Cyanotoxin Introduction & Introduction to toxins: who, what, why, and how?

Barry H. Rosen, Ph. D.
Florida Gulf Coast University
World-Class Scholar and Professor

brosen@fgcu.edu

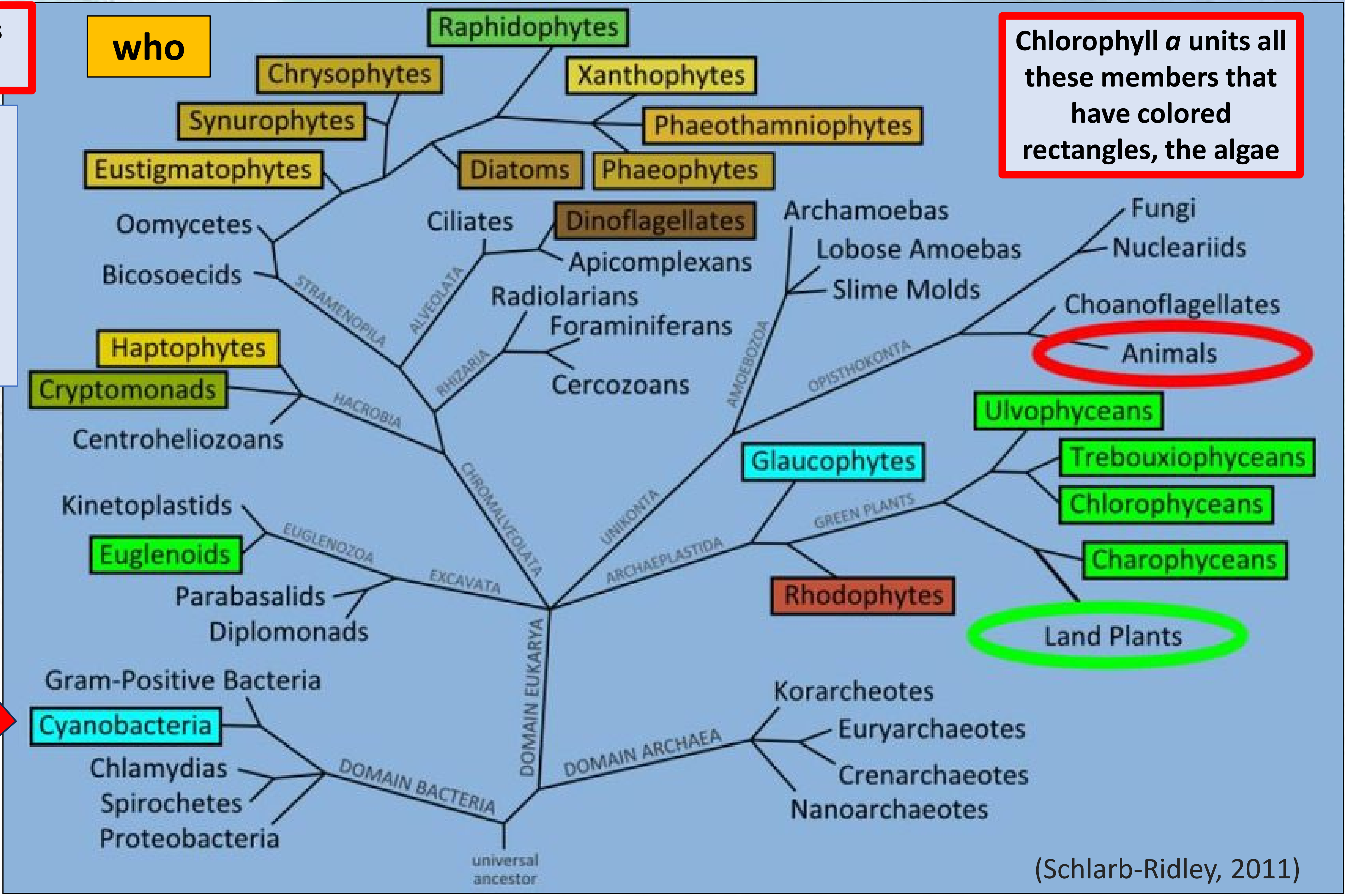
239-745-4589

THE WATER SCHOOL AT **FGCU**

What distinguishes these groups are:

- **Pigments**
- Cell Wall
- Motility
- Photosynthesis Storage Compounds
- Morphology

Today's talk

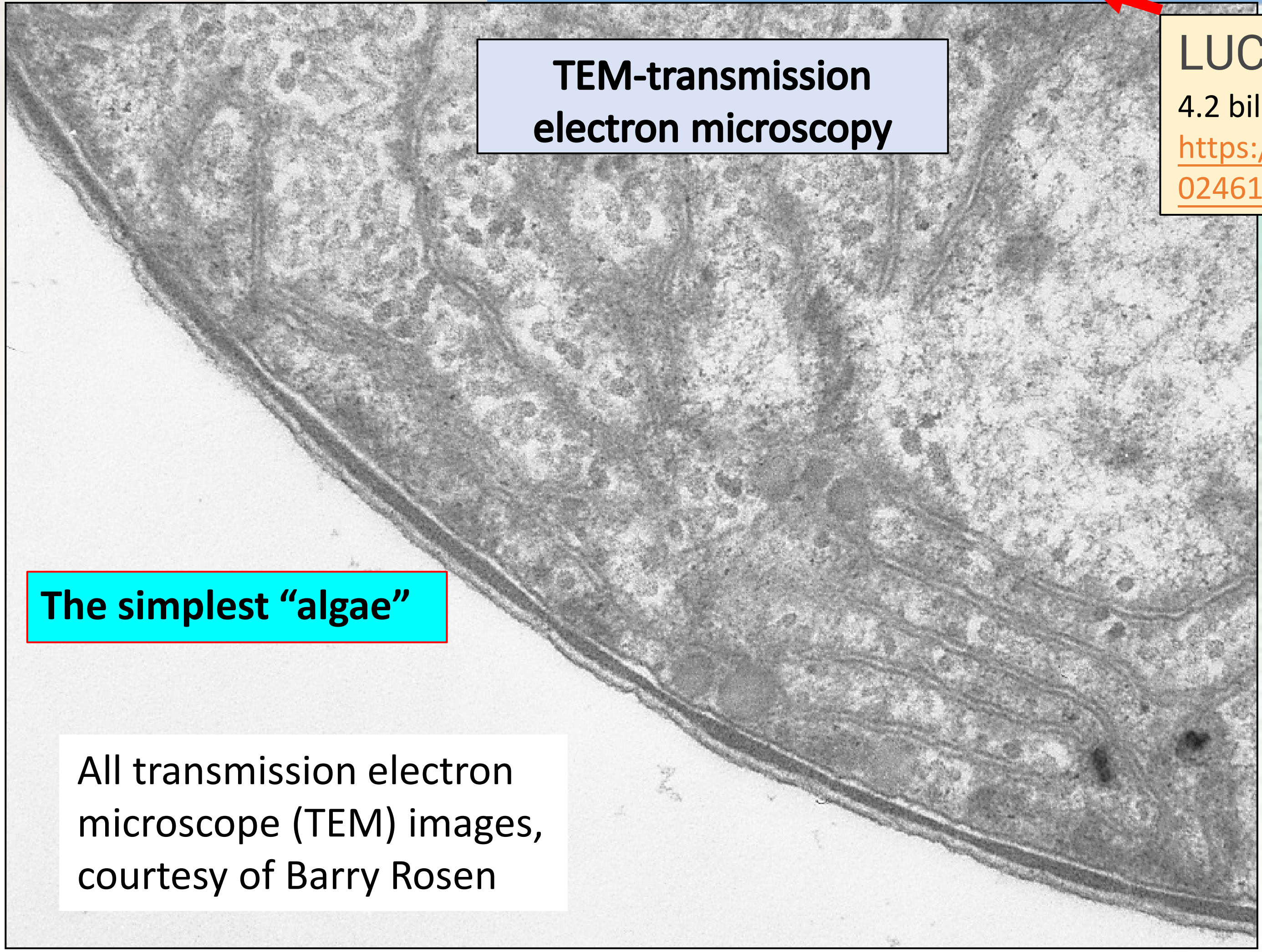


(Schlarb-Ridley, 2011)

Cyanobacteria are Prokaryotes

who

LUCA- last universal common ancestor
4.2 billion years ago (arrived from?....)
https://doi.org/10.1038/s41559-024-02461-1



TEM-transmission electron microscopy

The simplest "algae"

All transmission electron microscope (TEM) images, courtesy of Barry Rosen

- *AKA:* blue-green algae
- Gram-negative bacteria
- No *membrane-bound* organelles, but they do have membranes...like the thylakoids-(chlorophyll-*a*)

The Great Oxidation Event (2.4 bya)

Photosynthetic "Waste Product"

who

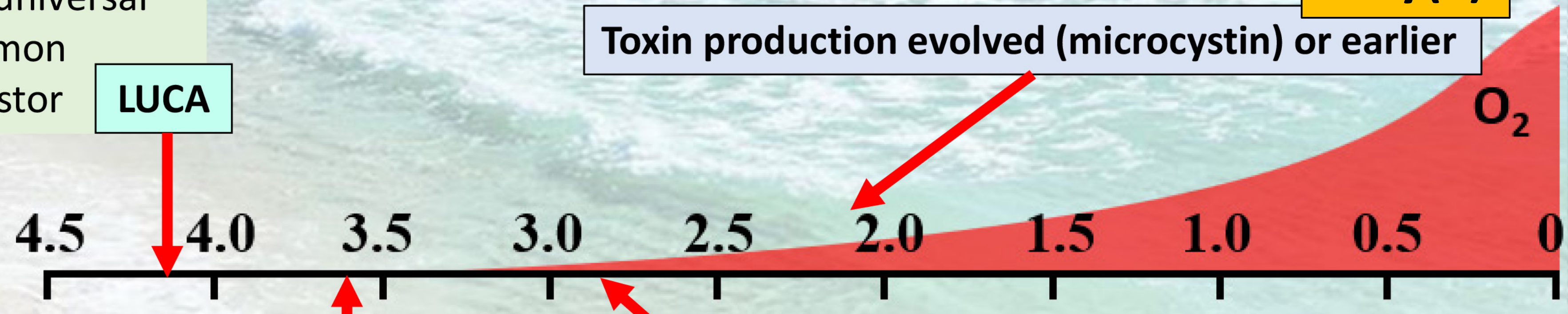
We owe them! **This allowed the more complex lifeforms on Earth**

why(?)

Last universal common ancestor **LUCA**

Toxin production evolved (microcystin) or earlier

Billion Years Before Present



Precambrian Time: The Time of Prokaryotes

Archaean Era: Era of Archaea	Proterozoic Era: Era of Cyanobacteria	Phanerozoic Time Eukaryotic Time

First eukaryotes (organelles from prokaryotes)

The genes have coevolved with cyanobacterial housekeeping genes, suggesting they have been present for a significant portion of the phylum's history

First 3.8 bya Cyanobacteria

cyanobacterial oxygen wiped out most of life on Earth

https://pmc.ncbi.nlm.nih.gov/articles/PMC327188/

So you have a bloom, is this an issue?

day vs. night

photosynthesis & respiration

vs. respiration only

what

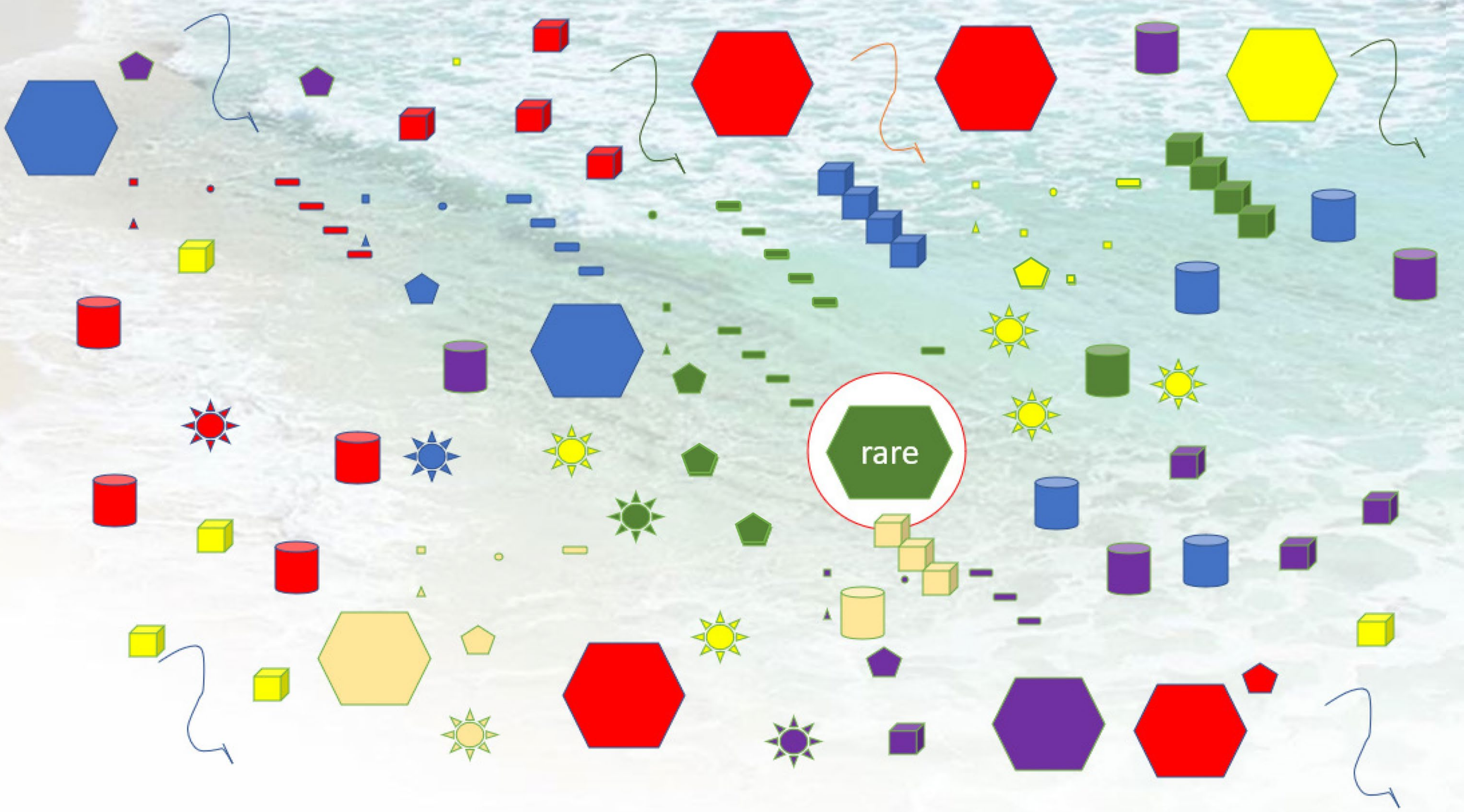
Cyanobacterial Harmful Algal Blooms (CyanoHABs)

why

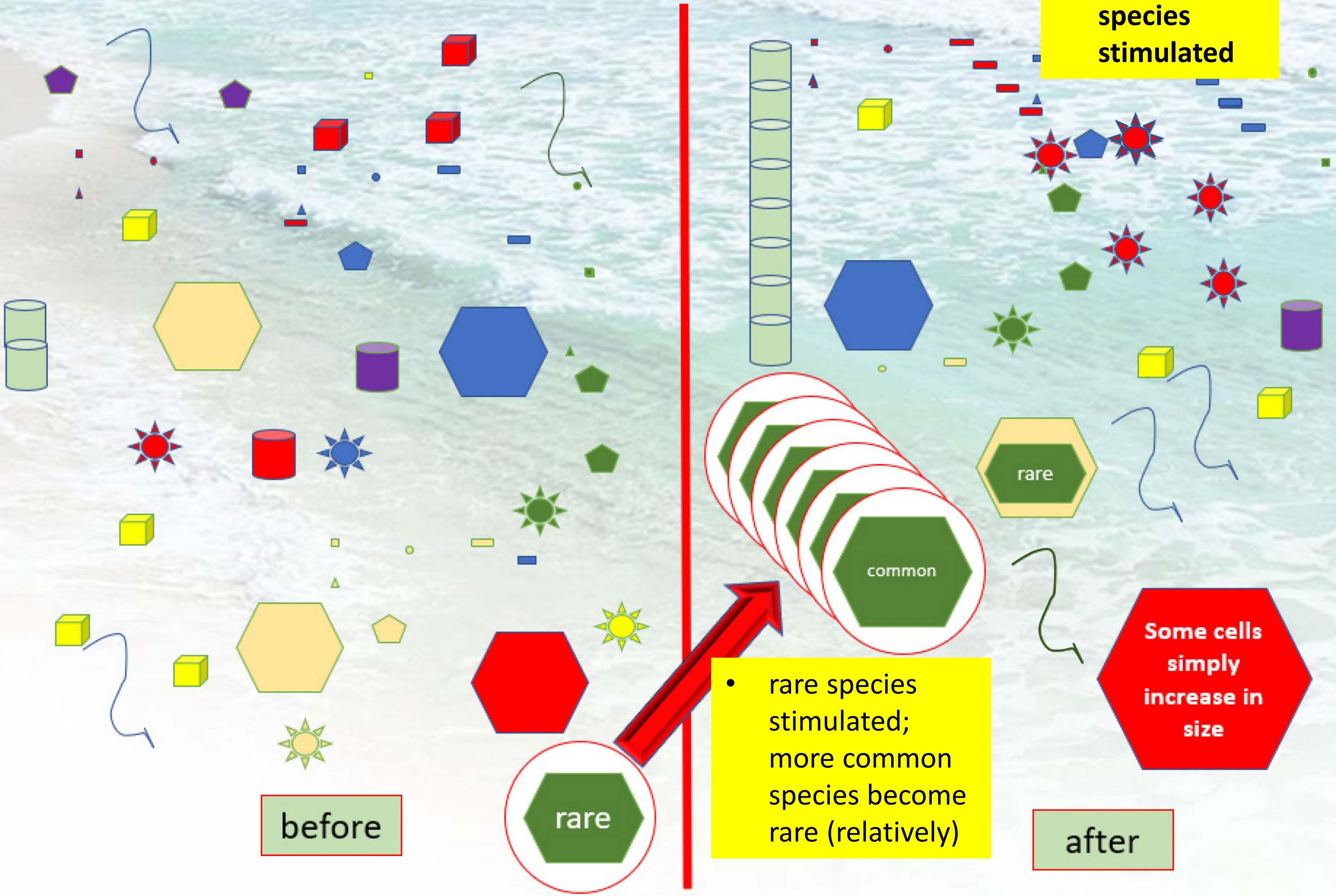
First: Understanding why cyanobacteria are successful- their eco-physiological strategies

Paradox of the plankton

Depiction of the primary producers (eukaryotic algae and cyanobacteria)



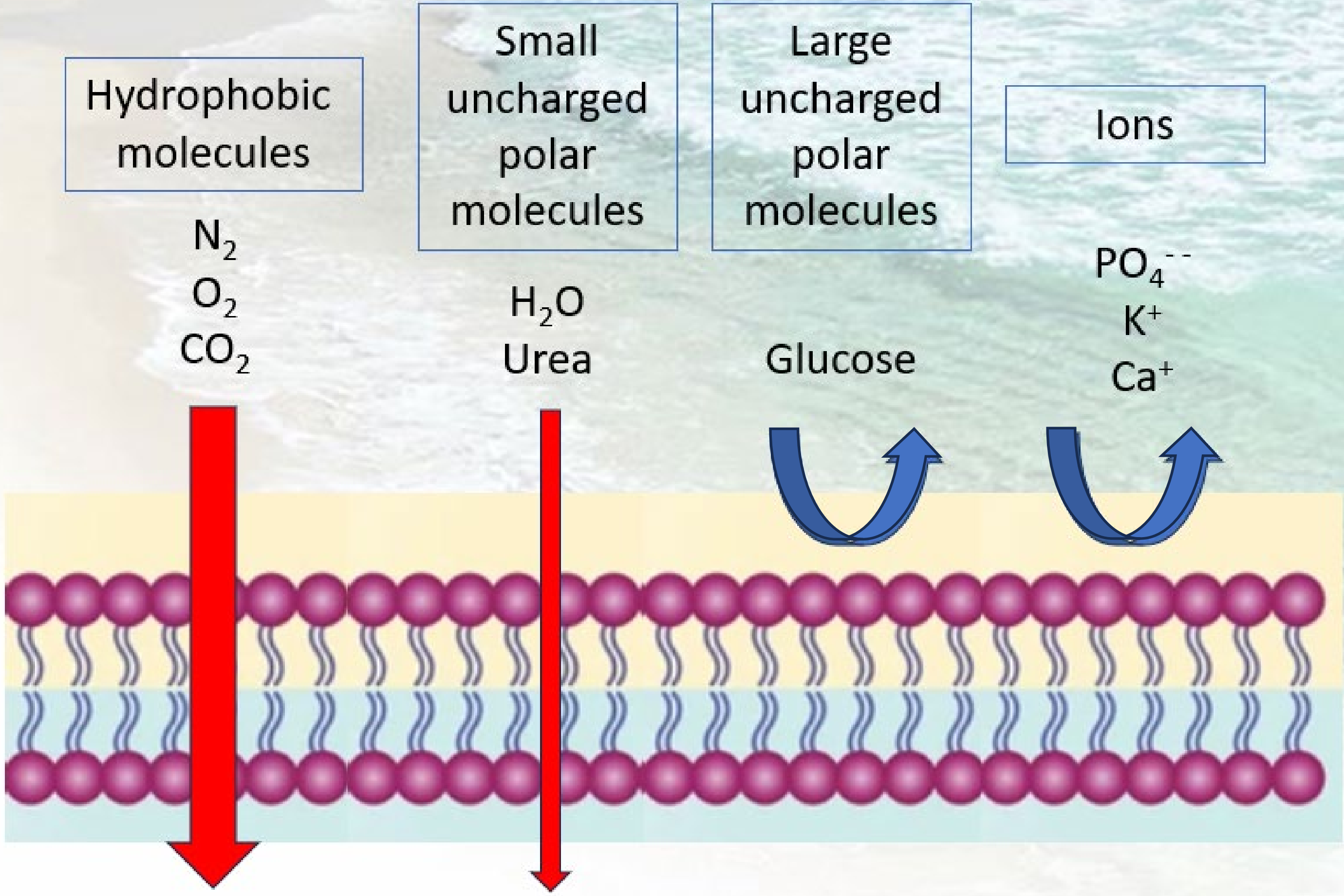
Add a limiting factor



- quiescent (subsistent) species stimulated

- rare species stimulated; more common species become rare (relatively)

Example: inorganic phosphorus uptake



Michaelis constant (Km) is defined as a measure of the affinity of an enzyme for its substrate

Michaelis-Menten Parameters:

V_{max}: The maximum P uptake rate, specific to each organism and its history (nutrient-deprived organisms will take it up faster).

