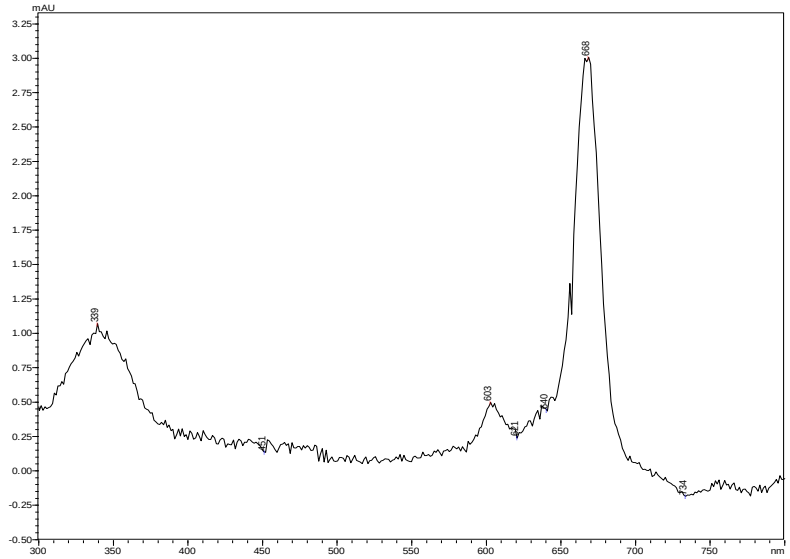


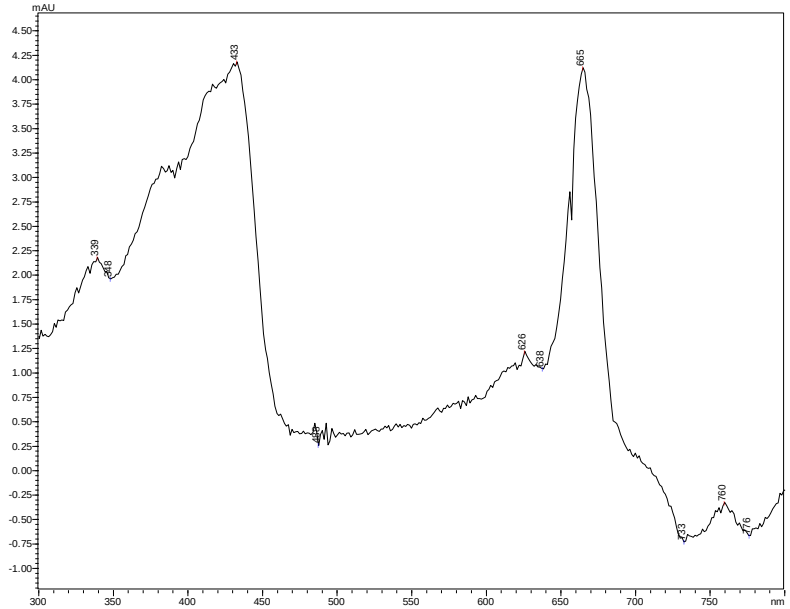
2

R_1 = CHO : chlorophyll a

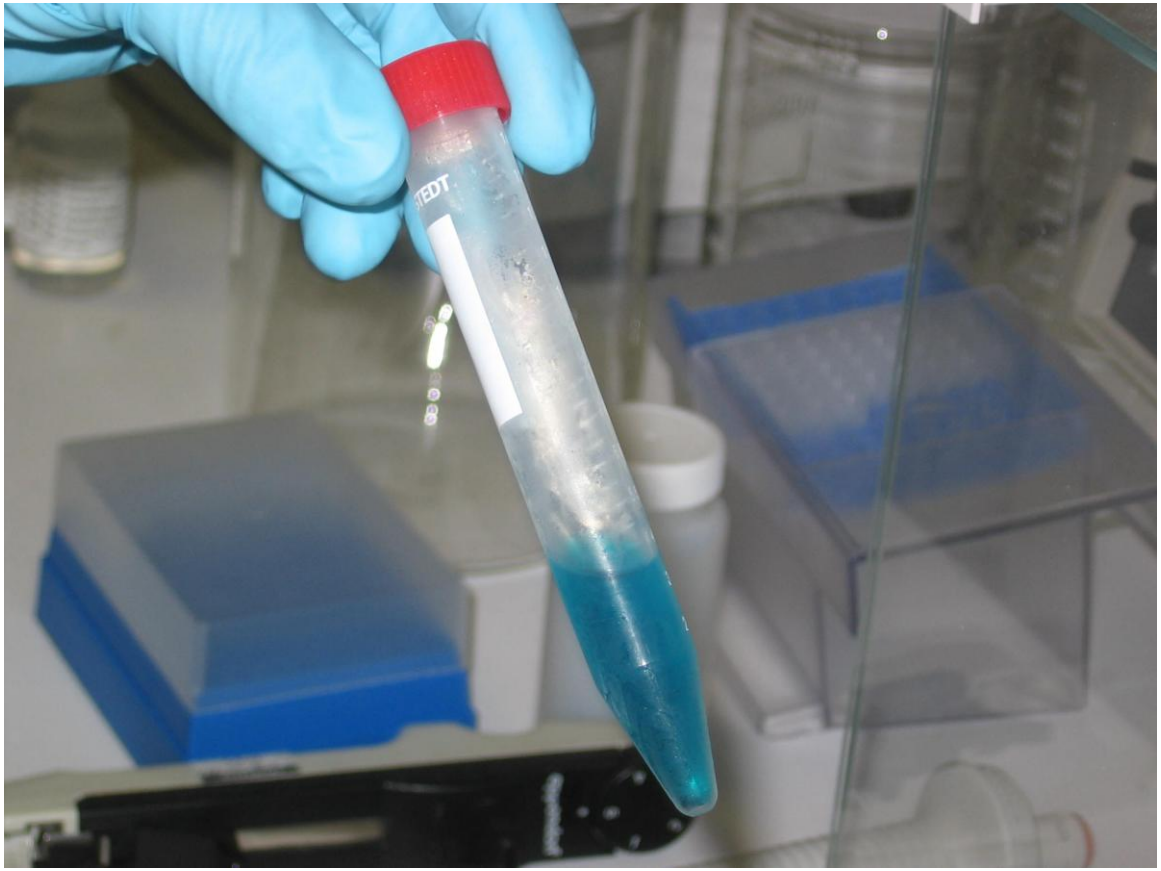
R_1 = CH_3 : chlorophyll b

R_2 = phytyl 

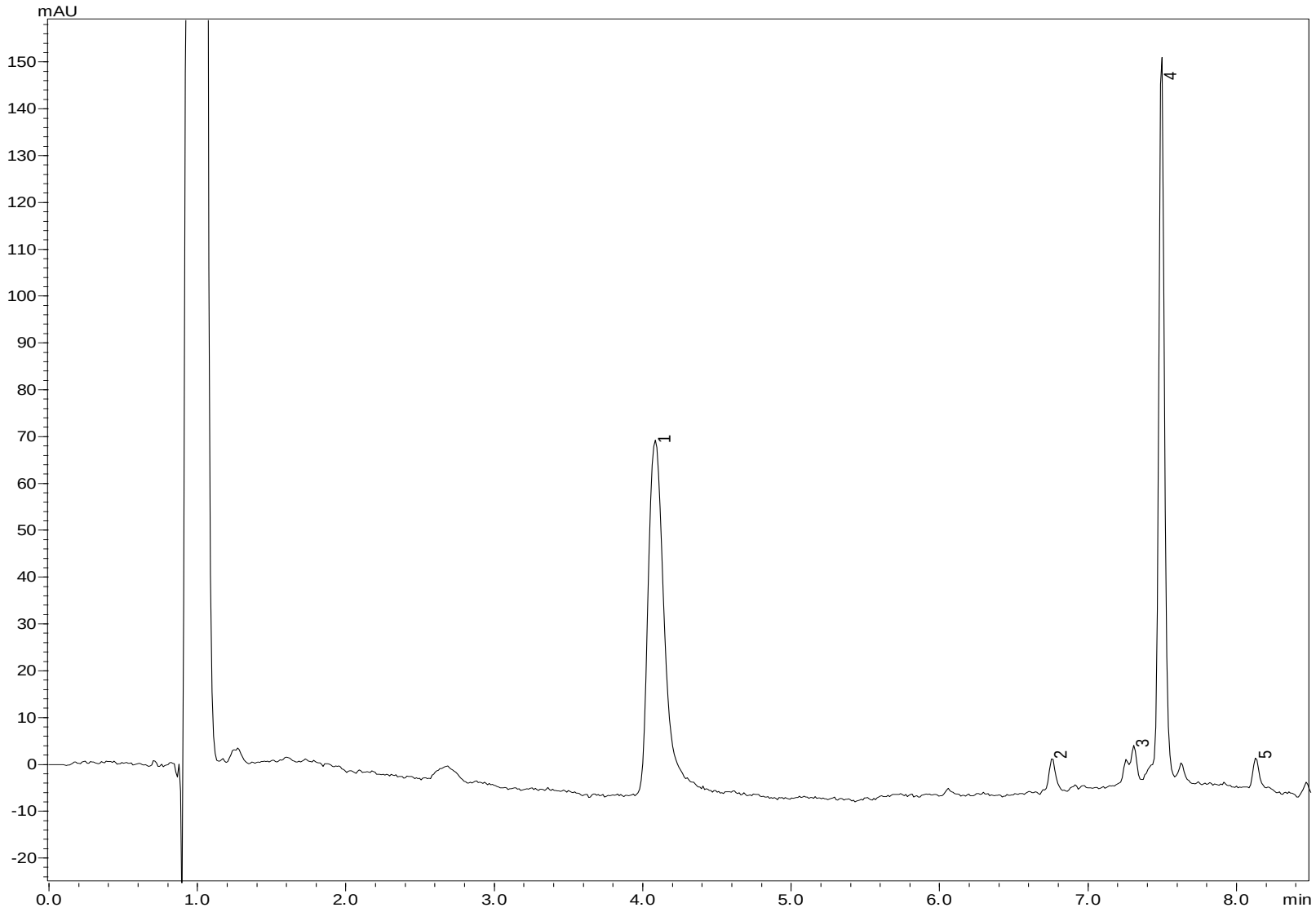




Comparison of the absorption spectra of ZnPc (top) versus Chlorophyll a (bottom). Both compounds have a strong absorption band around 665 nm



Sample extract containing internal standard and chlorophylls extracted from phytoplankton



Chromatogram of sample extract in DMF. (1) ZnPc, (2) Chl b, (4) Chl a

How Chlorophyll Concentrations are Corrected by using an Internal Standard

- For unknown samples add a known amount of internal standard prior to extraction and correct the results for the amount of internal standard that is recovered during analysis

Degradation Studies

- In order to evaluate how the internal standard behaves relative to chlorophylls a and b we wanted to purposely degrade the compounds and measure the effect on them
- Repeated sonication is a reproducible method to degrade chlorophylls in a systematic way
