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Advancing Environmental Solutions

Strategies for Preventing and Managing ***Benthic*** Harmful Cyanobacterial Blooms (HCB-2)

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Meet The Trainers

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Ben Holcomb
Utah Department of Environmental Quality

Advancing Environmental Solutions

★ Introduction to Benthic Cyanobacteria

Cyanotoxins

Monitoring

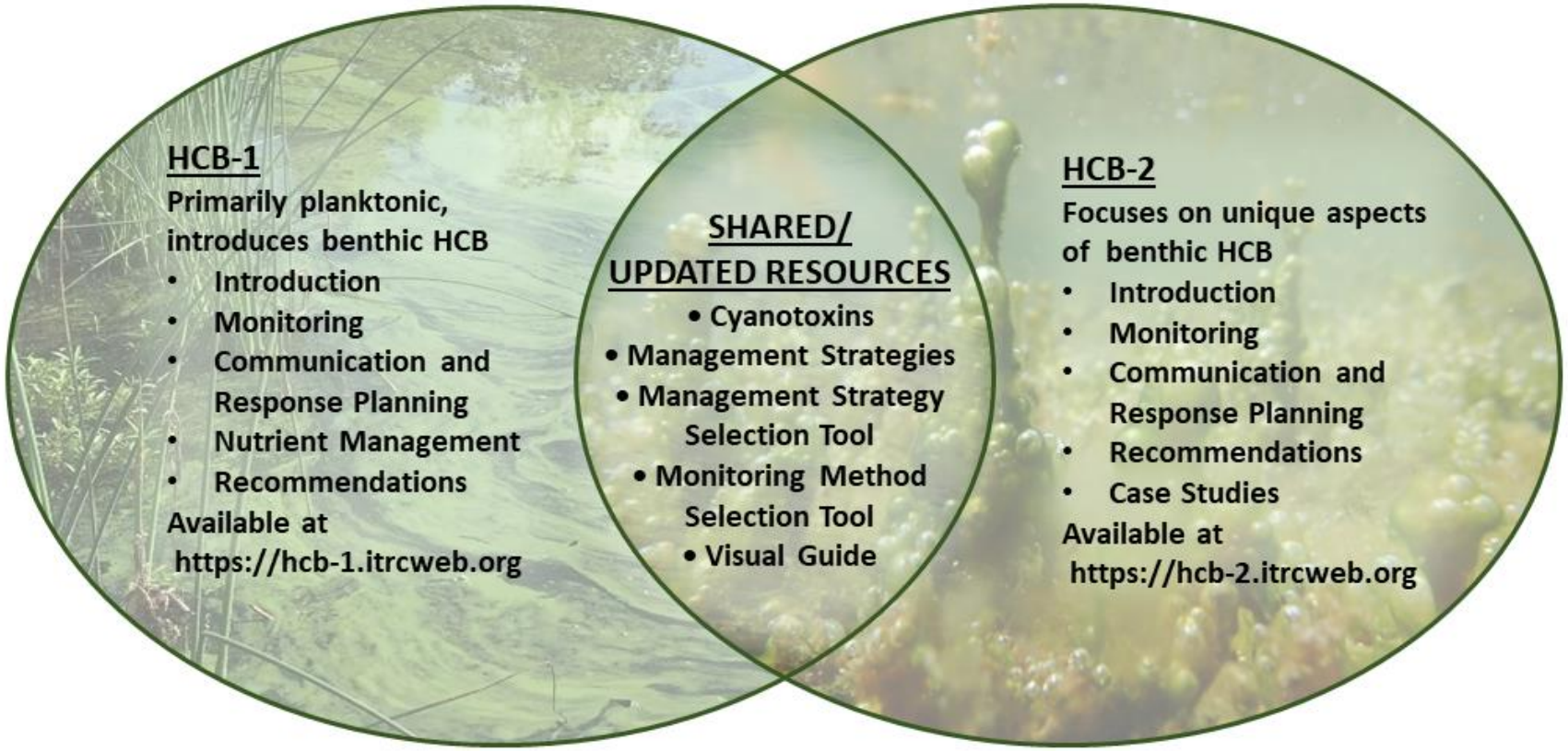
Management

Communication

See Section 1 of the HCB-2 Guidance Document

Why Separate Information For Benthic HCBs?

Framework of HCB Guidance Documents



HCB Introduction

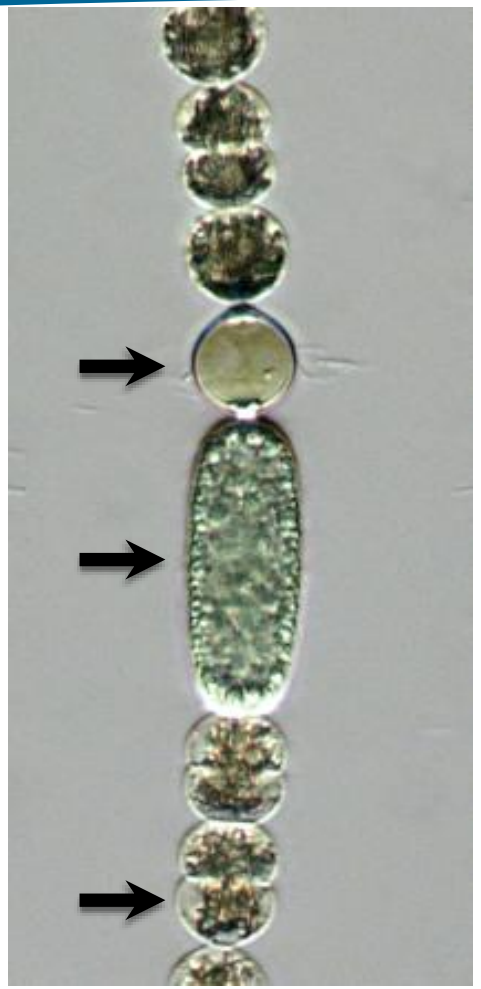
- Benthic vs. planktonic HCBs
  - Visual Identification
  - Ecology
  - Habitats

Source: Robyn Henderek

See Section 1 of the Benthic HCB Guidance Document

Cyanobacteria Basics

- Naturally occurring in both aquatic and terrestrial environments
- Beneficial roles
  - Acting as food source
  - Producing oxygen via photosynthesis
  - Some types also fix nitrogen
- Unique characteristics
  - Resting/dormant stages
  - Buoyancy control in planktonic species



Source: Gina LaLiberte

What Do They Look Like And How Can I Tell Them Apart?