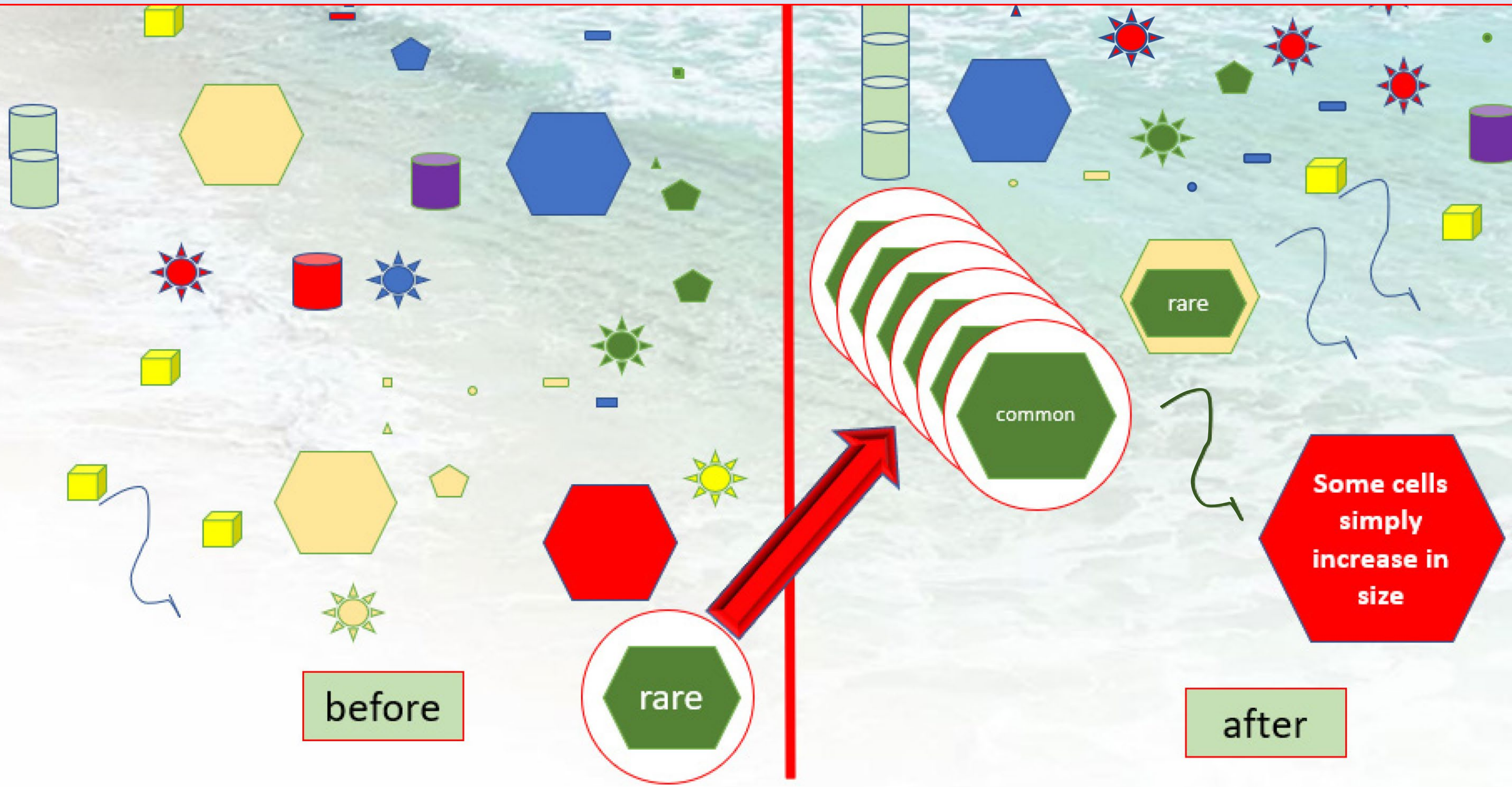
K_m: Half-saturation constant; lower values indicate higher affinity.

C_{min}: The threshold concentration below which no net uptake occurs.

- Species-specific, in general
- Intracolonial/filament variation
- Current cell content-”needs”
- Interplay with other cellular processes
- Interplay with other elements

It’s more complicated than rocket science!

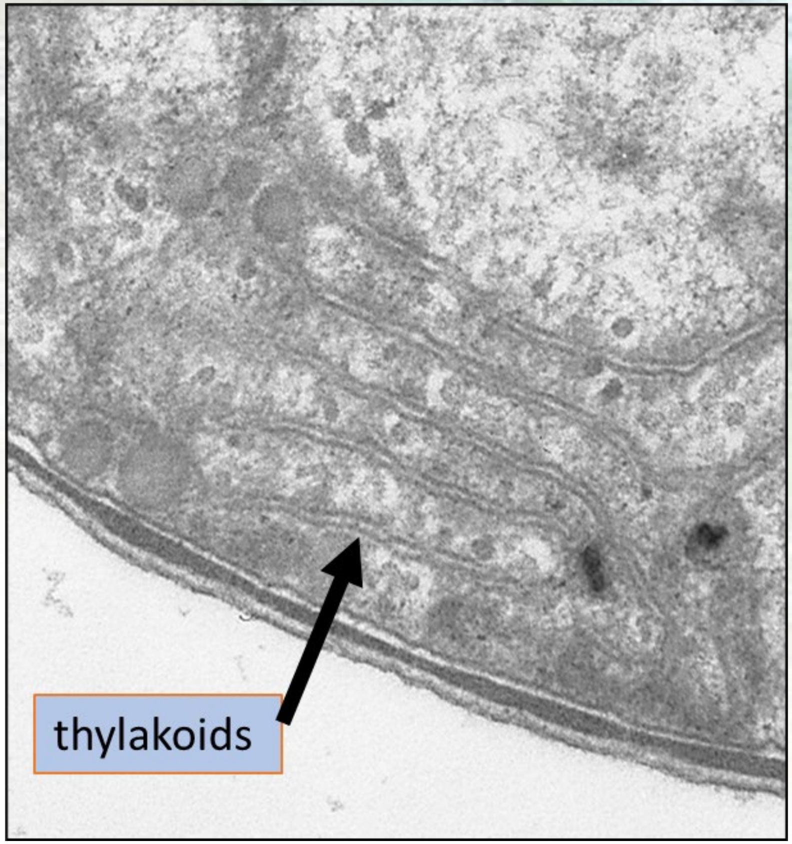
- **Daily**, **weekly**, **monthly**, **seasonal** forcing functions (**temp**., **light quantity** and **quality**, rainfall/dilution, water body stability)
- Each organism has an optimum **rate** of nutrient uptake; and optima for **all other factors**
- Each organism has a **concentration** threshold efficiency to take up that nutrient

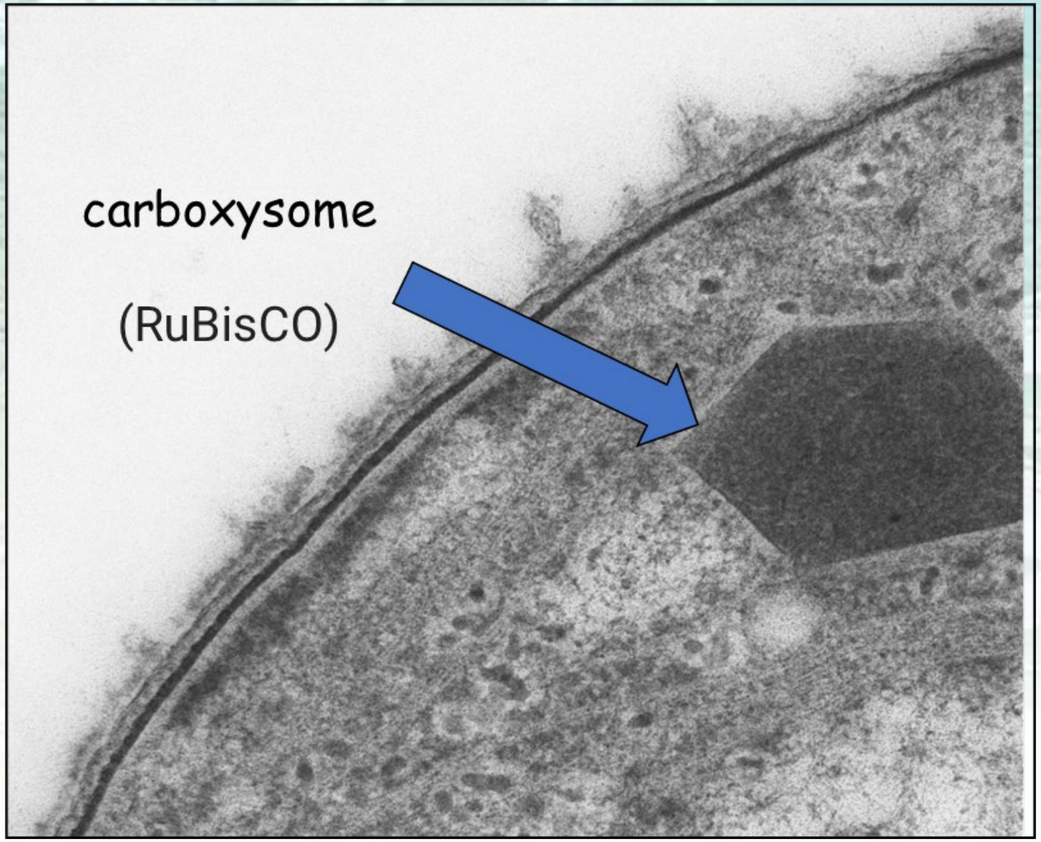


Cyanobacteria Top Priority: Photosynthesis

why/how

1) Energy captured in biochemical form (ATP, NADPH)
2) H_2O is split (**oxygen waste product to the cyano's**)
3) "eventually" CO_2 fixed into "sugars" needed for metabolism





why/how

Why are they so successful?

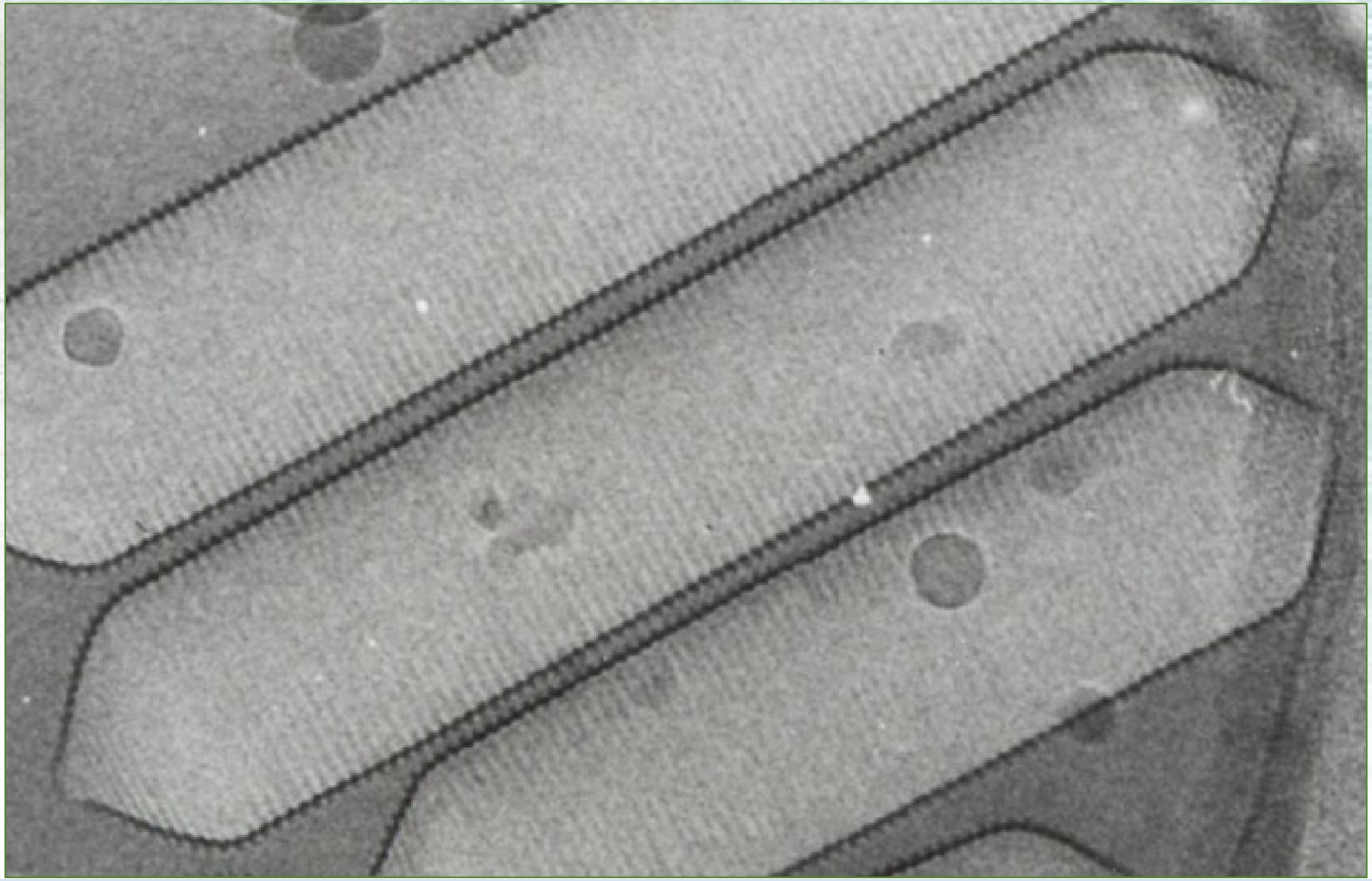
Ecological Strategy: Staying in the light (photic zone), but much more; Gas Vesicles: Buoyancy regulation and vertical migration- competitive advantage

Select genera

why/how

Anatomy of a Gas Vesicle

- hollow protein shells
- almost exclusively of gas vesicle protein A (GvpA)
- assembled and disassembled to change their density



Herzfeld J. Chapter 10 in *The Cell Biology of Cyanobacteria*, Caister Academic Press (Norfolk, UK, 2014).