- Recoveries are calculated for each compound after each sonication cycle

Recoveries after Repeated Sonication Cycles, Average of 4 Samples

Cycles	ZnPc	Chl a	Chl b
AVG (1c)	99%	100%	100%
AVG (2c)	74%	93%	102%
AVG (3c)	60%	85%	97%
AVG (4c)	56%	80%	95%
AVG (5c)	58%	75%	89%

Current Conclusions from the Research Project

- ZnPc does have potential for use as an internal standard
- Due to solubility problems and the formation of aggregates in solution, acetone cannot be used for extractions with ZnPc as internal standard (We spent many hours in the lab trying to make it work!!)
- An alternative solvent, dimethyl formamide (DMF) was needed for extractions when using ZnPc as internal standard although smaller injection volumes must be used (We found this solvent worked well after trying <u>many</u> alternatives)
- Further research by the team will continue to fully evaluate ZnPc

Questions?

Project Team Members at SFWMD

- Yelena Guthman
- David Struve

References

BarentsPortal. (2009). Phytoplankton. Retrieved April 18, 2011, from http://www.barentsportal.com/barentsportal09/index.php?option=com_content&view=article&id=194%3Aphytoplankton&catid=60%3Abiotic-components&Itemid=184&lang=en

Bohn, T., & Walczyk T. (2004). Determination of Chlorophyll in plant samples by liquid chromatography using zinc-phthalocyanine as an internal standard. Journal of Chromatography, 1024, 123-128.

DT's Plankton Farm. (1996). Premium Reef Blend. Retrieved April 18, 2011, from http://www.dtplankton.com/phytoplankton.html

Energy Engineering Blog. (2008, December 19). X-Algae: Mutant algae for biofuel production? Message posted to http://energy-eng.blogspot.com/2008/12/mutant-ninja-algae-for-biofuel.html

Falkowski, P., & Oliver M. (2007). Mix and match: how climate selects phytoplankton. Nature Review Microbiology, 5, 813-819.

Florida Department of Environmental Protection. (2011). Watershed and River Basin Stats. Retrieved April 18, 2011, from http://www.protectingourwater.org/watersheds/map/ocklawaha/

New Hampshire Lakes Association. (2009). Cyanobacteria Update. Retrieved April 18, 2011, from http://www.nhlakes.org/algae.htm