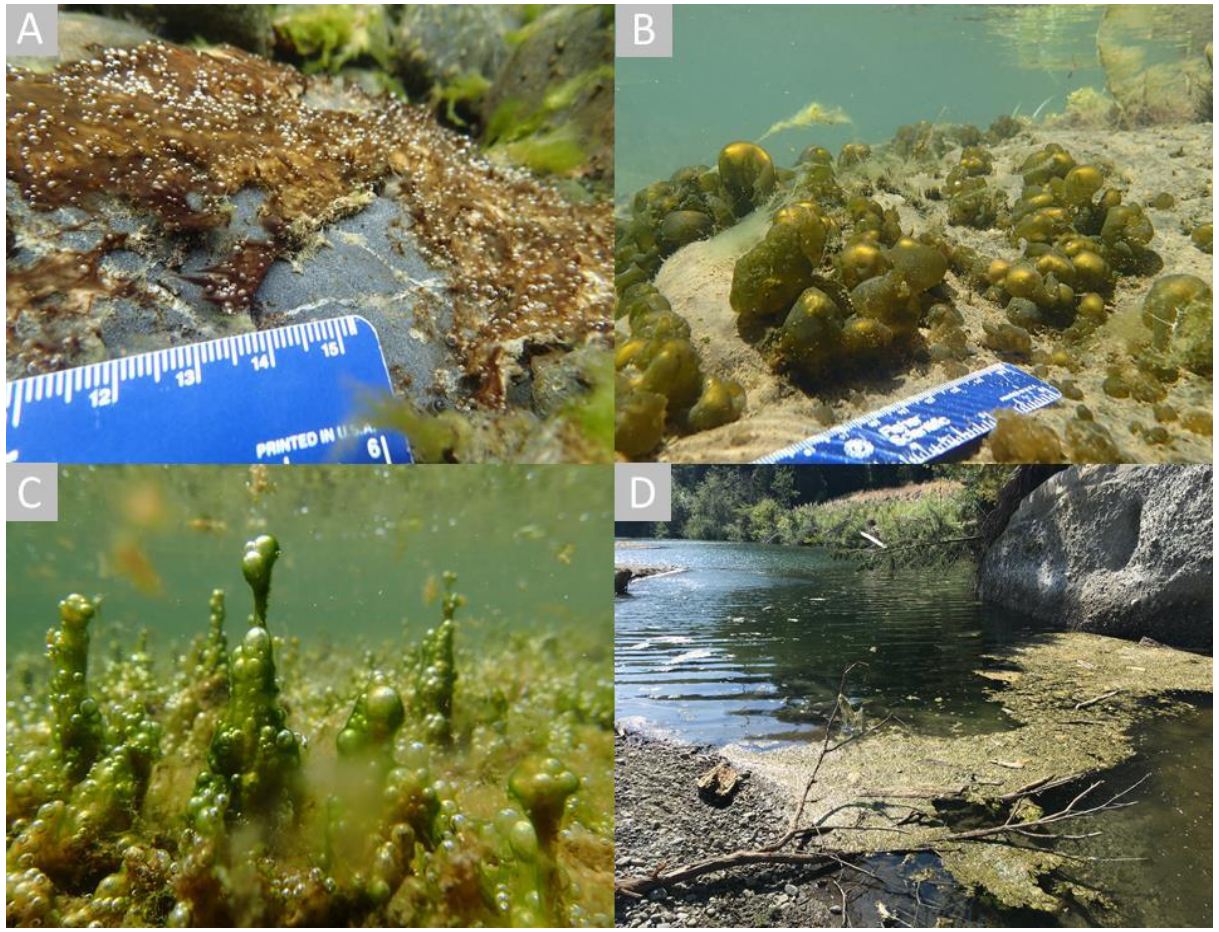
- New Visual Guide & Video!
  - Field photos, microscopic images
  - Cyanobacteria assemblages & taxa by:
    - Form (colonial or filamentous)
    - Planktonic and benthic habitats
  - And more! Non-cyanobacterial examples – aquatic plants, filamentous algae, other algae for comparison

Source: Robyn Henderek

Source: Elizabeth Fabri Smith

Benthic HCB Appearance

- Attached or loosely associated mats or colonies
  - Grow on bottom sediments or other substrates
  - Tend to be filamentous species
  - Many species are motile
- May be darker colors – black or brown