why/how

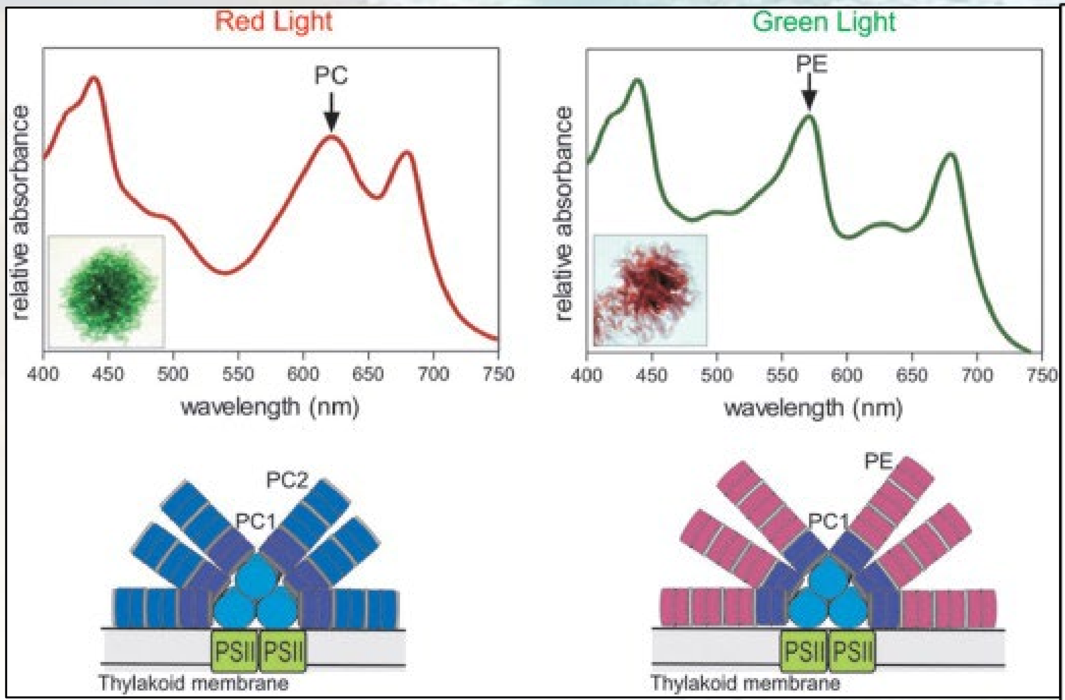
Gas Vesicle under the microscope

why/how

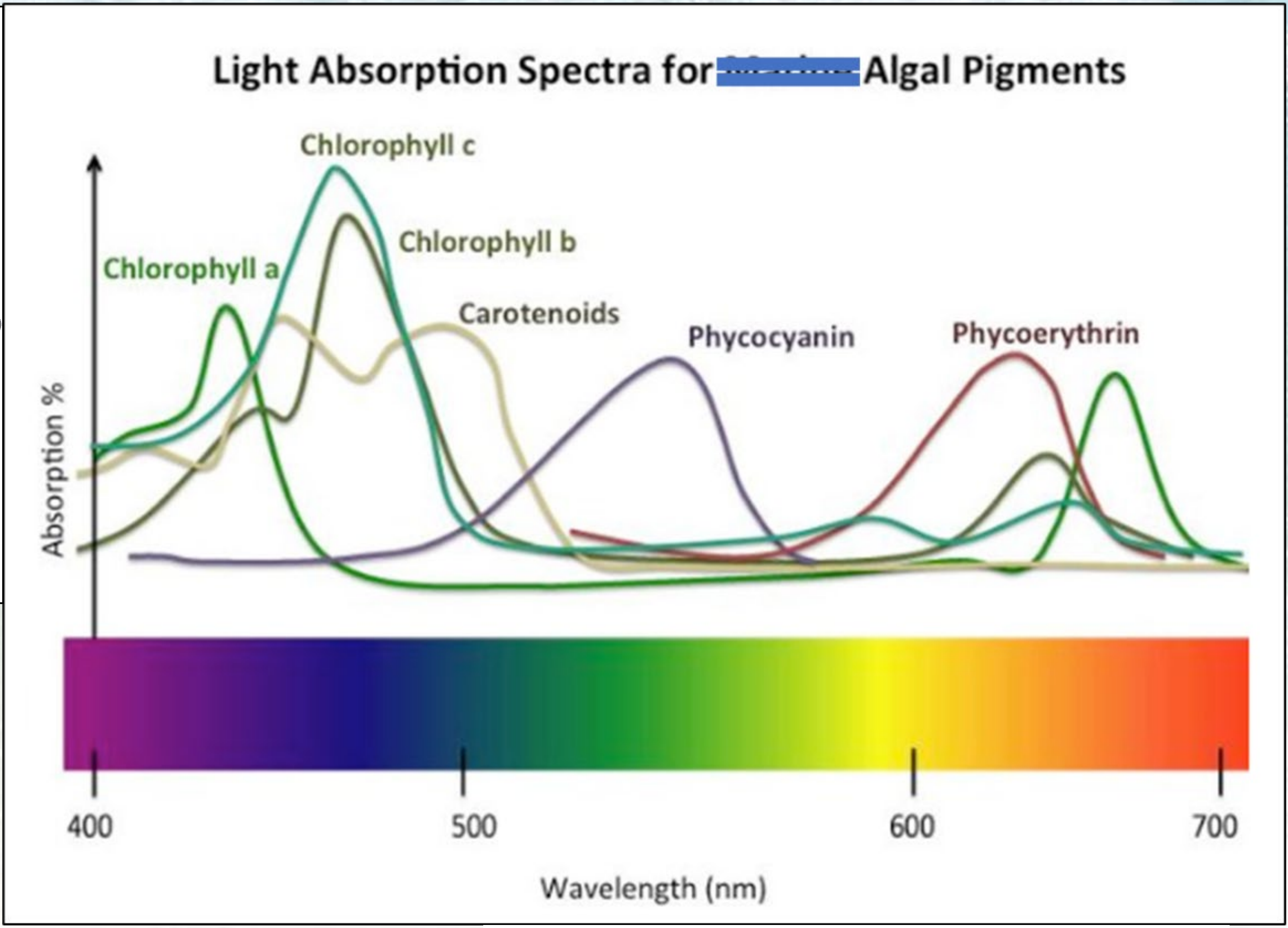
Gas Vesicle under the microscope

Algal pigments

why/how



https://www.sciencedirect.com/science/article/pii/S1674205214606432



Phycoerythrin & Phycocyanin

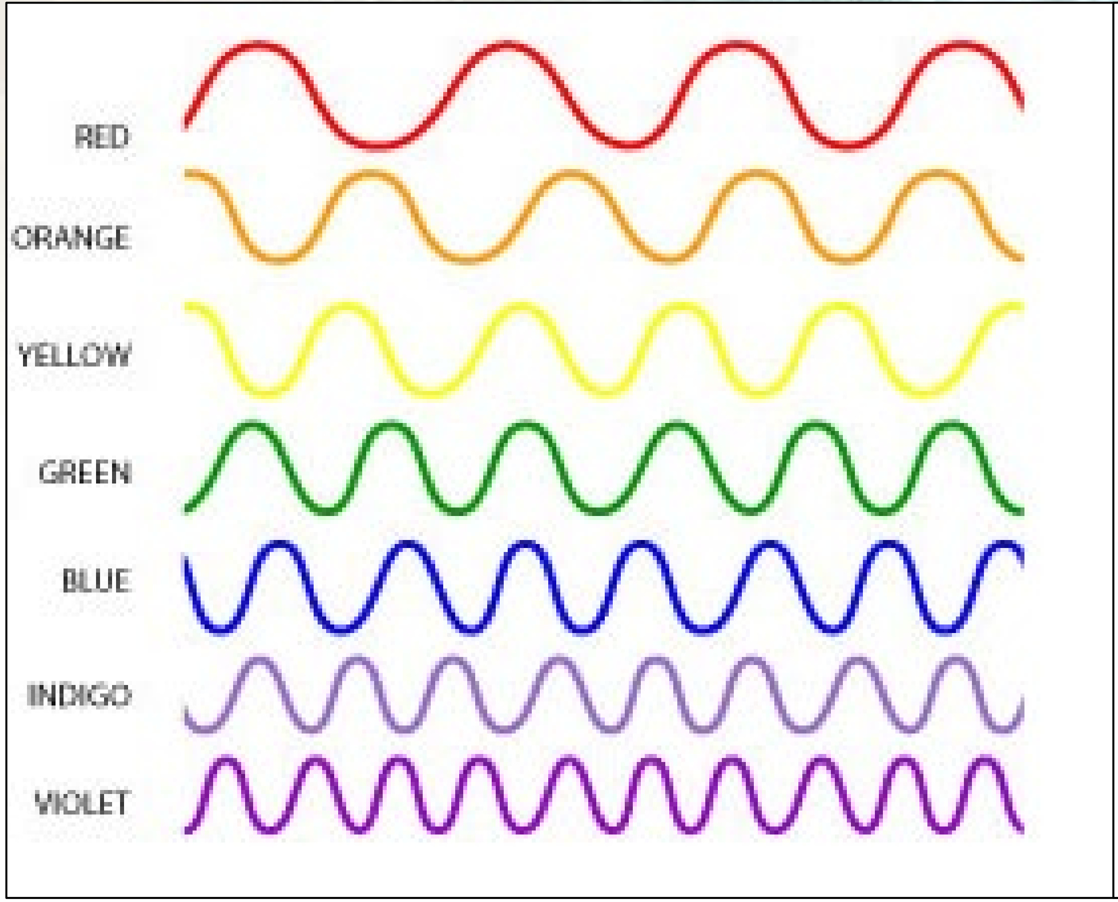
This figure was uploaded by Charles Yarish

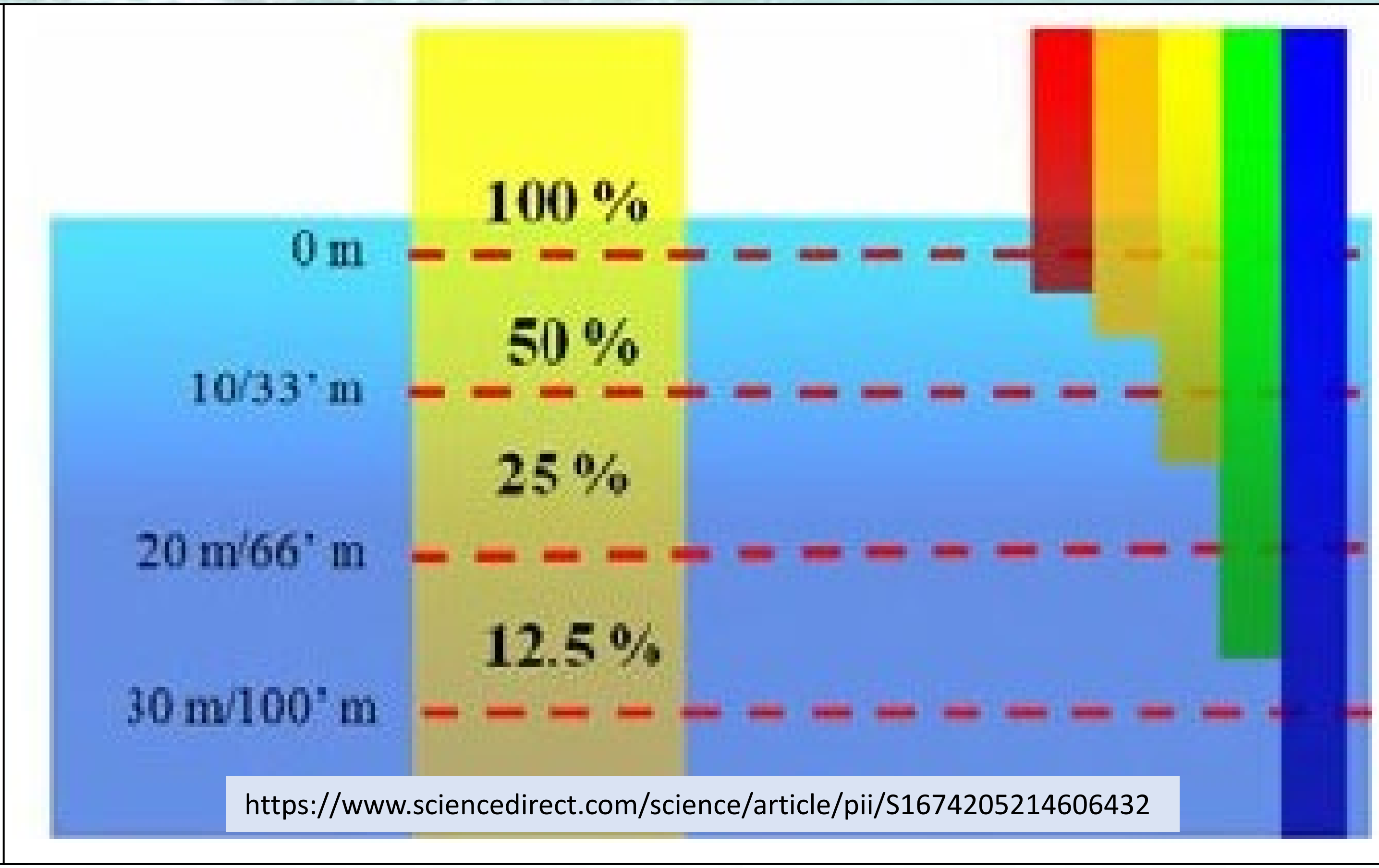
why/how

Why have so many types of pigments?

optimize light capture by creating a diverse “antennae” and photoprotection of chlorophyll

Penetration of light of various wavelengths through water; blue light is the strongest and red light is the weakest





https://www.sciencedirect.com/science/article/pii/S1674205214606432

why/how

Chl *a* & *d* & *f*

Cyanobacteria

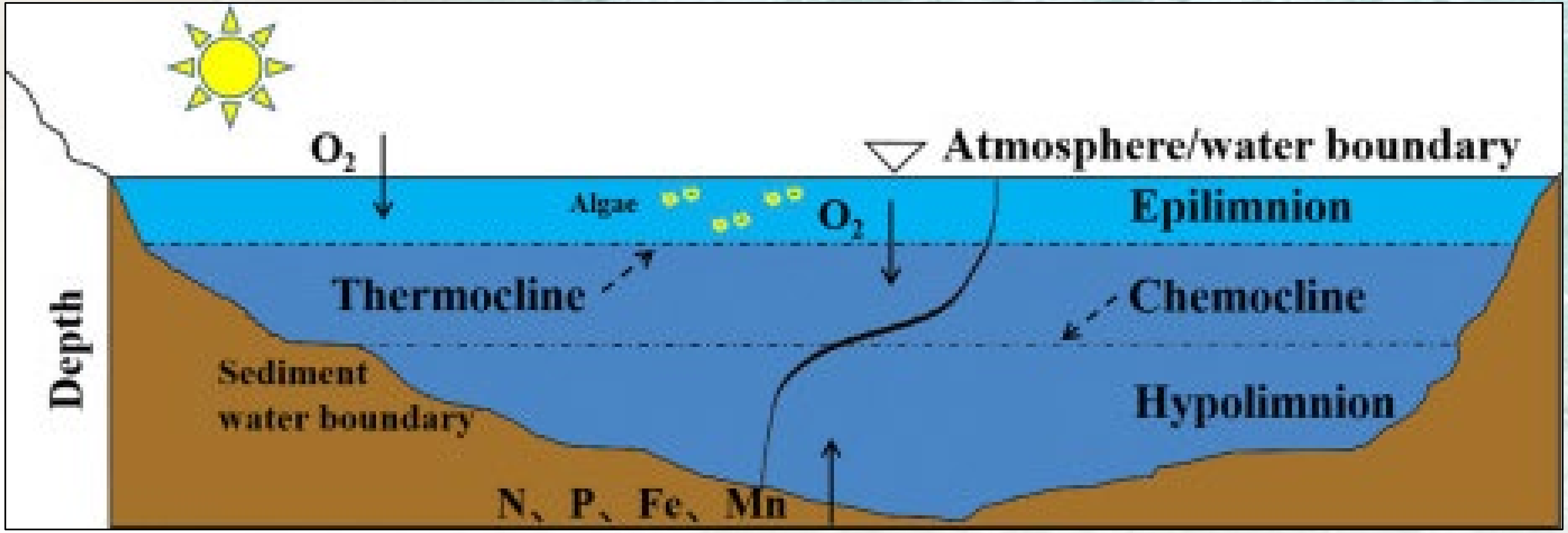
Chl *d and f:* low-light, far-red environments, such as microbial mats

Buoyancy can also to maximize nutrient uptake

why/how

Stratification
Thermocline

Nutrient Release & recycling



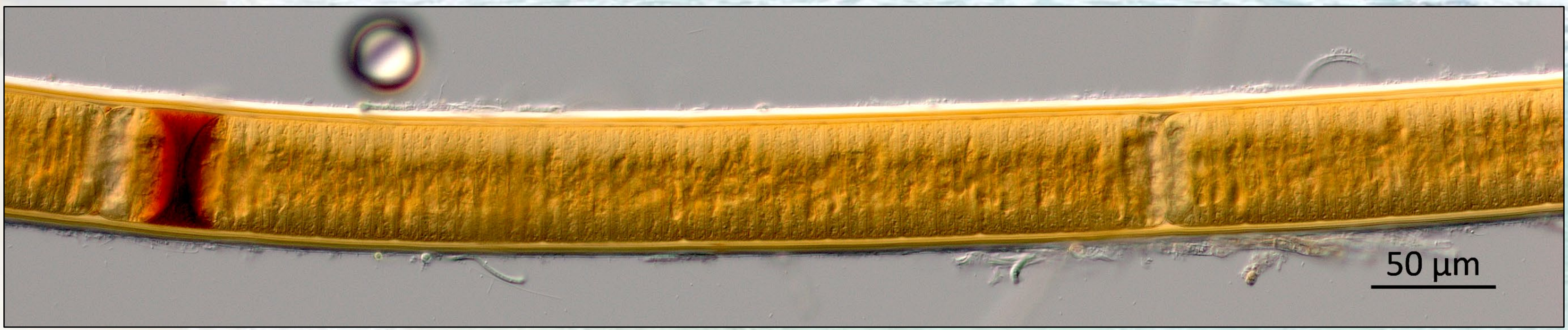
http://dx.doi.org/10.1016/j.scitotenv.2022.157787

Photosynthesis
$(C_6H_{12}O_6)_n$
ballast

$(C_6H_{12}O_6)_n$
utilization
respiration

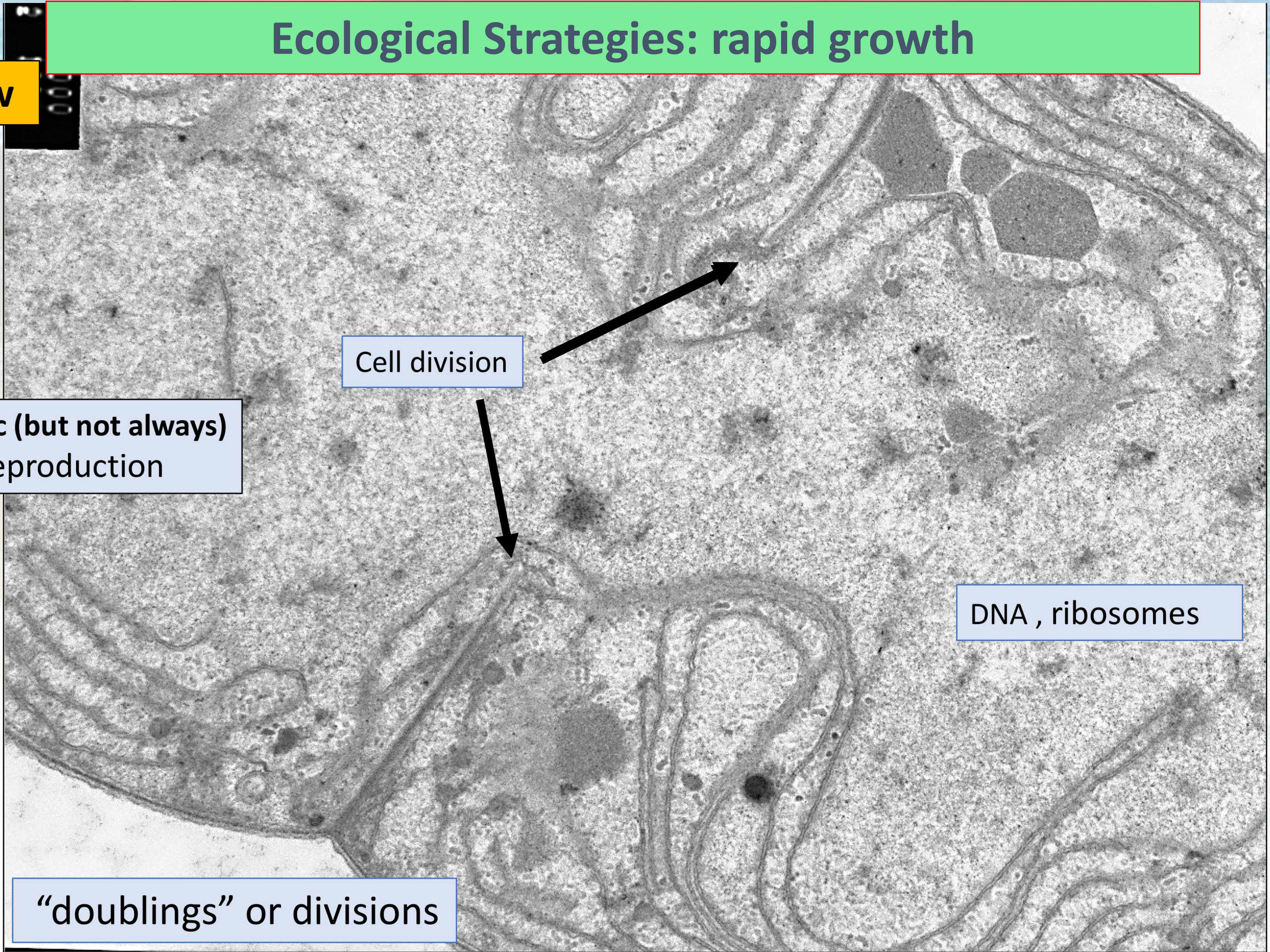
why/how

Ecological Strategies: an array of pigments for maximizing photosynthesis



Ecological Strategies: rapid growth

why/how

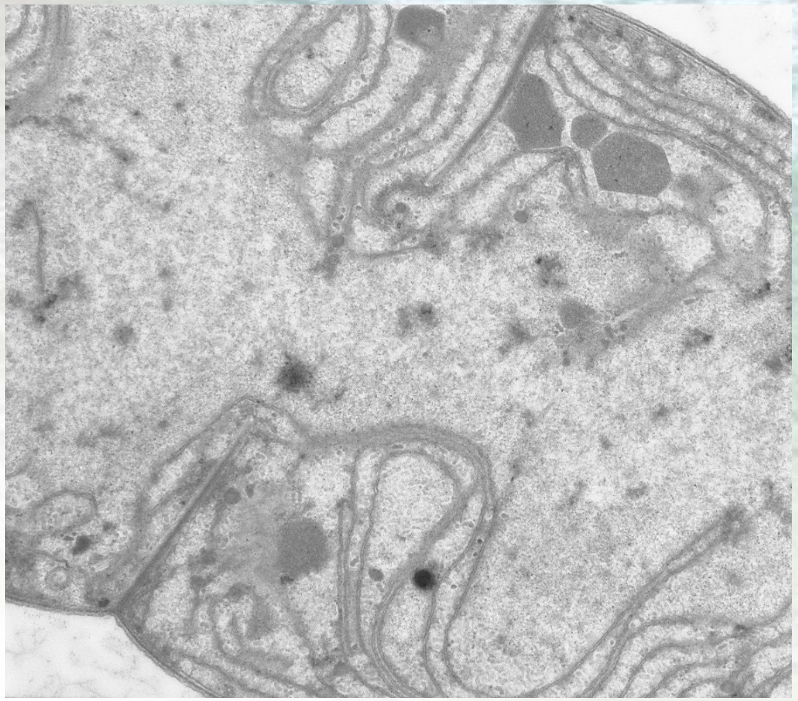


Thermophilic (but not always)
Rapid reproduction

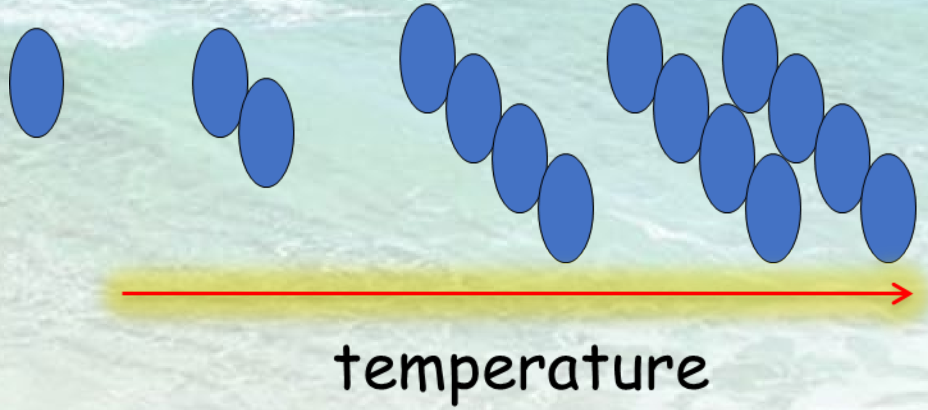
“doublings” or divisions

why/how

Ecological Strategies: bacteria in a eukaryotic world- thermophiles grow faster



Rapid Growth



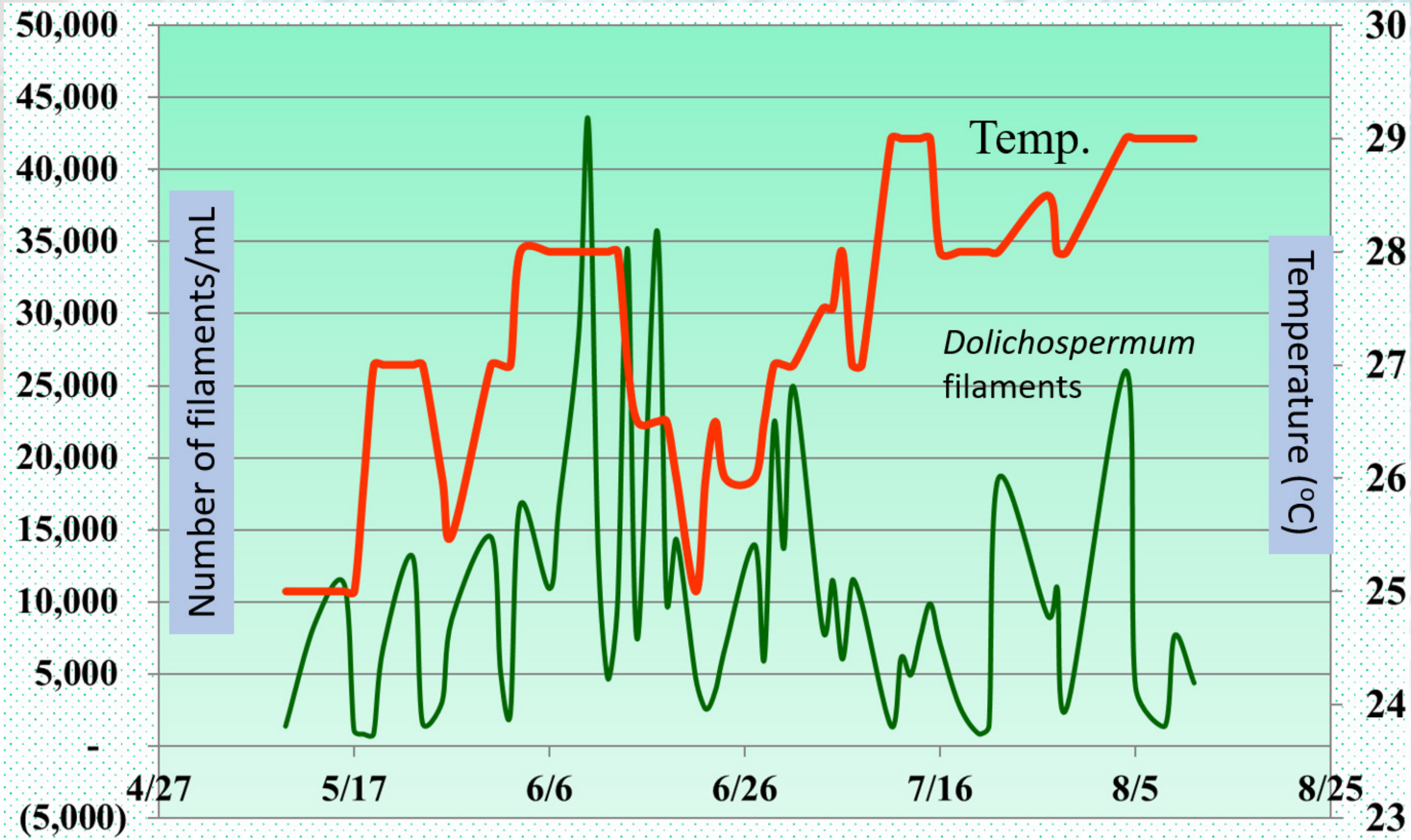
3 “doublings” or divisions per day!

Caveats: light, temperature, nutrients must not be limiting

Ecological Strategies: Temperature Case Study

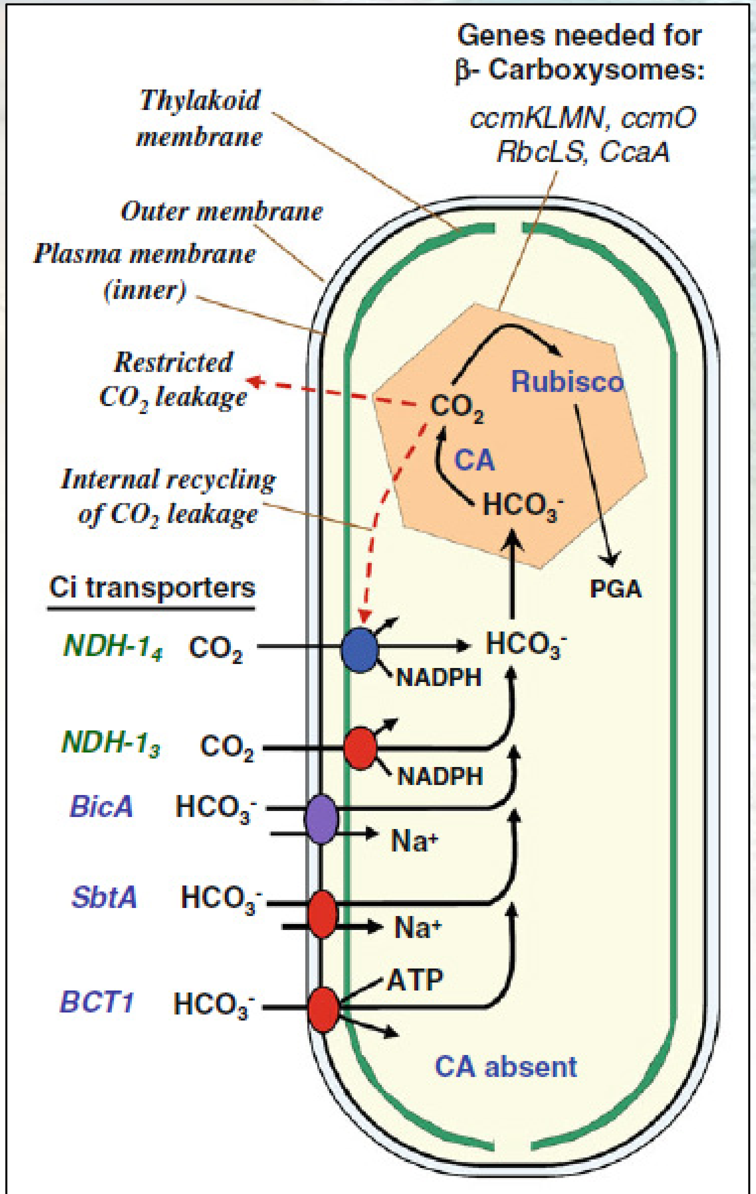
Dolichospermum circinale in the Hillsborough River, FL

why/how



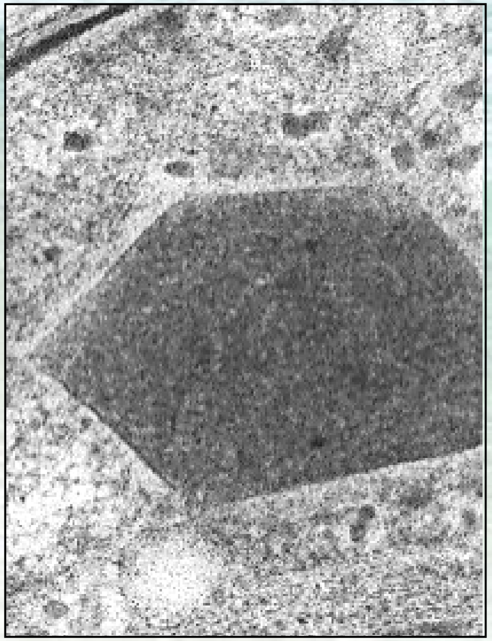
why/how

Ecological Strategies: carbon dioxide concentrating mechanism



Take in CO_2 and bicarbonate, up to **1000-fold** over that in the surrounding water.

- 5 separate transport systems; some require ATP



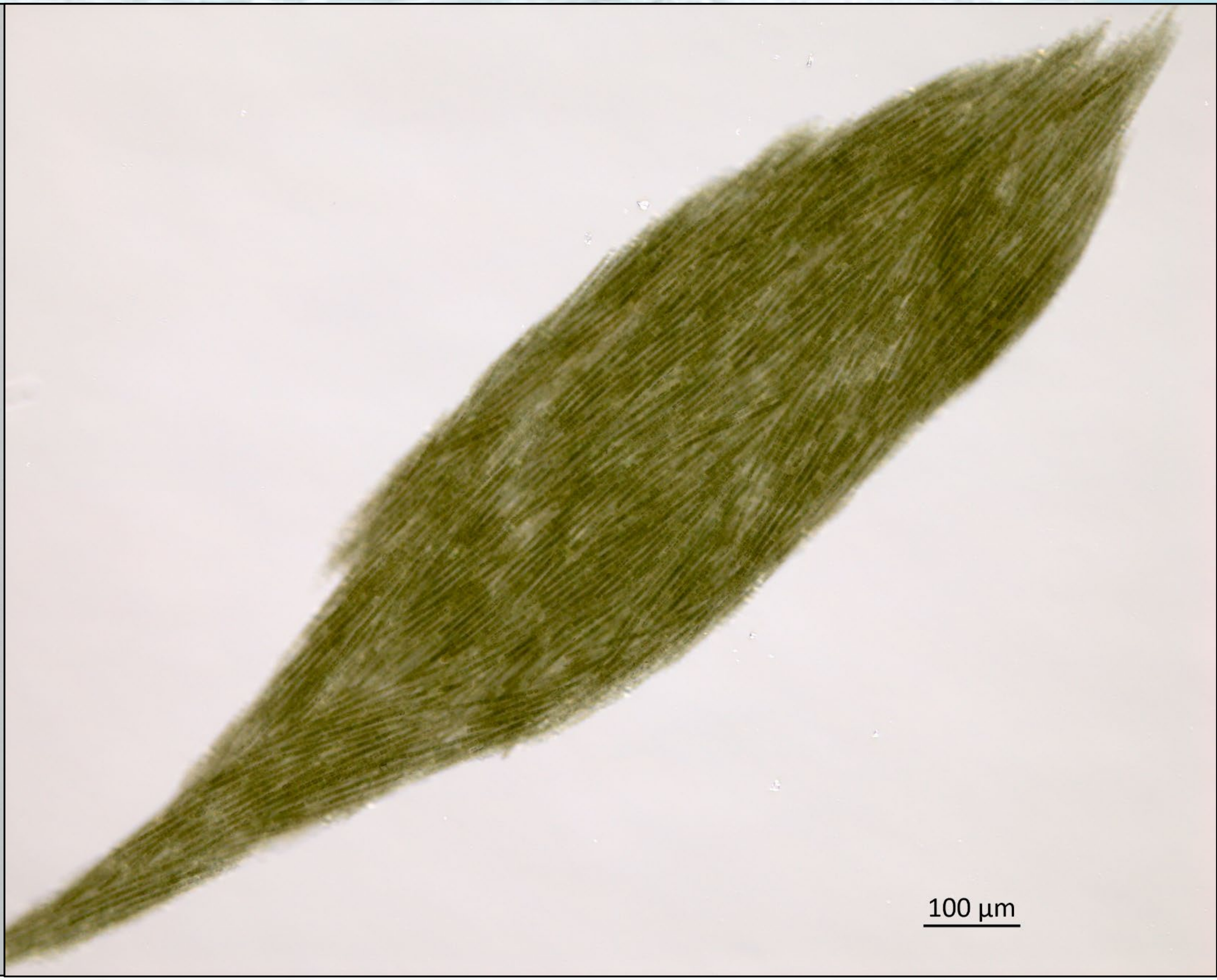
Inorganic carbon dioxide transports in conjunction with the carboxysome

Ribulose-1,5-bisphosphate carboxylase-oxygenase

https://doi.org/10.1007/s11120-010-9608-y

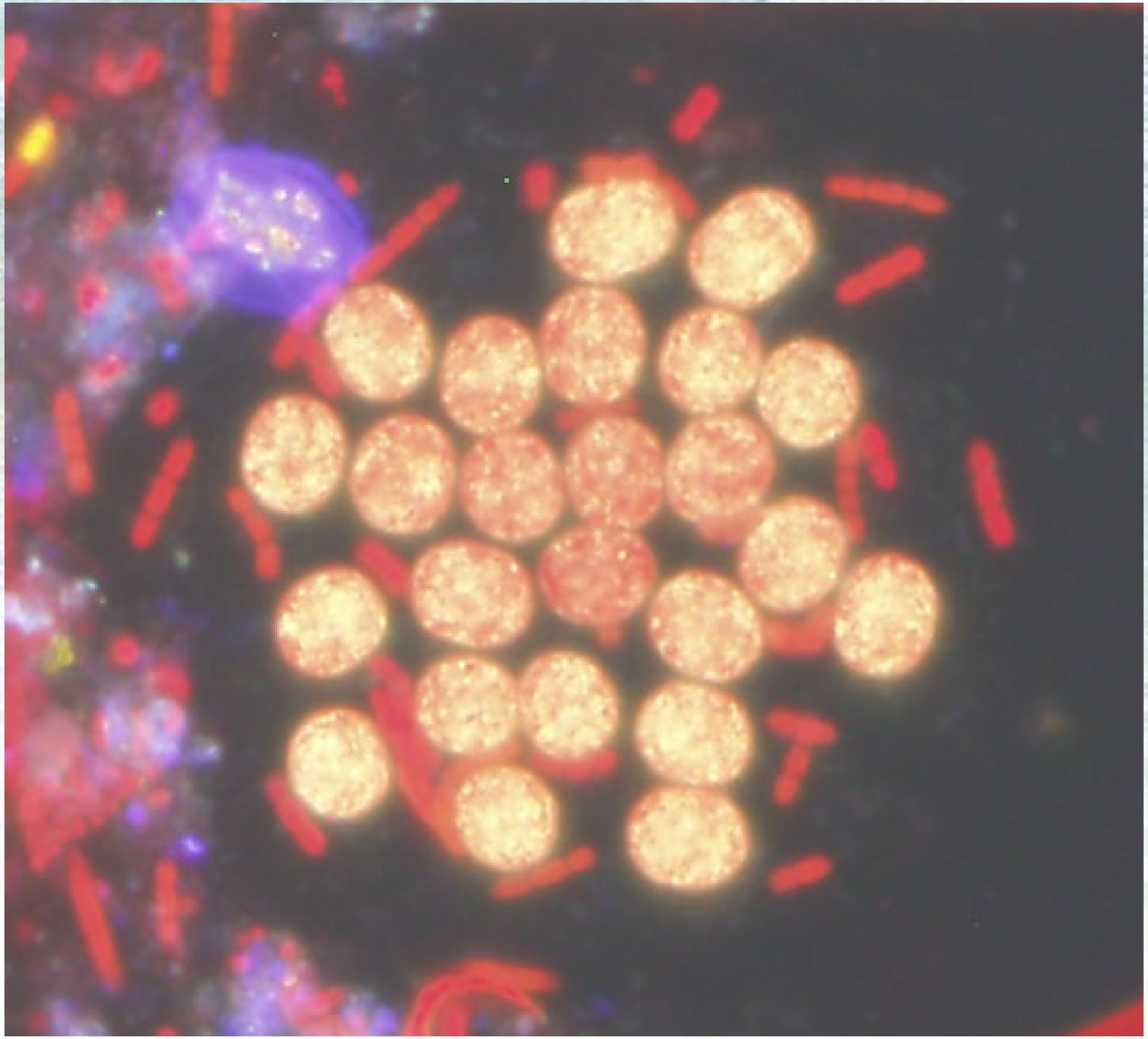
why/how

Morphology for staying in the water column



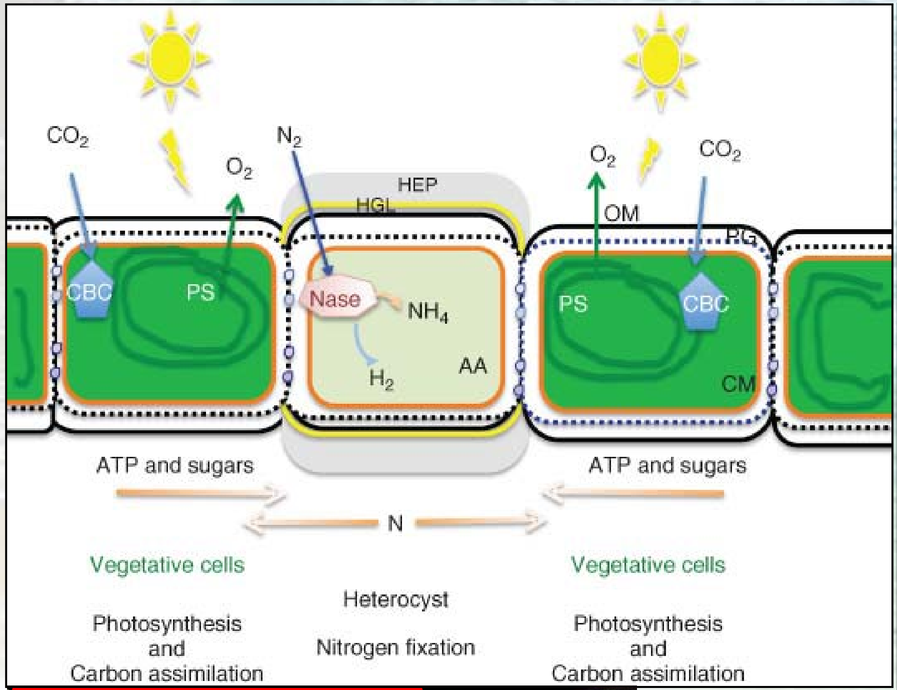
Commensalism?: organisms staying in the water column

observation



why/how

Some genera can overcome a limiting factor, like nitrogen limitation: heterocytes, etc.