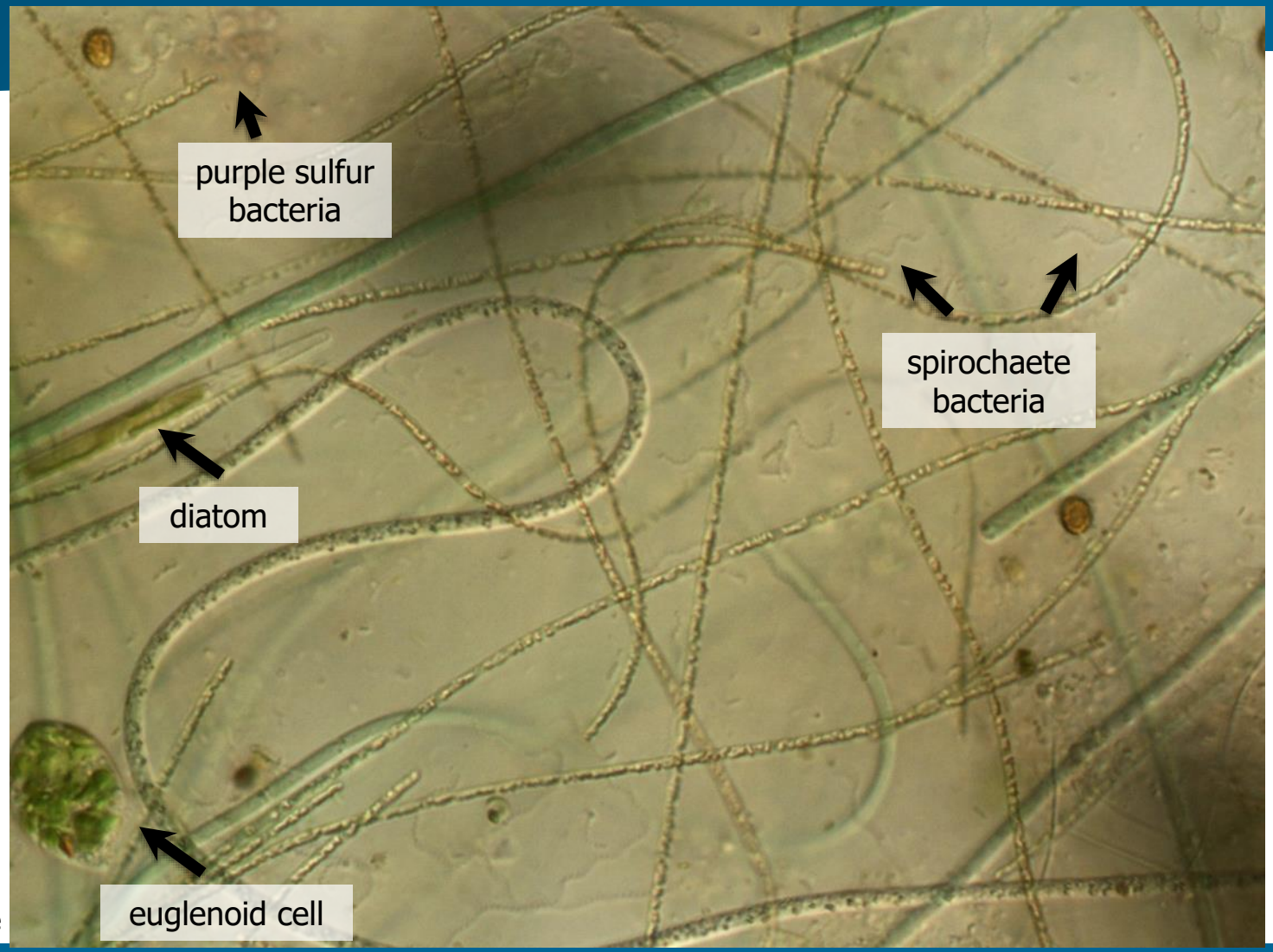


Source: Keith Bouma-Gregson

See Appendix A Visual Guide to Common Harmful Cyanobacteria and Learn to Identify Cyanobacteria Blooms video

Benthic HCB Communities

- Mat communities may be uniform or contain diverse microbial assemblage including multiple cyanobacterial species
  - Other bacteria common
  - Diverse algae & protists



Source: Gina LaLiberte

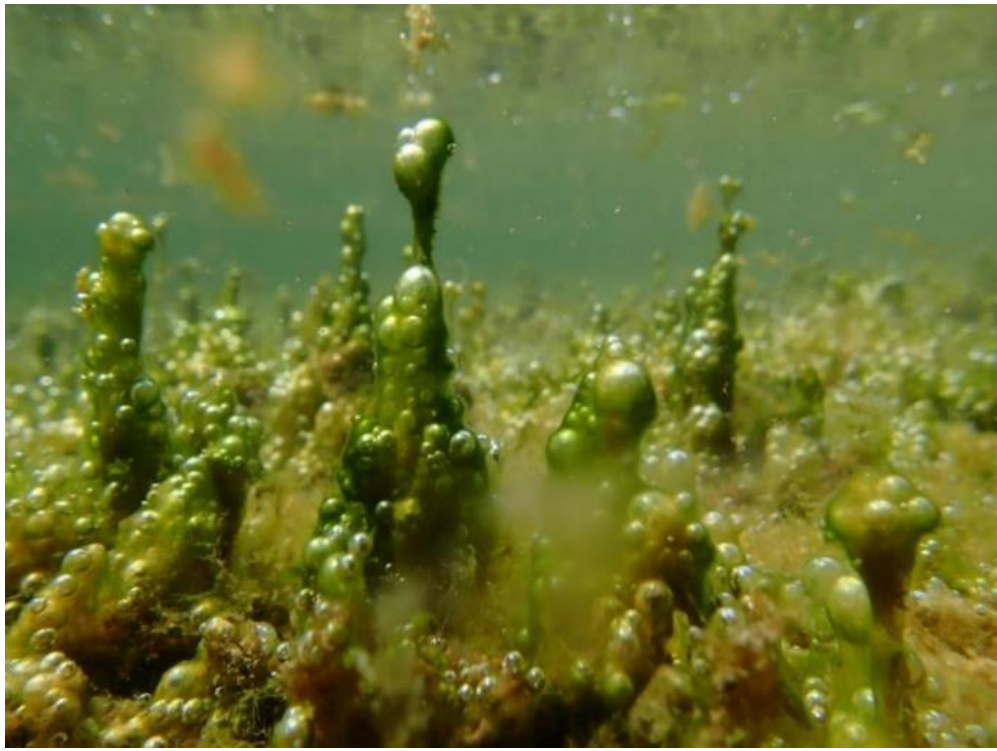
Benthic HCBs And Light

- Require light for photosynthesis
  - Clear water
  - Adaptations for low light at greater depths
- Compare to planktonic HCBs growing in turbid water