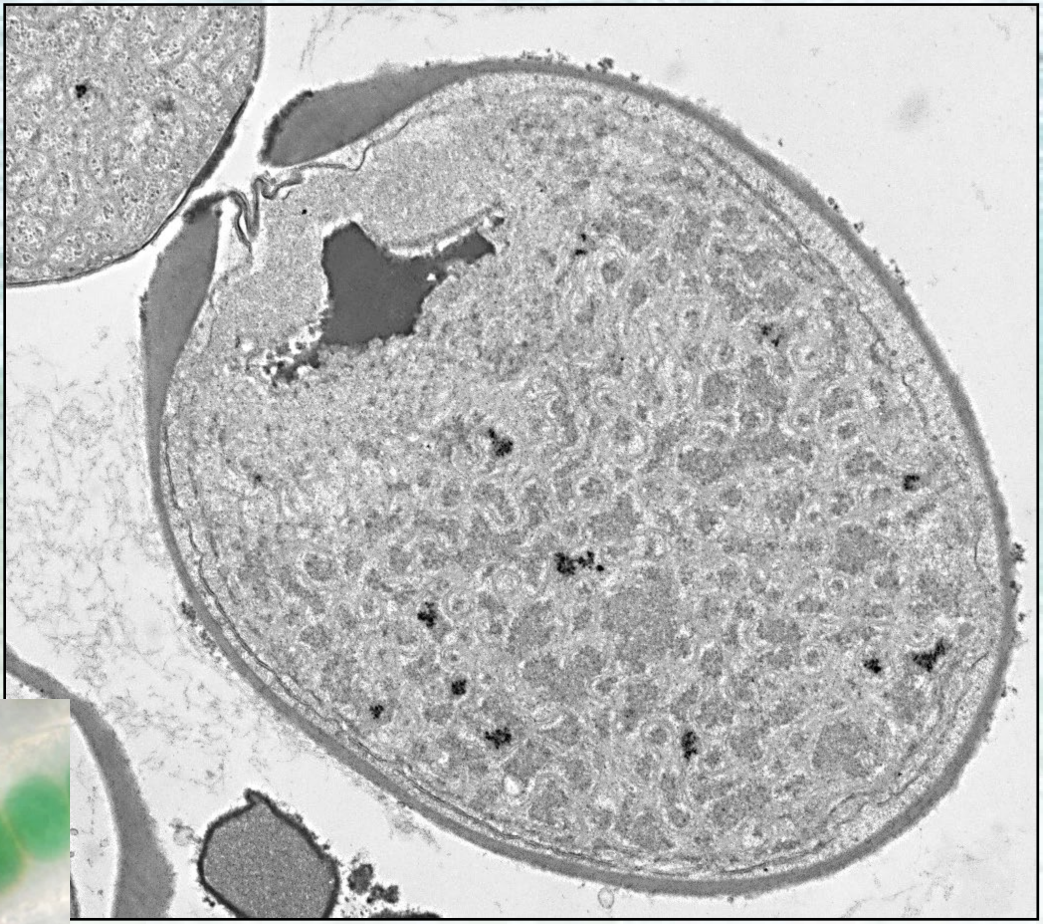
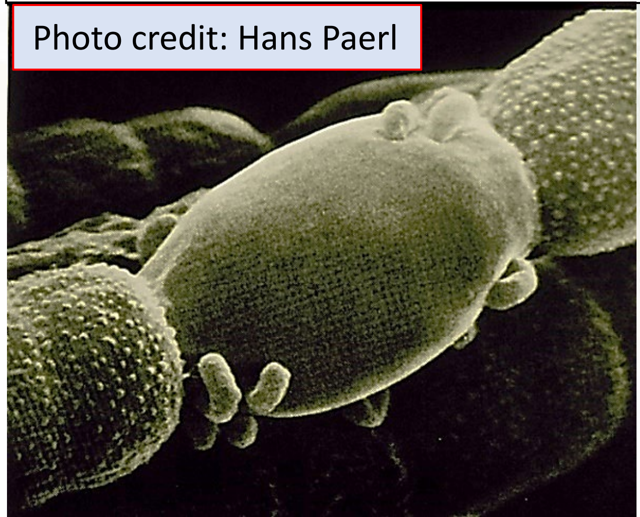
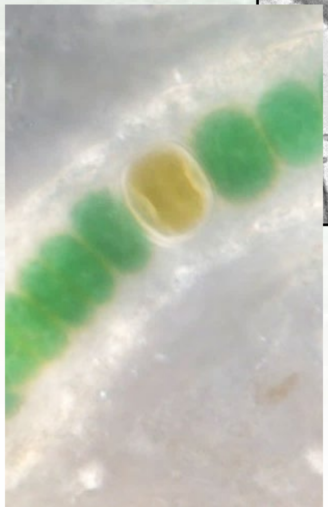


Photo credit: Hans Paerl





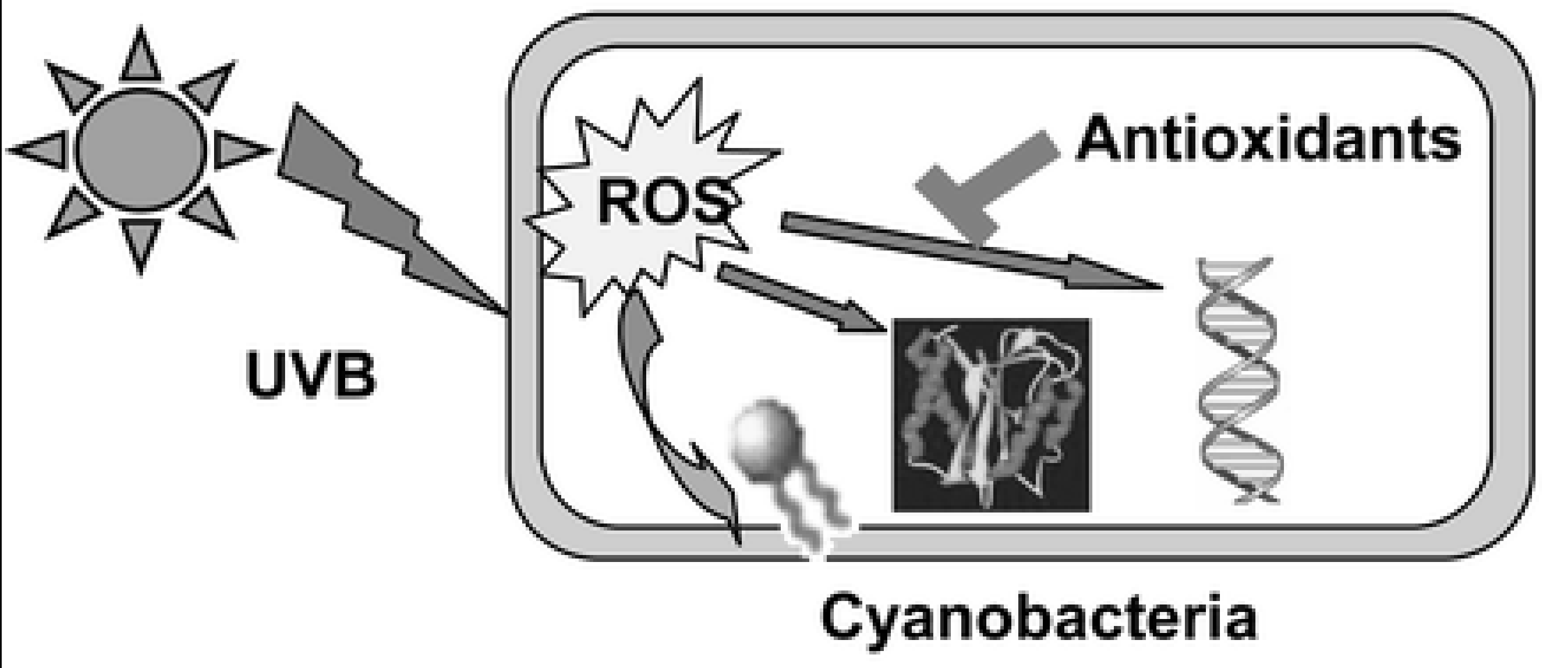
- Lipid layer around cell wall
- Loss of oxygenic-photosystem II
- Visible pigment difference

Light capture has its hazards: reactive oxygen species

why: some functions of cyanotoxins for the cyanobacteria

Oxidative Stress Protection: Cyanotoxins, particularly microcystins, help cyanobacteria cope with high light intensity and oxidative stress. They bind to redox-sensitive proteins, protecting them from damage.

The damage to photosynthetic apparatus induces the inhibition of photosynthesis that is mediated partially by ROS. UV-B-induced oxidative stress and oxidative damage increases with irradiation time and can be reversed after long-term irradiation.



https://pubs.rsc.org/en/content/articlelanding/2002/pp/b110365m

why: some functions of cyanotoxins for the cyanobacteria

Iron Scavenging and Homeostasis

Microcystins (MCs) are believed to act as iron-scavenging molecules, assisting in iron uptake, which is vital for photosynthesis, respiration, and nitrogen fixation. They are produced in higher concentrations under iron-deficient conditions.

Chelation

implicated in an intracellular role that involves metal chelation

"the relative order of stability for the simulated metal–microcystin complexes was, for metal complexation by microcystin-LR: Zn > Cu ≥ Fe ≥ Mg > Ca, metal complexation by microcystin-RR: Zn ≥ Cu ≥ Mg > Ca > Fe"

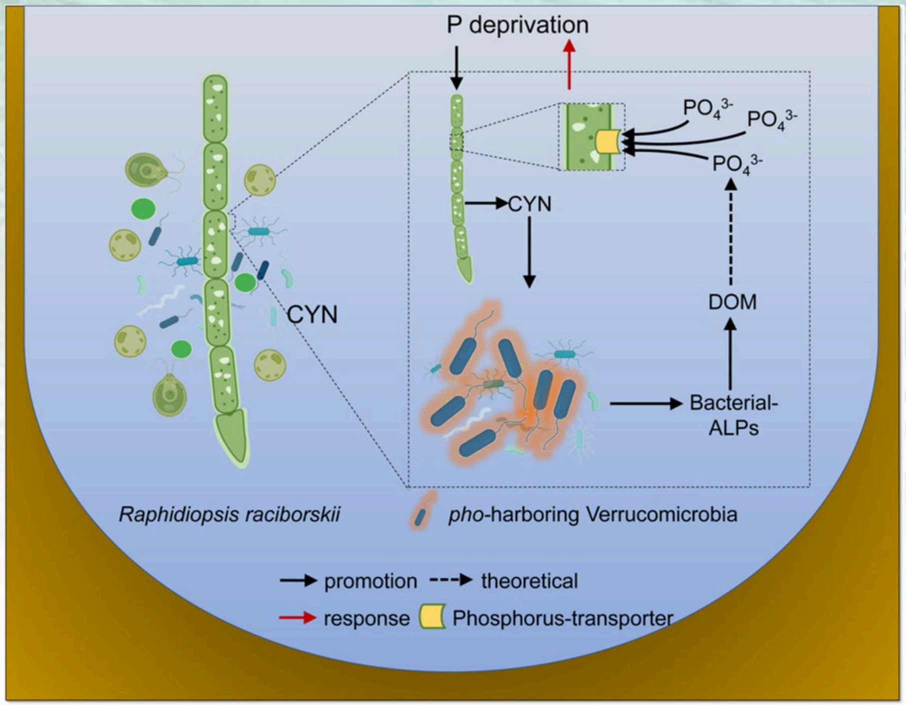
https://doi.org/10.1007/s10311-017-0639-x

why: some functions of cyanotoxins for the cyanobacteria

Nutrient Uptake and Metabolism

Cyanotoxins (e.g., cylindrospermopsin) can aid in nutrient acquisition, specifically phosphorus, by causing surrounding organisms to secrete alkaline phosphatases, which the cyanobacteria can then exploit. They are also linked to the regulation of nitrogen metabolism.

Cylindrospermopsin (CYN) induces the secretion of alkaline phosphatase in surrounding phytoplankton and bacterioplankton, particularly under low-phosphorus conditions. This mechanism is a key survival strategy for the bloom-forming cyanobacterium *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis*), which uses CYN to trigger other microorganisms to hydrolyze organic phosphorus into inorganic phosphate for nutrient uptake.



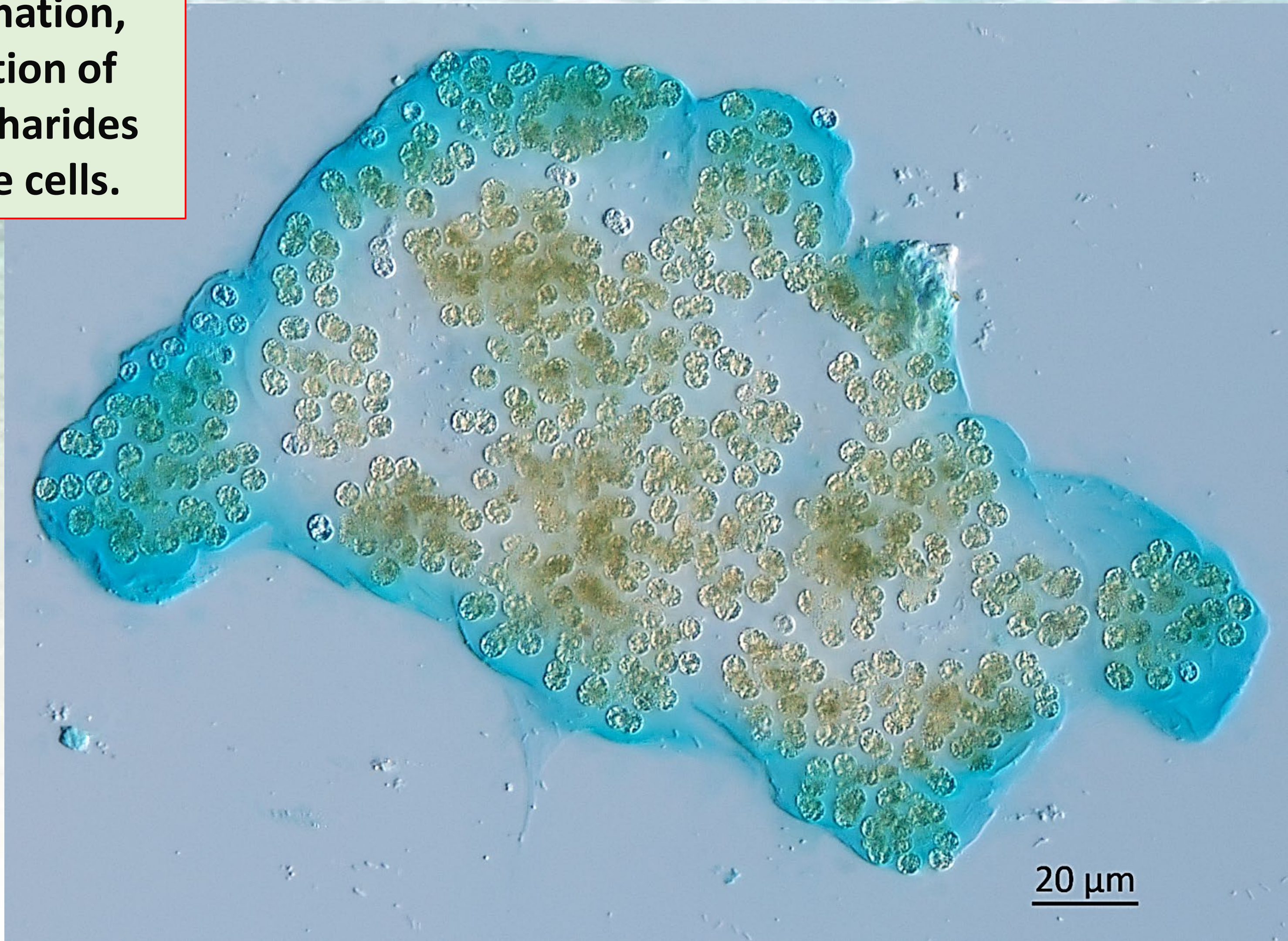
https://doi.org/10.1016/j.watres.2023.121010

Signaling and Colony Formation

why: some functions of cyanotoxins for the cyanobacteria

Extracellular microcystins act as signaling molecules that can influence colony formation, aiding in the production of extracellular polysaccharides (EPS) that protect the cells.

Extracellular Polymeric Substances: Carbohydrates stained with Alcian Blue



why: some functions of cyanotoxins for the cyanobacteria

Chemical Defense

Saxitoxin: Reduced feed rates for zooplankton like *Daphnia*

Allelopathy: Some cyanotoxins are released into the environment to inhibit the growth of competing algae and aquatic plants, securing resources.

Nitrogen Storage

Bottom line, we as humans can’t possibly know all the roles of the cyanotoxins

But

Why would such an expensive metabolic pathway have evolved and survived for so long if it had just few purposes.

why we as humans care

Families of cyanotoxins

- **Hepatotoxins**
  - Disrupt proteins that keep the liver functioning, may act slowly (days to weeks)
- **Neurotoxins**
  - Cause rapid paralysis of skeletal and respiratory muscles (minutes)
- **Dermatotoxins**
  - Produce rashes and other skin reactions, usually within a day (hours)
- **b-N-methylamino-L-alanine**
  - Excitotoxin, killing neurons
  - potentially linked to ALS, Parkinson's, Alzheimer's
- **Cyanopeptides beyond microcystins**
  - Protease inhibitors
  - TBD

microcystins (300+ variants)
nodularin
cylindrospermopsin

anatoxin-a
guanitoxin (formerly anatoxin-a (s))
saxitoxin
neosaxitoxin

lyngbyatoxin

BMAA & DABA

anabaenopeptins

why we as humans care

Potency of Cyanotoxins

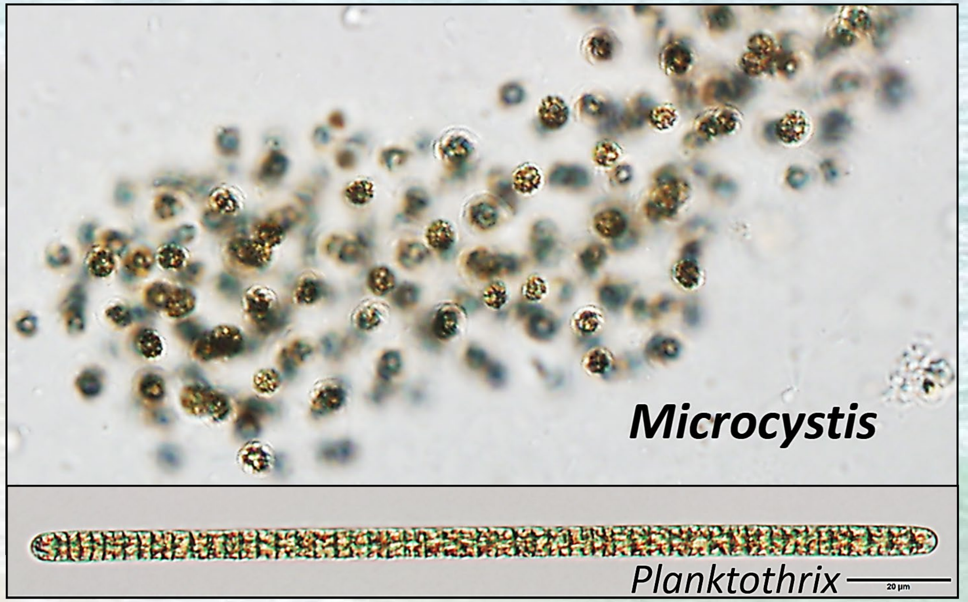
*Lethal Dose$_{50}$ (µg/kg=parts per billion)

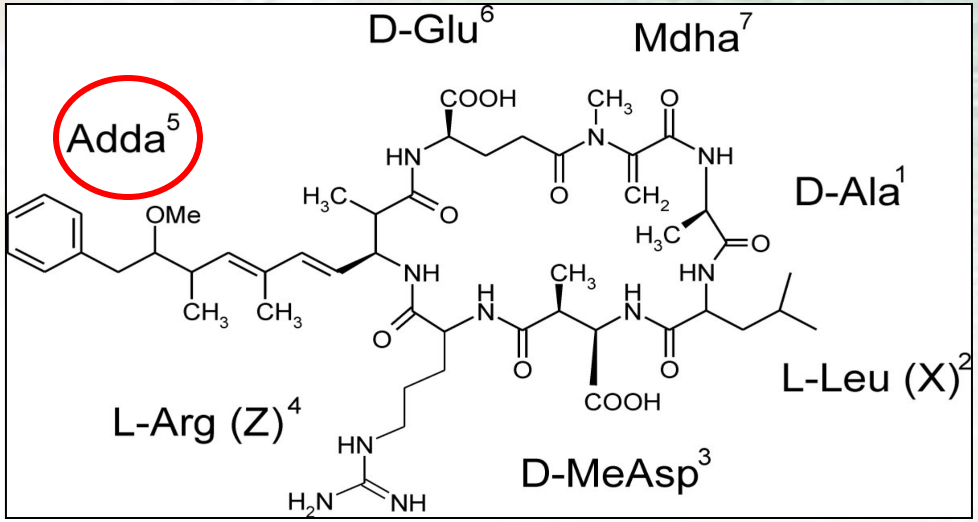
Saxitoxin	9	Ricin	0.02
Guanitoxin Anatoxin-a(s)	20	Cobra toxin	20
Microcystin LR	50	Curare	500
Anatoxin-a	200-250	Strychnine	2000
Nodularin	50		
Cylindrospermopsins	200		

***Exposure routes*: data is based on ingestion/direct dosing. Little has been done on inhalation effects**

Microcystins

why we as humans care





Microcystis aeruginosa

- non-N fixer
- Very common
  - also produced by a number of other species.
- Peptide Toxins:
  300+ structural variants
- Microcystins are hepatotoxic
  LD-50: 25-60 $\mu g\ kg^{-1}$
- Called "fast death factor"
  Potent tumor promotor

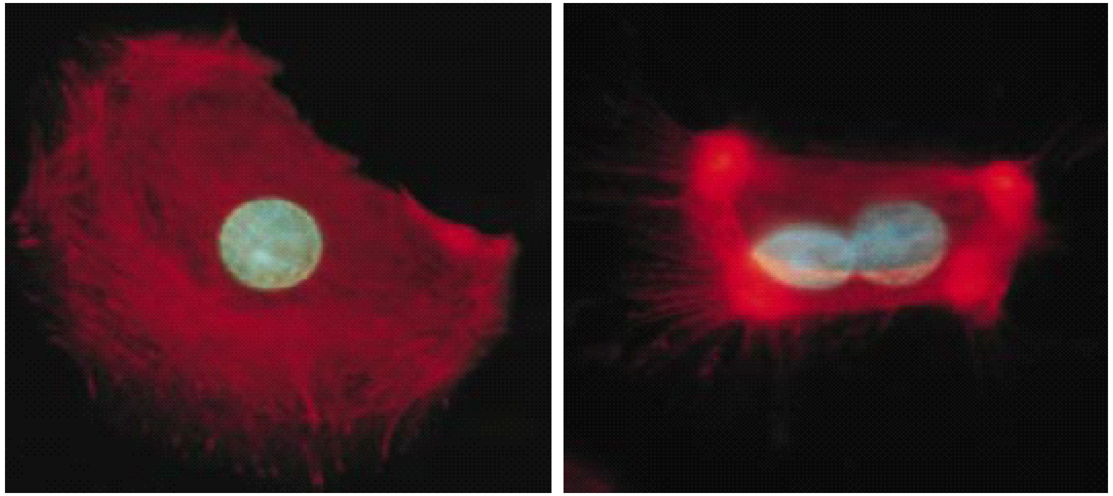
why we as humans care

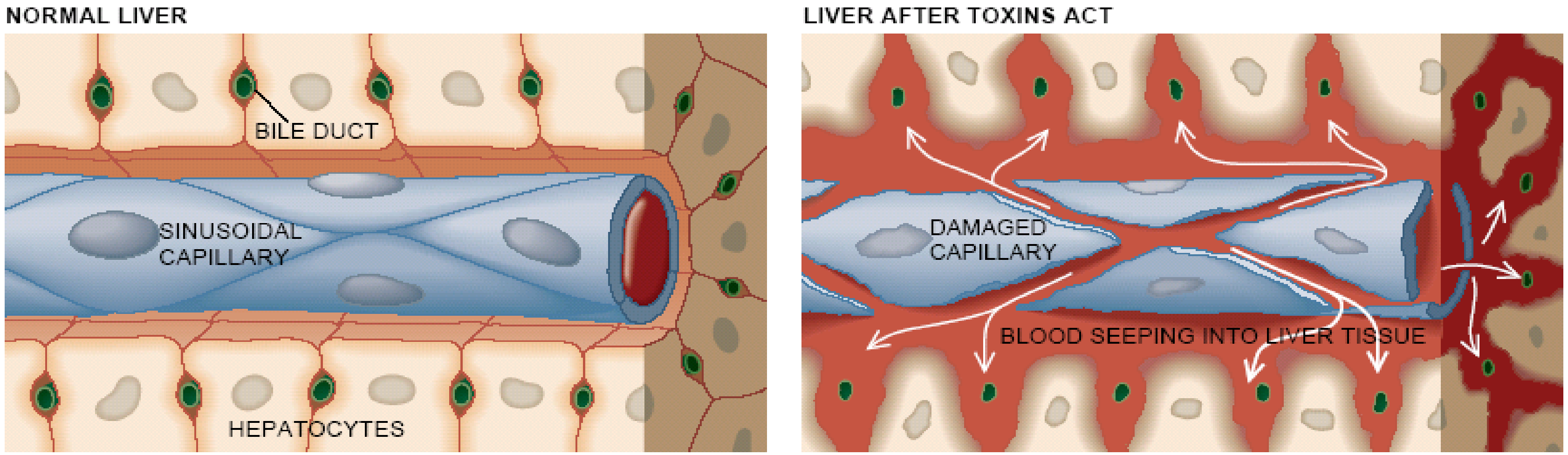
Microcystin exposure: response

- Uptake by bile acid transporter
- Inhibit protein phosphatases 1 and 2A
- Affects cytoskeleton, cell cycle, general metabolism, apoptosis

Hepatotoxicity

MICROFILAMENTS (*red threads in micrographs*), structural components of cells, are usually quite long, as in the rat hepatocyte at the left. But after exposure to microcystins (*right*), microfilaments collapse toward the nucleus (*blue*). (This cell, like many healthy hepatocytes, happens to have two nuclei.) Such collapse helps to shrink hepatocytes—which normally touch one another and touch sinusoidal capillaries (*left drawing*). Then the shrunken cells separate from one another and from the sinusoids (*right drawing*). The cells of the sinusoids separate as well, causing blood to spill into liver tissue. This bleeding can lead swiftly to death.





Wayne Carmichael ISOC-HAB Ch. 4, Scientific American, January, 1994

why we as humans care

Drinking Water Guidelines

0.3 micrograms per liter for **microcystin**

0.7 micrograms per liter for **cylindrospermopsin**

- children younger than school age
- exposure for 10 days.

1.6 micrograms per liter for microcystin

3.0 micrograms per liter for cylindrospermopsin

- all other ages

EPA Issued Health Advisories for Algal Toxins in Drinking Water *Release Date: 05/06/2015*

why we as humans care

Recreational Guidelines

8 micrograms per liter for **microcystins**
15 micrograms per liter for **cylindrospermopsin**

- All age groups

EPA Issues Recommendations for Recreational Water Quality Criteria and Swimming Advisories for Cyanotoxins

Release Date: 05/22/2019

https://www.epa.gov/newsreleases/epa-issues-recommendations-recreational-water-quality-criteria-and-swimming-advisories

why we as humans care

The public has been alerted

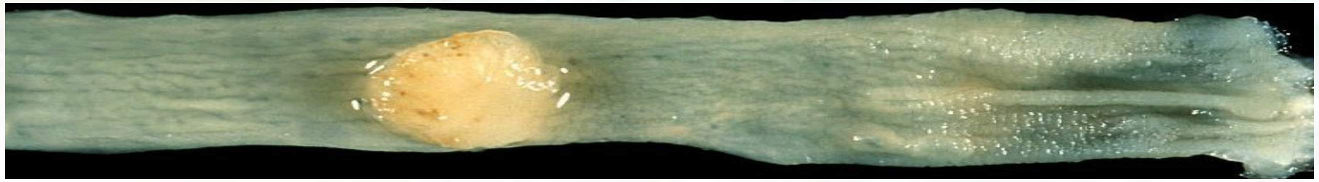
"The algal blooms are growing larger and appearing earlier and earlier each summer. These harmful algal blooms (HABs) often produce toxins that make humans and animals sick. The most infamous of these toxins is microcystin, which has reached levels three times above the recommended limit for swimming beaches in areas of Lake Erie."

why we as humans care

Microcystin exposure: tumor promotion

- Epidemiology in China:
  - Contaminated drinking water ↔ primary liver and colon cancer.
- Injection of toxin ± initiator:
  - Increased size/number of liver cancer precursors.
- Oral *M. aeruginosa.* extract:
  - Skin papillomas larger/heavier
  - No effect on duodenal tumours or lymphoma.

Colon cancer precursors

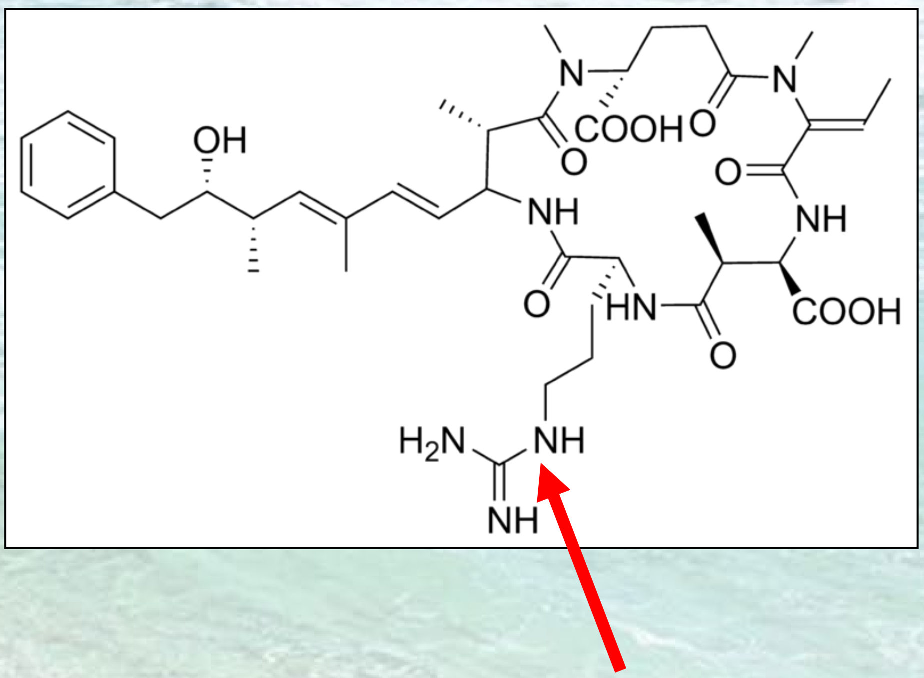


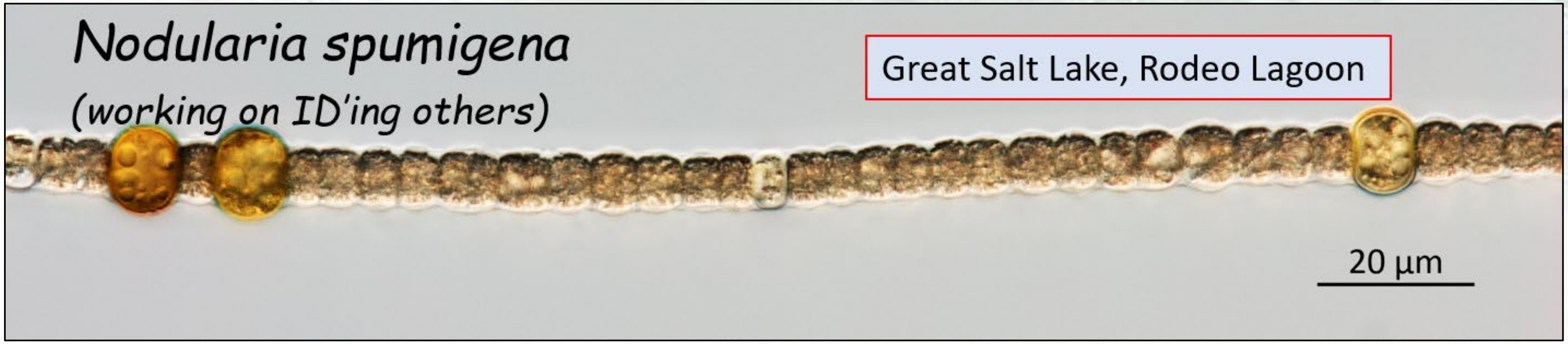
Andrew Humpage ISOC-HAB Ch. 16

Nodularin-R

why we as humans care

- Cyclic nonribosomal peptide
- Brackish; world-wide
- Hepatotoxin (same as microcystin LR)

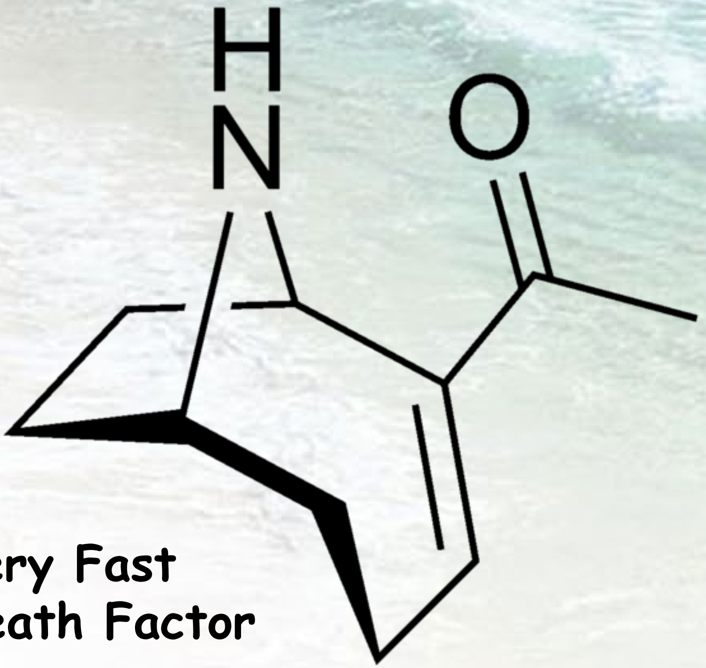




why we as humans care

Anatoxin-a

actylcholine agonist



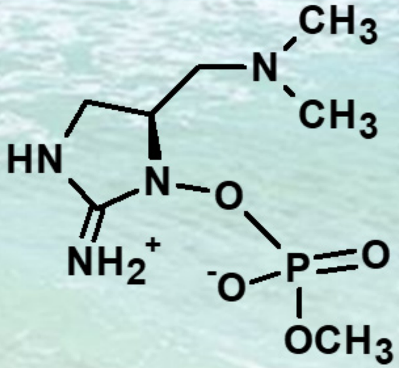
Very Fast
Death Factor

Dolichospermum flosaquae &
D. lemmermannii

Guanitoxin

formally called anatoxin-a(s) (2020)

acetylcholinesterase inhibitor

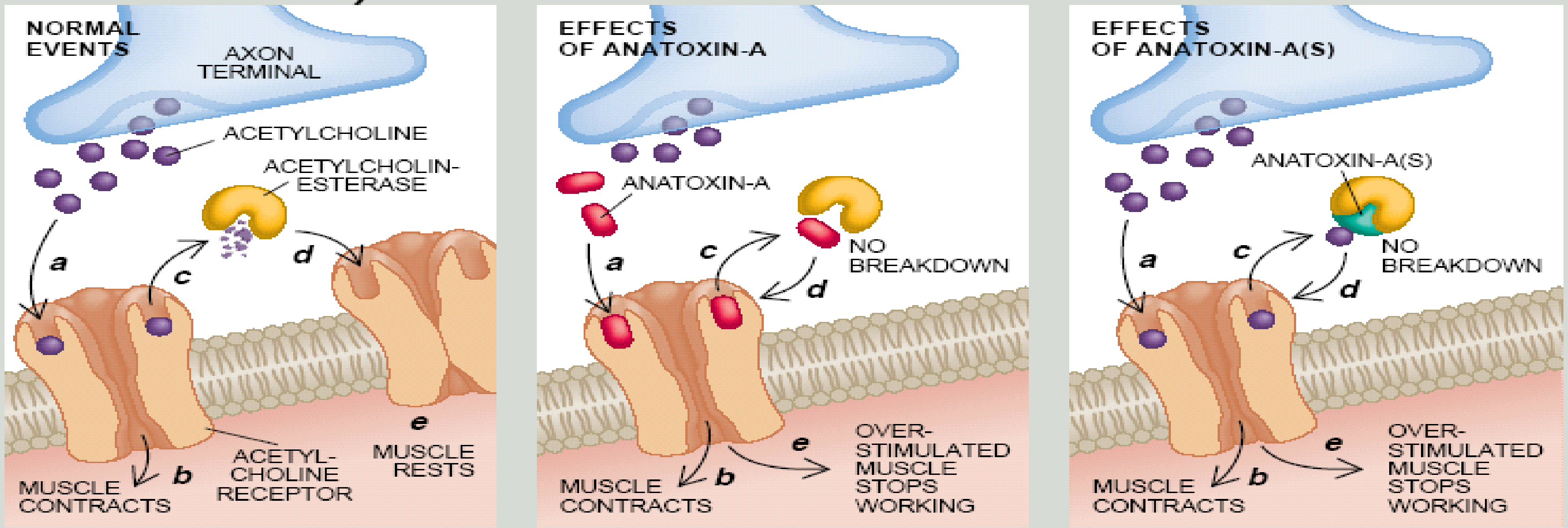


why we as humans care

Anatoxin-a and Guanitoxin

Anatoxin-a: Acetylcholine receptor agonist
Guanitoxin: Acetylcholinesterase inhibitor

Neurotoxicity



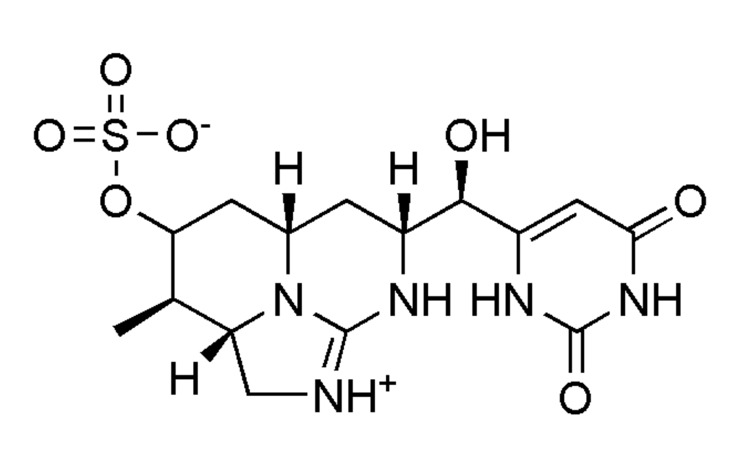
Anatoxin-a and anatoxin-a(s) (*center and right panels*) overexcite muscle cells by disrupting the functioning of the neurotransmitter acetylcholine. Normally, acetylcholine molecules (*purple*) bind to acetylcholine receptors on muscle cells (*a in left panel*), thereby inducing the cells to contract (*b*). Then the enzyme acetylcholinesterase (*yellow*) degrades acetylcholine (*c*), allowing its receptors and hence the muscle cells to return to their resting state (*d* and *e*). Anatoxin-a (*red in center panel*) is a mimic of acetylcholine. It, too, binds to acetylcholine receptors (*a*), triggering contraction (*b*), but it cannot be degraded by acetylcholinesterase (*c*). Consequently, it continues to act on muscle cells (*d*). The cells then become so exhausted from contracting that they stop operating (*e*). Anatoxin-a(s) (*green in right panel*) acts more indirectly. It allows acetylcholine to bind to its receptors and induce contraction as usual (*a* and *b*), but it blocks acetylcholinesterase from degrading acetylcholine (*c*). As a result, the neurotransmitter persists and overstimulates respiratory muscles (*d*), which once again eventually become too fatigued to operate (*e*).