Source: Dr. Christen Thieman

Benthic HCBs And Nutrients

- Often grow in low nutrient waters
  - Take up nutrients from sediments, macrophytes, etc.
  - Benthic species tend to be those with lower nutrient preferences
- Compare to high nutrient water bodies which support the proliferation of planktonic HCBs



Source: Keith Bouma-Gregson

Benthic HCBs And Habitat

- In streams and rivers
  - On sediments in pools – slow water
  - On rocks in riffles – fast water
  - Compare to planktonic bloom development in stagnant water

- In lakes and reservoirs
  - Protected or deep areas

Source: Kurt Carpenter

Source: Scott Caven

Benthic HCBs And Dispersion In Water Bodies

- Role of photosynthetic oxygen production
  - High photosynthesis rates in clear water
  - O_2 bubbles accumulate in mat
  - Mats lift off substrate

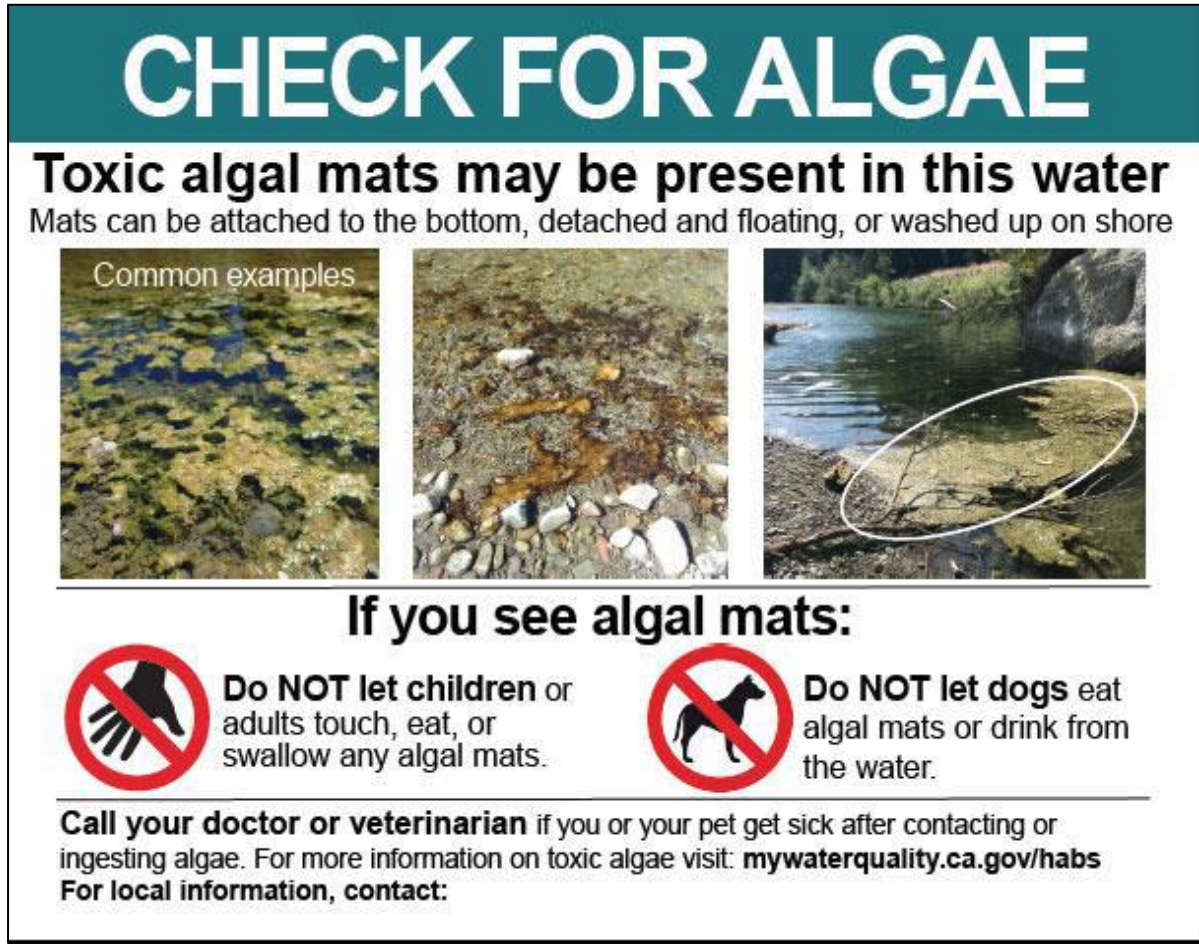
Source:
Keith Bouma-Gregson

- Role of disturbance
  - Hydrodynamic forces – drag and turbulence
  - Mechanical disturbance – wind, waves, boat wakes, wading

Source: Ann St. Amand

Benthic HCB Risk Communication & Identification Challenges For The Public

- Not the "blue-green algae" they are familiar with
- Appearance
  - Mats & colonies vs. particles & scums
  - Muted dark colors vs. vivid colors
  - Lower apparent density
- Habitat
  - Flowing water
  - Clear, low-nutrient water



Source: CA Cyanobacteria and Harmful Algal Bloom Network

Toxins And Risk Assessment

- May produce toxins
  - Mats present a potentially higher ingestion risk
  - High cell densities & high toxin concentrations
- Risks higher for animals
  - More likely to intentionally consume mats

Source: Gina LaLiberte

Advancing Environmental Solutions

Introduction to Benthic Cyanobacteria

★ **Cyanotoxins**

Monitoring

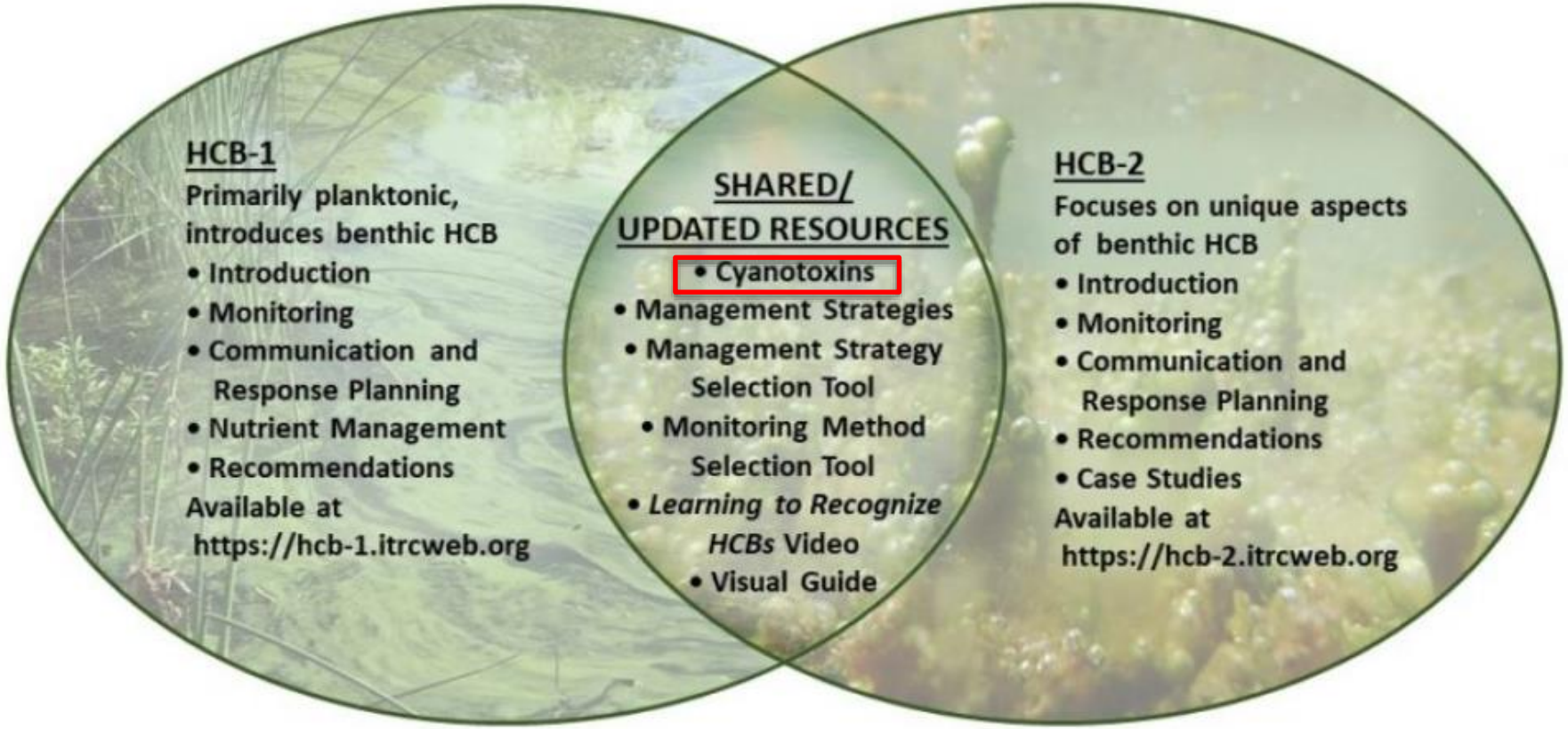
Management

Communication

See Section 2 of the HCB-2 Guidance Document

Cyanotoxins

- Cyanotoxins and irritants produced by planktonic and benthic cyanobacteria



See Section 2 of the HCB-2 Guidance Document

Cyanotoxins

- Cyanotoxins and irritants produced by planktonic and benthic cyanobacteria
- Exposure routes and health impacts to humans and animals
- Cyanotoxin distribution, stability, and exposure considerations
- Classes of cyanotoxins
- Cyanotoxins thresholds for humans & domestic animals

How Can Humans Be Exposed?

- In or around affected water bodies:
  - Accidentally swallowing affected water
  - Breathing in aerosols in water spray or mist
  - Direct water contact with skin
- Ingestion of contaminated drinking water, food, or supplements

Source: CDC website

How Can Animals Be Exposed?

- Animals are especially at risk because of:
  - Higher exposure while drinking and swimming in affected waters
  - Lack avoidance behavior of blooms and mat material
  - Feeding directly on cyanobacteria or other prey (shellfish, fish, macroinvertebrates) containing cyanotoxins (bioaccumulation)
  - Incidental ingestion from grooming cyanobacteria that has accumulated on their fur/feathers

Source: Andrew Chapman

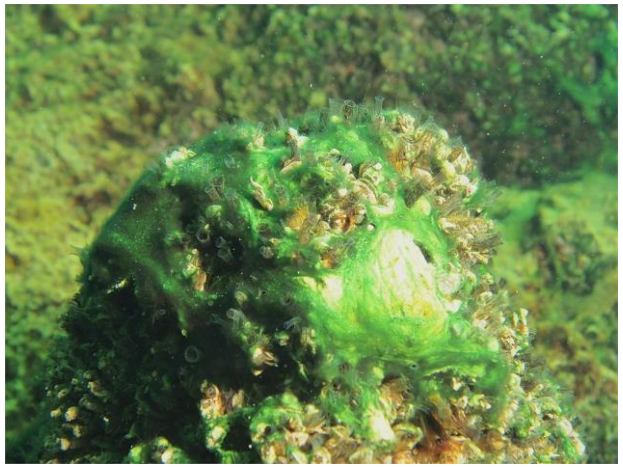
Source: HCB-2 Guidance Document

What Are The Health Effects Of Cyanotoxins?