Wayne Carmichael ISOC-HAB Ch. 4, Scientific American, January, 1994

why we as humans care

Cylindrospermopsin

Raphidiopsis



Alkaloid Toxin

- Gastrointestinal effects
- Hepatotoxicity
- Liver necrosis
- Kidney effects
- Inhibition of protein synthesis

- Covalently modify DNA and/or RNA
- Resistant to degradation by pH and temp-persistent

why we as humans care

Saxitoxins

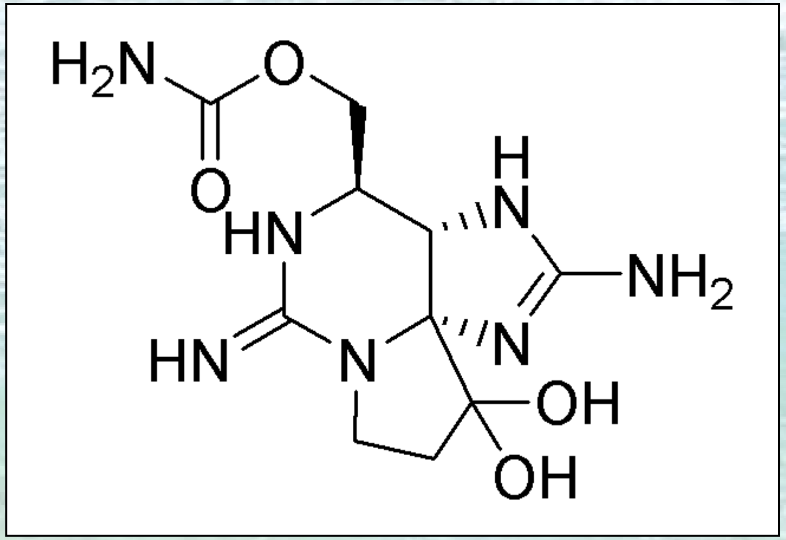
Dolichospermum

Aphanizomenon

Cylindrospermopsis

Lyngbya

Planktothrix



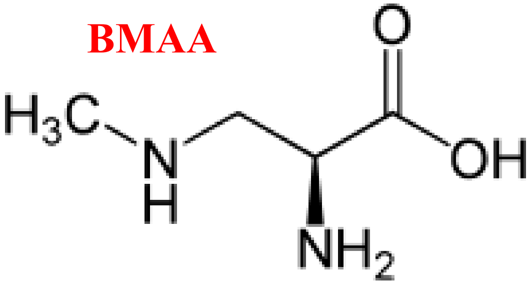
- Neurotoxin (voltage-gated sodium channels); respiratory failure
- Paralytic Shellfish Poisoning (PSP)
- Numerous marine dinoflagellates

why we as humans care

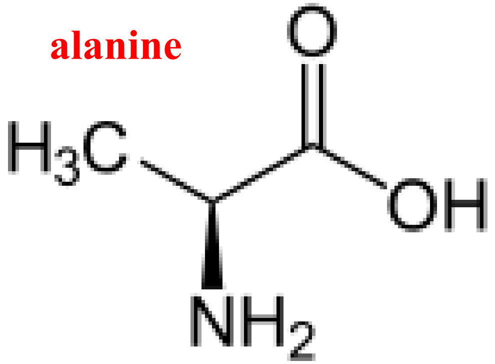
β-methyl amino alanine *(BMAA)* & 2,4-diaminobutyric acid *(DABA)*

- **Non-proteinogenic amino acid**
- **Made by almost all cyanobacteria**
- **DABA is a metabolite of BMAA**

BMAA



alanine



Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2013; Early Online: 1–9

informa healthcare

REVIEW ARTICLE

Is exposure to cyanobacteria an environmental risk factor for amyotrophic lateral sclerosis and other neurodegenerative diseases?

WALTER G. BRADLEY[1], AMY R. BORENSTEIN[2], LORENE M. NELSON[3], GEOFFREY A. CODD[4], BARRY H. ROSEN[5], ELIJAH W. STOMMEL[6] & PAUL ALAN COX[7]

why we as humans care

Some things we don’t know yet

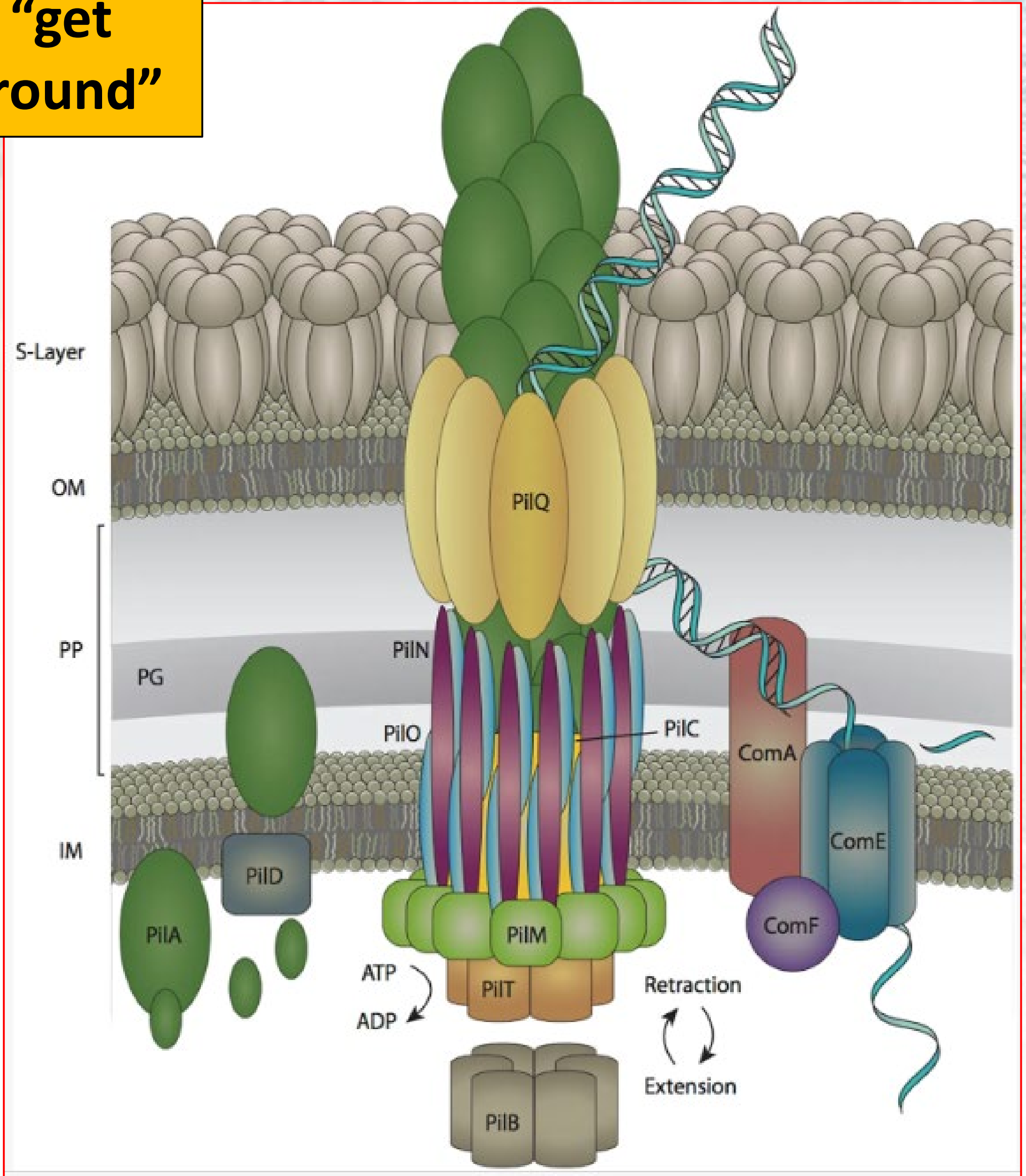
- **Environmental triggers for toxin production***
- **Reasons for high variability of impact on fish and invertebrates**
- **Actual degree of impact on humans**
- **Are more algae producing toxins, or are we just now detecting it?**

* Pound HL, Wilhelm SW (2020) Tracing the active genetic diversity of *Microcystis* and *Microcystis* phage through a temporal survey of Taihu. PLoS ONE 15(12): e0244482. https://doi.org/10.1371/journal.pone.0244482

1) “In a mixed population of *Microcystis flos-aquae* and *Microcystis wesenbergii* accounted for ~86% of total *Microcystis* transcripts, while the more commonly studied *Microcystis aeruginosa* only accounted for ~7%....
2) Study indicated that expression patterns were likely dictated by competition driven by environmental factors, not phylogeny
3) ...revealed 24 *Microcystis-specific* genotypes
4) ...pH best described the temporal shift in the Microcystis community genotypic composition, promoting hypotheses regarding carbon concentration mechanisms and oxidative stress.

how toxins "get around"

Genomics Approaches to Deciphering Natural Transformation in Cyanobacteria



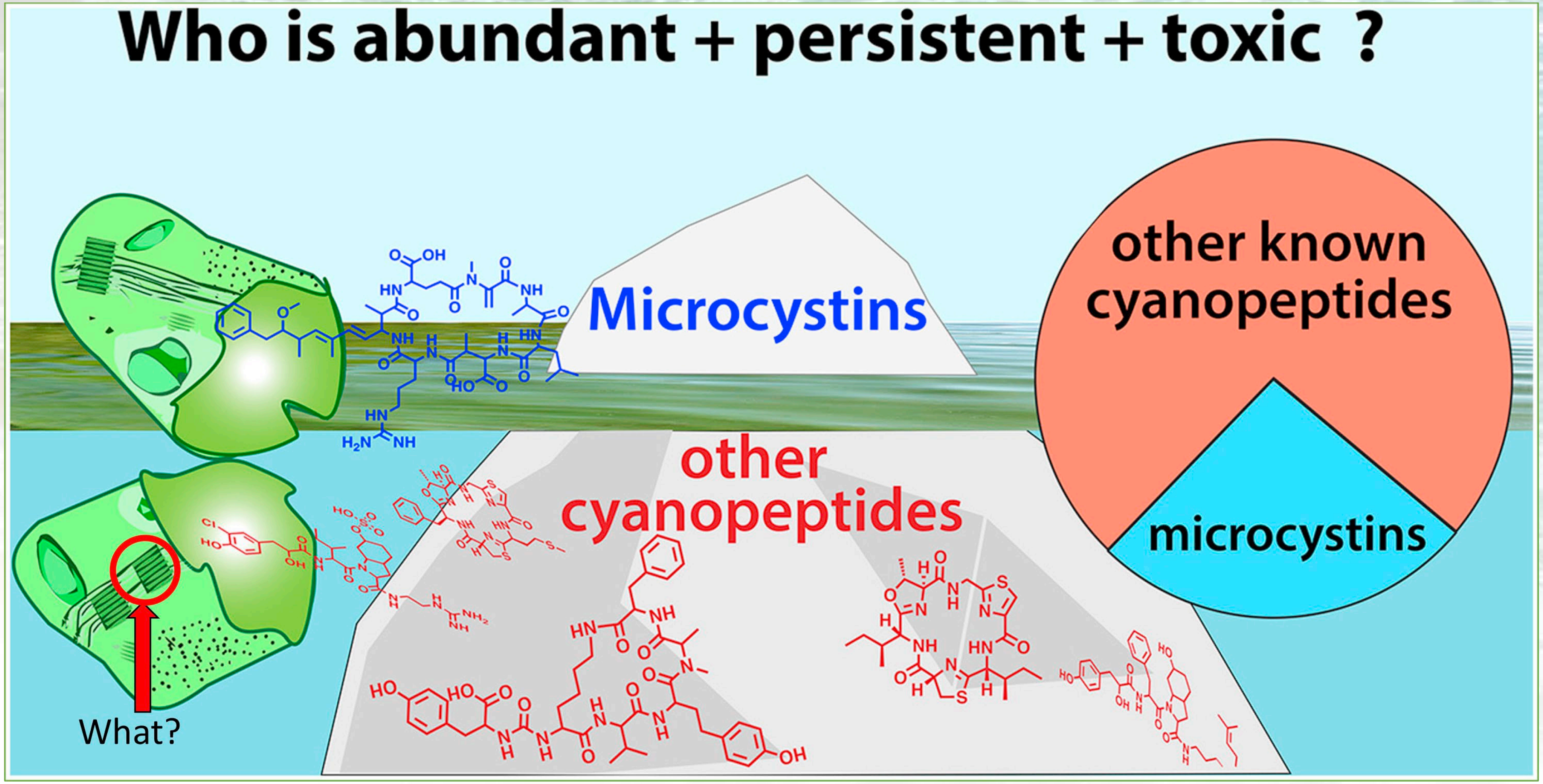
The DNA uptake apparatus in *Synechocystis* sp. PCC 6803. DNA enters the periplasm through an outer membrane pore composed of PilQ subunits. The double stranded DNA is then converted to single-stranded form prior to traversing the inner membrane pore, which is composed of ComE subunits. S-Layer is the surface layer, OM is the outer membrane, PP is the periplasm, PG is the peptidoglycan layer, and IM is the inner membrane.

aka, space invaders

https://doi.org/10.3389/fmicb.2019.01259

why we as humans care

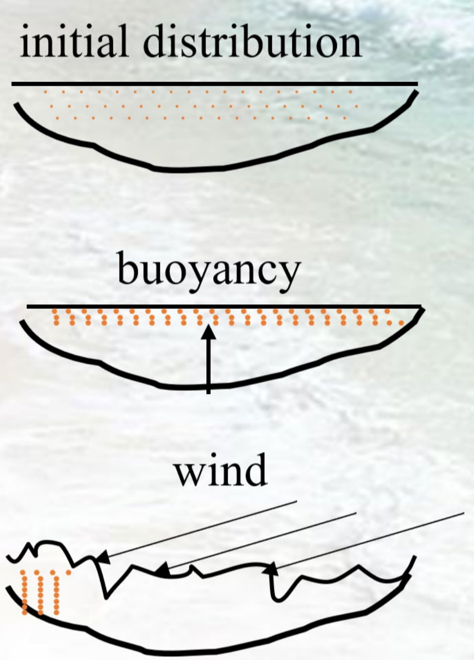
Cyanopeptides such as anabaenopeptides (600+)

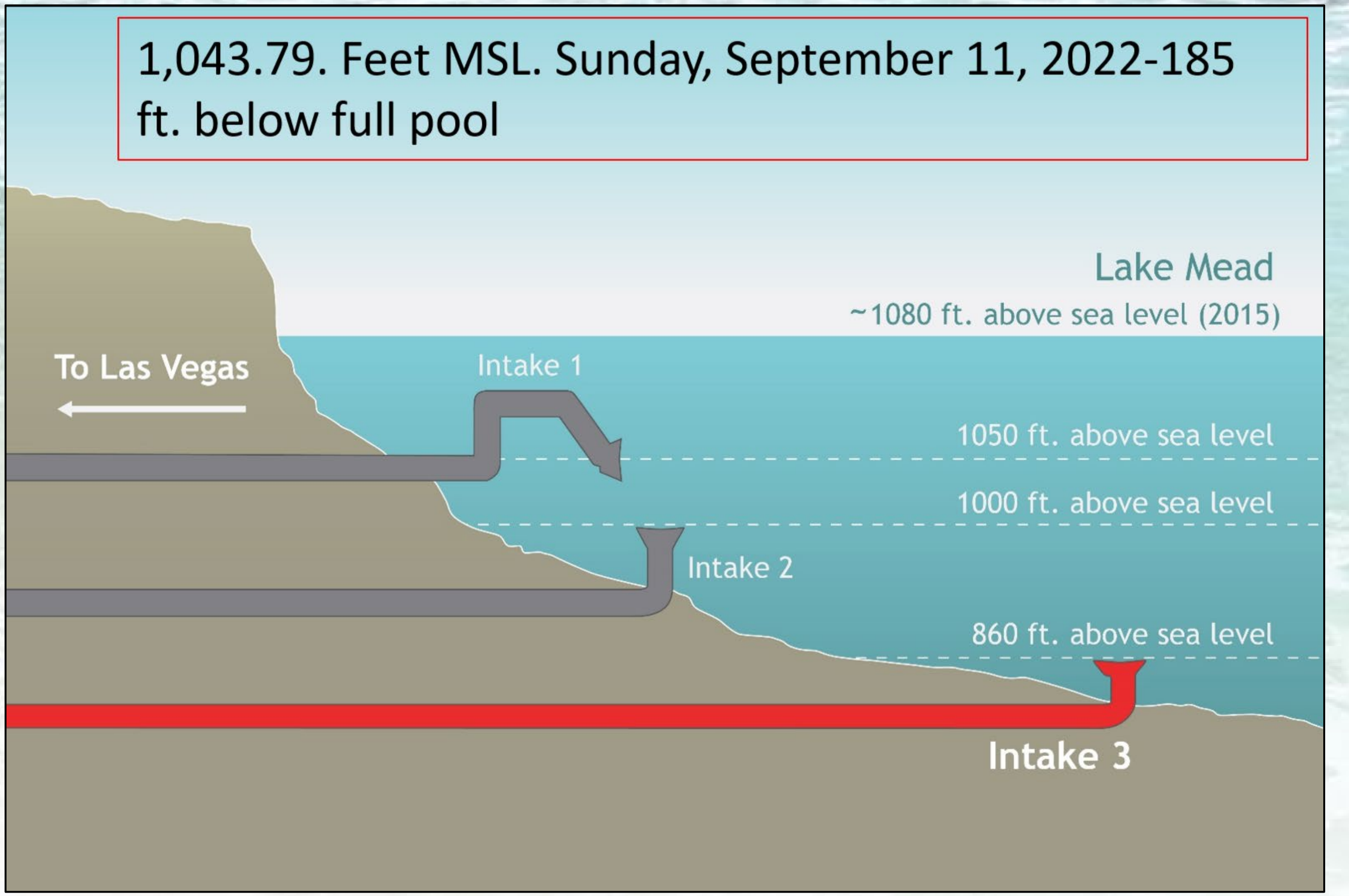


https://www.sciencedirect.com/science/article/pii/S0043135418310662#!