- Cyanobacteria can produce cyanotoxins and other irritants that cause serious health effects in people and animals:
  - Liver (hepatotoxin)
  - Nervous system (neurotoxin)
  - Skin and mucous membranes (dermatoxin)
  - General irritation/allergic reaction
- Commonly reported symptoms include gastrointestinal, lethargy, skin irritation
- Documented cases of animal illness and death, particularly for dogs, due to cyanotoxins
- Bioaccumulation of cyanotoxins in aquatic food web

Cyanotoxin Distribution And Stability

- Toxin production varies over time and space
- Multiple toxin types may be produced by single species and not all cyanobacteria capable are actively producing toxins



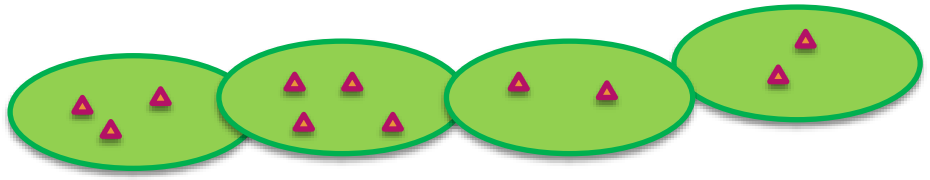
Source: Kelly Lorenz

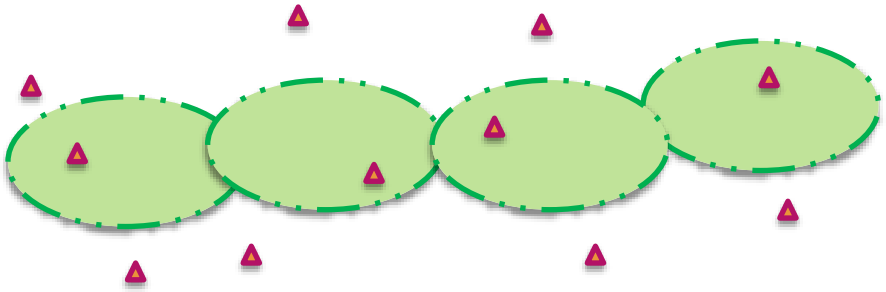
Source: Keith Bouma-Gregson

Source: Robyn Henderek

Cyanotoxin Distribution And Stability

- Toxins generally held within the cyanobacterial cell (**intracellular**) except for cylindrospermopsin
- Toxins are released to water (**extracellular, dissolved**) as the cell dies/lyses
  - When the bloom naturally decays
  - When a chemical treatment is applied
  - When cells are ingested
- Degradation rates range from days to months for extracellular cyanotoxins
- Intracellular cyanotoxins can persist while cell is intact





Classes Of Cyanotoxins

Hepatotoxins

- **Microcystins and Nodularins**
- **Cylindrospermopsins**

Other classes

Dermatoxins and skin-irritating compounds

Secondary metabolites

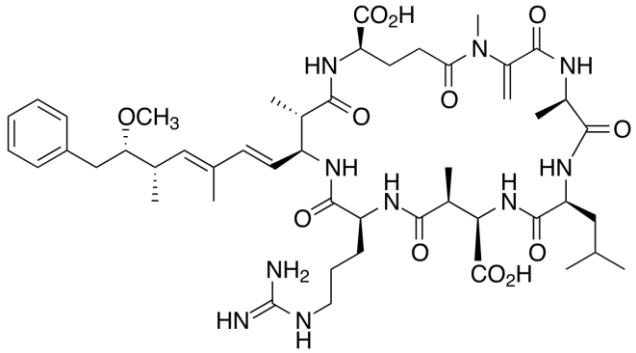
Neurotoxins

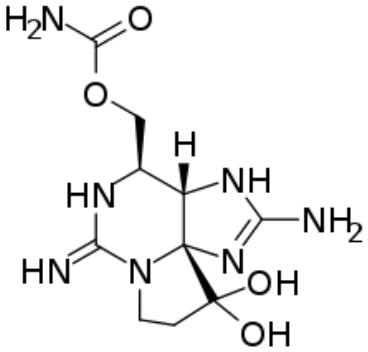
- **Saxitoxins**
- **Anatoxins**

Guanitoxin

BMAA

Aetokthonotoxin





- Cyanotoxin with published threshold values

Cyanotoxin Thresholds

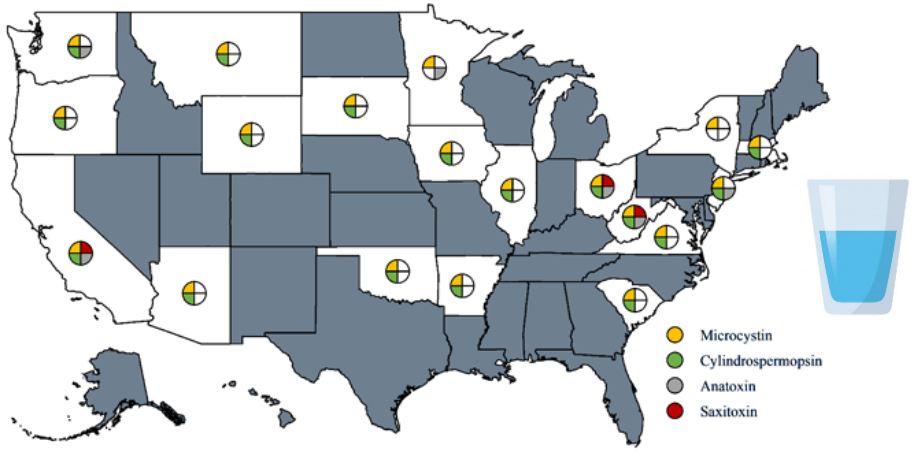
- Concept of level with no adverse health effects during typical exposure scenario
- Concentration of cyanotoxin per volume or biomass (µg/L; ng/g)
- Components of threshold equation:
  - Reference dose (toxicity)
  - Uncertainty factors
  - Exposure assumptions
- Acute, short-term (10-day), lifetime

Source: HCB-2 Guidance Document

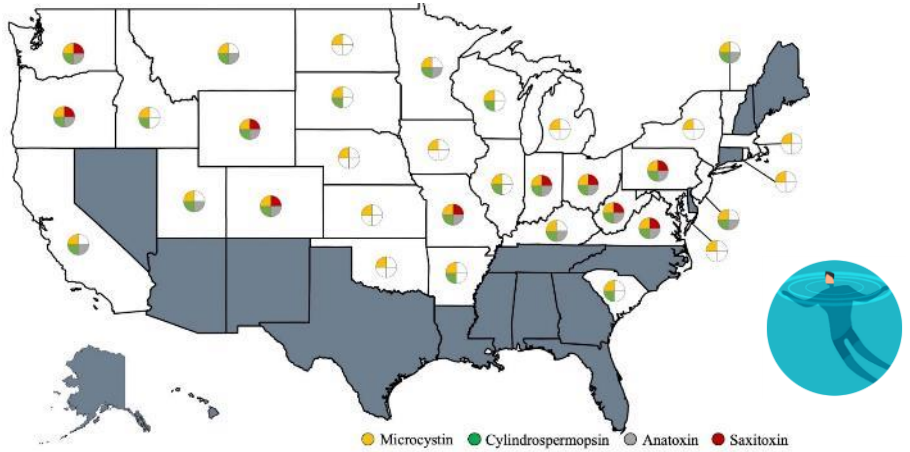
See Section 2.5 of the HCB-2 Guidance Document

Regulations And Guidance

- Currently no federal standards (e.g., MCL) for cyanobacteria or cyanotoxins
- USEPA published threshold values for two cyanotoxins (microcystin and cylindrospermopsin) in recreational and drinking water
- Many states have adopted USEPA thresholds and/or developed their own regulatory or guidance values for cyanotoxins
- WHO developed guidance for microcystin-LR, anatoxin-a, cylindrospermopsin, and saxitoxins



States with Drinking Water Threshold Values, Source: Tracy Lund



States with Recreational Water Threshold Values
Source: Adapted from *Mehinto et al. (2021)*

Source: CDC website

See Section 2.6 of the HCB-2 Guidance Document

Cyanotoxin Thresholds In Mat Material

- No thresholds for human consumption of mat material
- Integration of human recreational water and mat thresholds
  - Case study at Zion National Park
  - Modify sampling protocol "benthic disturbance" to represent worst-case for water sample
  - Passive sampling for presence of extracellular cyanotoxins
  - Tiered advisory based on cyanotoxin concentrations and other data
- CA has mat-based thresholds for dogs and cattle based on dry weight concentration

Source: Kelly Lorenz

Advancing Environmental Solutions

Introduction to Benthic Cyanobacteria

Cyanotoxins

★ **Monitoring**

Management

Communication

See Section 3 of the HCB-2 Guidance Document

Monitoring: Goals

- Benthic vs. Planktonic Monitoring
- Method Selection Tool
- Benthic Cyanobacteria Monitoring Program vs. Response Plan

Today's training will provide the basic understanding of how to monitor for benthic HCBs

Monitoring For Benthic Compared To Planktonic HCBs

- First step of monitoring for planktonic blooms = visual surface assessment
- Need specialized equipment for visual assessment for benthic HCBs (snorkel, scuba, and bathyscopes)

Planktonic Bloom
Source: Midge Elissan

Benthic Bloom
Source: Lower Colorado River Authority

Monitoring For Benthic Compared To Planktonic HCBs

- Sampling surface water does not characterize a benthic HCB
- Benthic HCB can grow on the bottom attached to sediment or other surfaces
- Need specialized equipment for sample collection (ponar dredge, rake)

Bathyscope

Source: J. Beskenis

Ponar Dredge

Source: Pat Braaten, USGS.

Monitoring For Benthic Compared To Planktonic HCBs

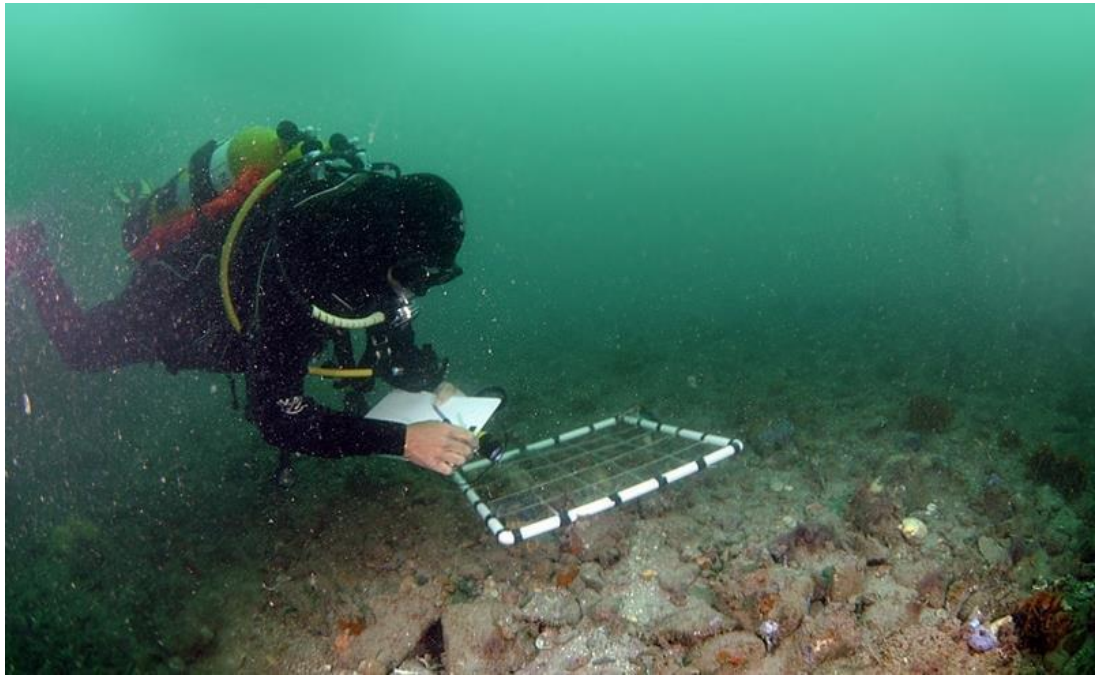
- Water/foam vs. mat/periphyton sample
- Specialized sampling such as artificial substrate or composite sample
- Different types of cyanotoxins may be present
- Underlying methods to analyze cyanotoxins remain the same (LC/MS/MS, ELISA)