why we as humans care

Drinking water example

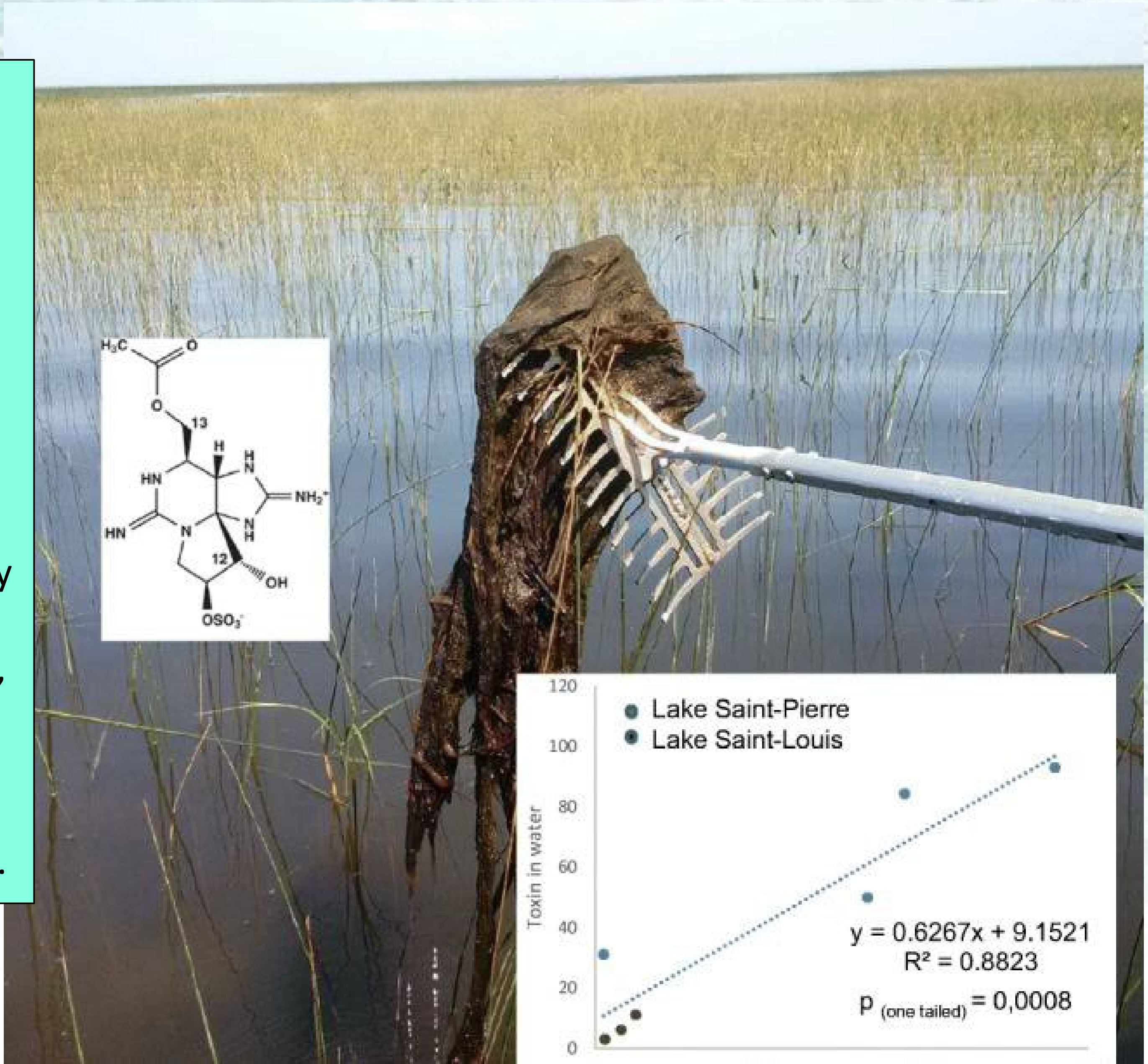




why we as humans care

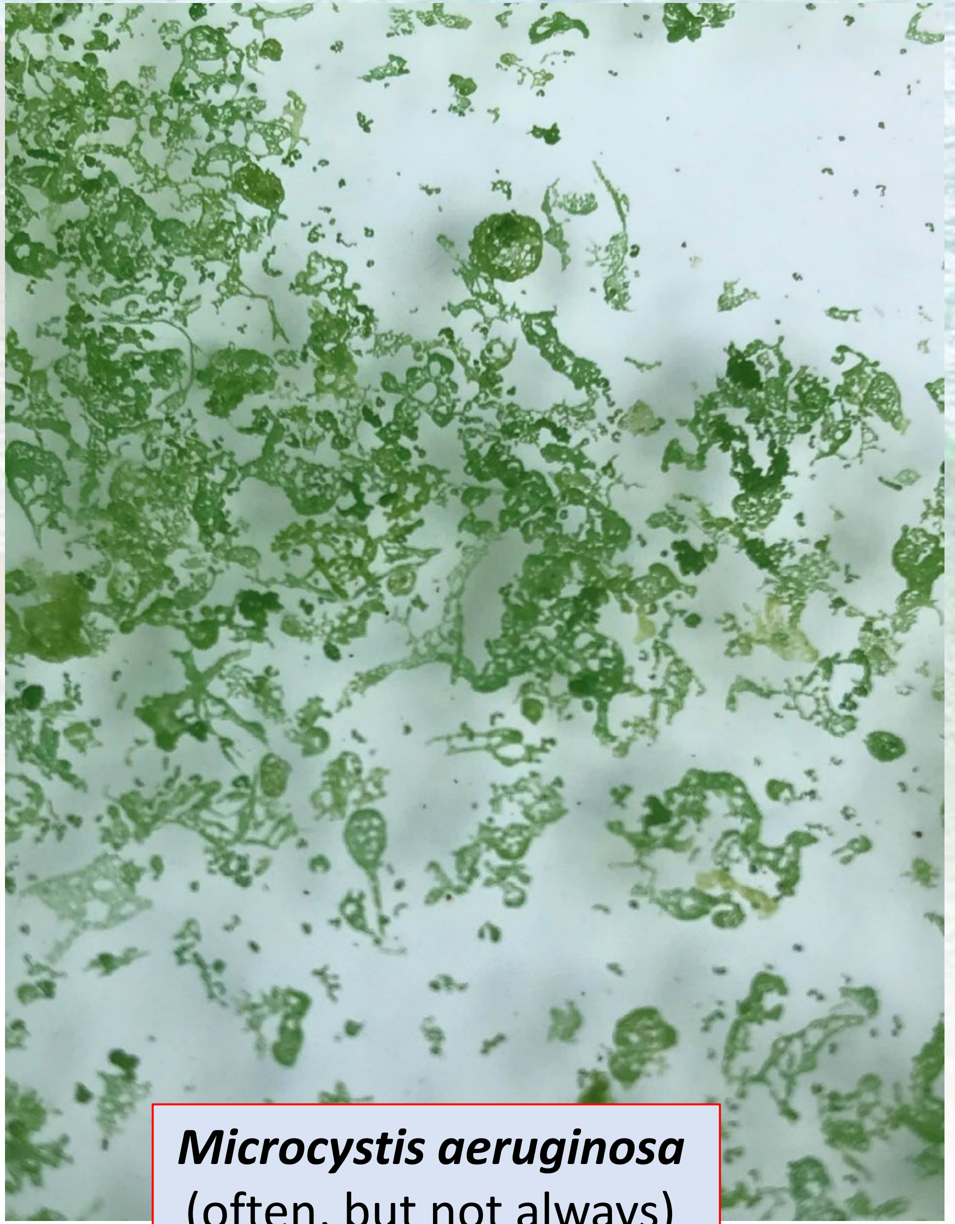
Microseira wollei

Paralytic shellfish poison (PSP) family as saxitoxin analogs (STX) such as mainly decarbamoylgonyautoxins 2 & 3 (dcGTX 2 & 3) and decarbamoylsaxitoxin (dcSTX), some other analogs, and *microseira*-specific LWTX 1–6 (Paerl and Huisman 2009). It may also produce cylindrospermopsin (CYN), deoxycylindrospermopsin (deoCYN), and some microcystins (O’Neil et al. 2012; Paerl et al. 2016).



who

Can not use visual cues to tell toxicity



Microcystis aeruginosa
(often, but not always)

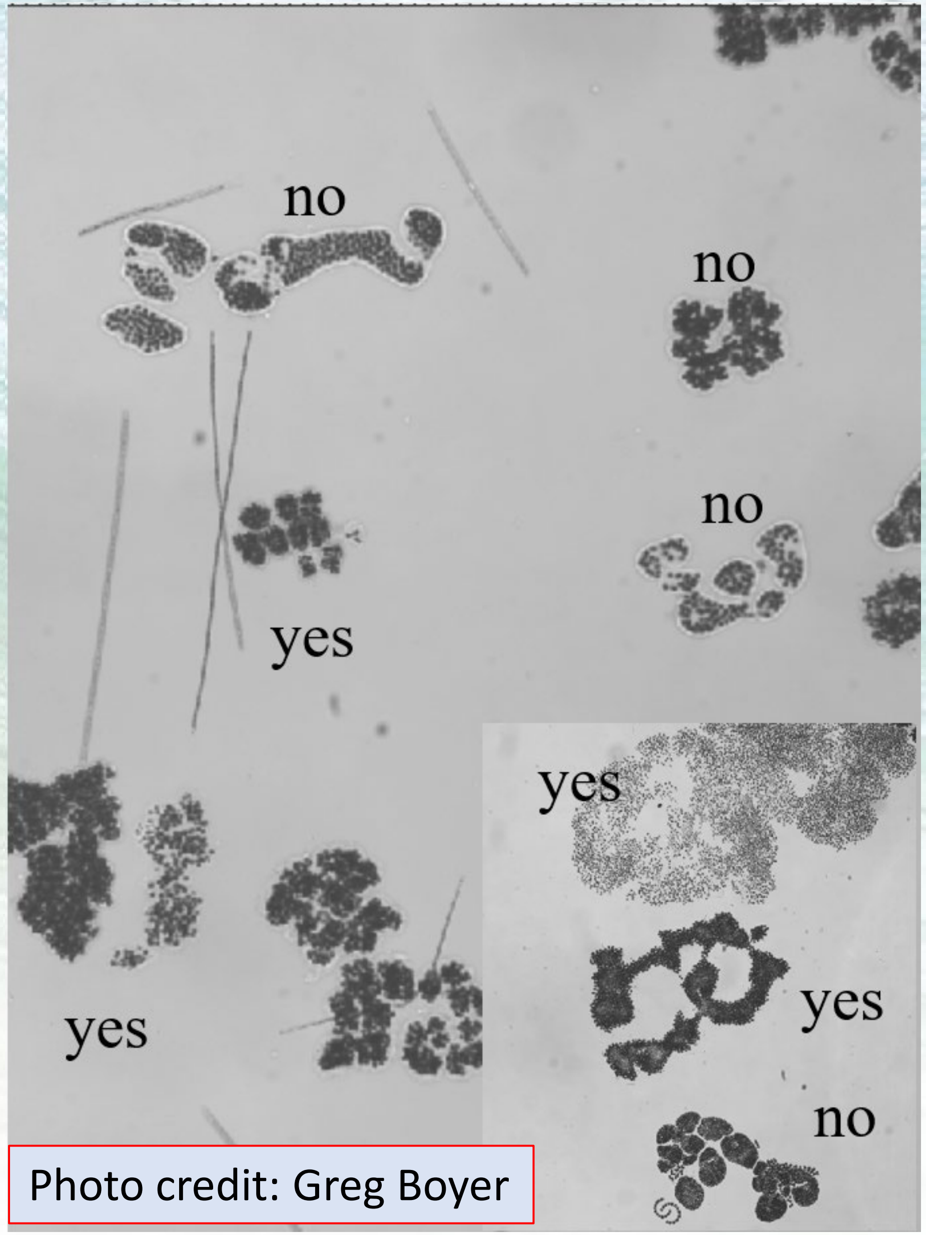


Photo credit: Greg Boyer

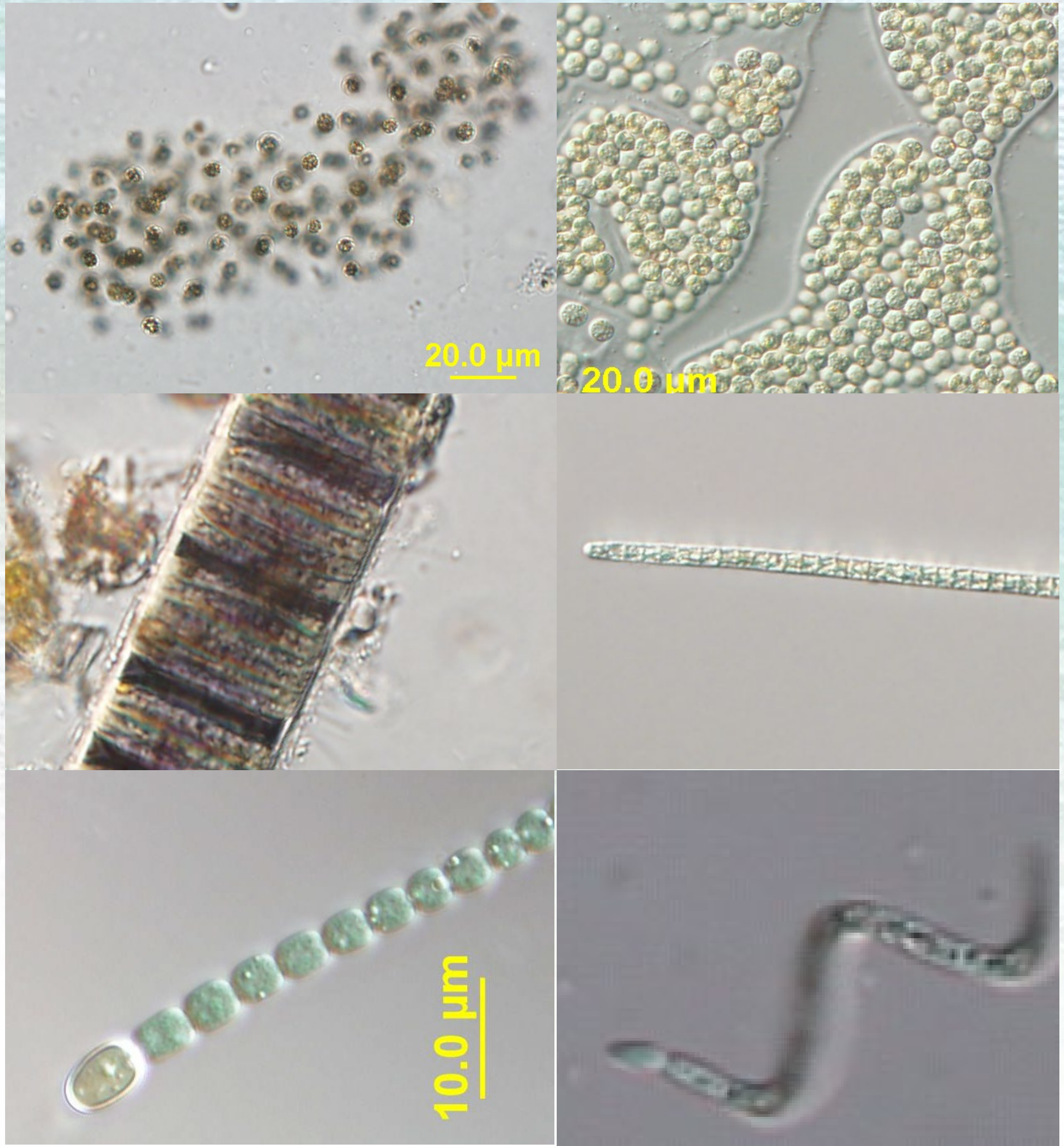
Key toxin-producing organisms: a phylogenetical diverse group

who

Unicellular forms
Microcystis

Filamentous
Lyngbya,
Oscillatoria
Planktothrix

Filamentous
Dolichospermum
Aphanizomenon
Raphidiopsis
Nodularia



How? No sextual reproduction: 1) fundamental to cyanobacteria metabolism since they evolved, 2) new genes acquired, such as the more recent origin of nodularin 3) some evidence lateral gene transfer?

how to observe and measure

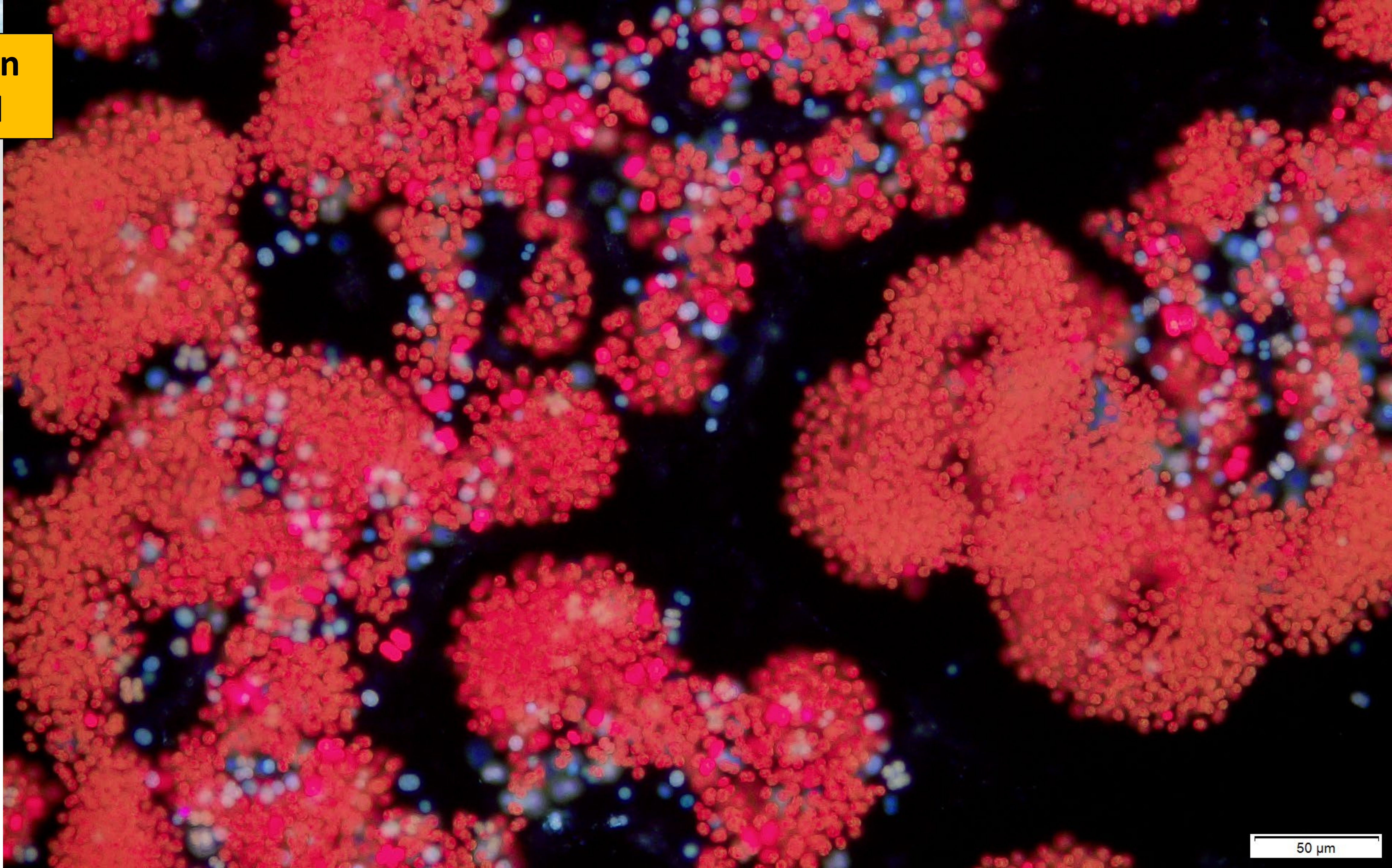
Cells must leak toxin to the water (naturally) or be forced to release toxin (for analytical techniques

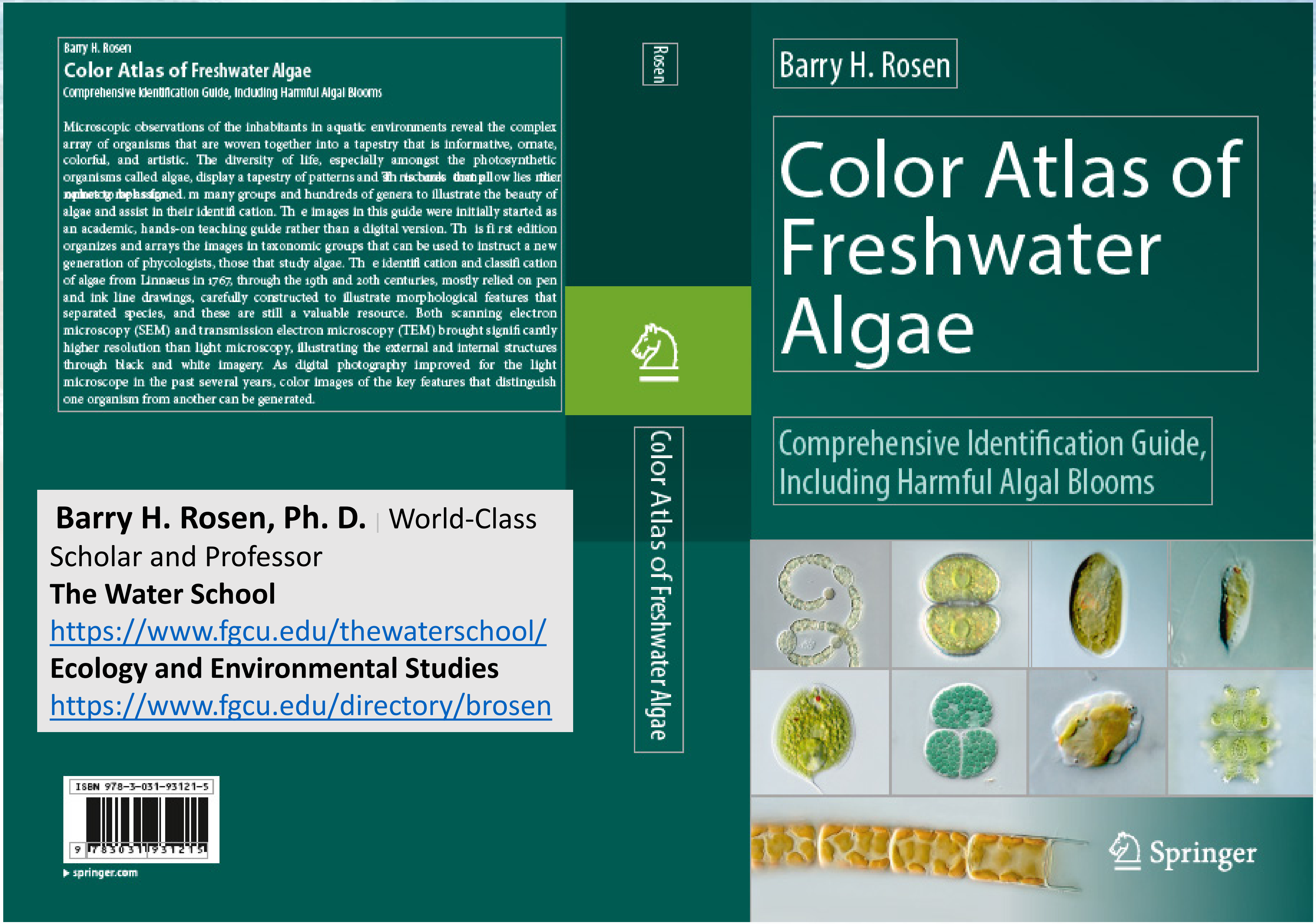
phycocyanin-covered shoreline

Physical or chemical lysis to release toxins:
Freeze-thaw, sonication, bead beating, etc.

What is in the cells, how much has leaked to the water, total toxin per liter?

keep in mind





Barry H. Rosen, Ph. D. | World-Class Scholar and Professor

The Water School

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Thank You!