Threshold values for planktonic water column samples ≠ threshold values for benthic mat samples

Source: Celeste Journey

Percent Cover As A Threshold Value

- Visual assessment to evaluate percent cover or mat extent of benthic HCBs
  - New Zealand uses Alert Level Framework
  - 20% coverage = surveillance mode
  - 20% – 50% = alert mode
  - 50% > = action mode



Source: James et al. 2016

Building A Monitoring Plan

- What is the overall goal of your monitoring plan?

  Public safety? Bloom trends? Management?

- What are you interested in monitoring for?

  Cyanotoxins? Cyanobacteria cells per m/L?

- Consider the sustainability

  Cost and training associate with monitoring

Source: Lower Colorado River Authority

Select your monitoring requirements:

Target Analyte	Lab Required	Turnaround Time
☑ Planktonic Cyanobacteria	☑ Yes	☑ Less than 24 hours
☑ Benthic Cyanobacteria	☑ No	☑ 1 to 3 days
☑ Cyanotoxin		

Symbols		Abbreviations	
🟢	Suitable	P/A	Presence/absence
🟡	Potential	ID	Identification
🔴	Not suitable	DEN/AB	Density/abundance
		CGN	Congener-specific concetrations
		TOT	Total cyanotoxin concetrations

Method	Cyanobacteria			Cyanotoxin			Result Type	Sample Type	Relative Cost	Level of Training
	P/A	ID	DEN/AB	P/A	CGN	TOT				
Visual Assessments - planktonic	🟢	🔴	🔴	🔴	🔴	🔴	Qualitative	Variable	$	Novice
Visual Assessments - benthic	🟢	🔴	🟡	🔴	🔴	🔴	Qualitative	Variable	$-$$	Novice to Expert
Jar and Stick Tests - planktonic	🟢	🔴	🔴	🔴	🔴	🔴	Qualitative	Point sampling	$	Novice
Pigments - planktonic	🟢	🔴	🟢	🔴	🔴	🔴	Quantitative	Point sampling	$$	Intermediate
Pigments - benthic	🟢	🔴	🟢	🔴	🔴	🔴	Quant./Qual.	Point sampling	$$	Intermediate

See https://hcb-2.itrcweb.org/monitoring-tool/

Select your monitoring requirements:

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☐ Planktonic Cyanobacteria	☑ Yes	☑ Less than 24 hours
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Method	Cyanobacteria			Cyanotoxin			Result Type	Sample Type	Relative Cost	Level of Training
	P/A	ID	DEN/AB	P/A	CGN	TOT				
Visual Assessments - benthic	🟢	🔴	🟡	🔴	🔴	🔴	Qualitative	Variable	$-$$	Novice to Expert
Pigments - benthic	🟢	🔴	🟢	🔴	🔴	🔴	Quant./Qual.	Point sampling	$$	Intermediate
Remote Sensing - benthic	🟡	🔴	🔴	🔴	🔴	🔴	Quant./Qual.	Indirect	$	Intermediate / Expert
Microscopy - benthic	🟢	🟡	🟢	🔴	🔴	🔴	Quant./Qual.	Point sampling	$$	Intermediate / Expert
Genetic Methods for Identification - benthic	🟢	🟢	🟡	🔴	🔴	🔴	Quantitative	Point	$$	Intermediate

See https://hcb-2.itrcweb.org/monitoring-tool/

Target Analyte	Lab Required	Turnaround Time
☐ Planktonic Cyanobacteria	☑ Yes	☑ Less than 24 hours
☐ Benthic Cyanobacteria	☑ No	☑ 1 to 3 days
☑ Cyanotoxin		

Method	Cyanobacteria			Cyanotoxin			Result Type	Sample Type	Relative Cost	Level of Training
	P/A	ID	DEN/AB	P/A	CGN	TOT				
Strip Tests / Dip Sticks	🔴	🔴	🔴	🟢	🔴	🟢	Semi-Quant.	Point sampling	$$	Novice
Protein Phosphatase Inhibition Assay (PPIA)	🔴	🔴	🔴	🟢	🔴	🔴	Quantitative	Variable	$$	Intermediate
ELISA	🔴	🔴	🔴	🟢	🔴	🟢	Quantitative	Variable	$$	Intermediate
Mass Spectrometry	🔴	🔴	🔴	🟢	🟢	🔴	Quantitative	Variable	$$$	Expert
Chromatography	🔴	🔴	🔴	🟢	🟢	🔴	Quantitative	Variable	$$$	Expert
Genetic Analysis for Cyanotoxins	🟢	🔴	🔴	🟢	🔴	🔴	Quantitative	Point sampling	$$	Intermediate

Symbols		Abbreviations	
🟢	Suitable	P/A	Presence/absence
🟡	Potential	ID	Identification
🔴	Not suitable	DEN/AB	Density/abundance
		CGN	Congener-specific concetrations
		TOT	Total cyanotoxin concetrations

Building A Monitoring Plan

- What field methods will support your overall goal?

  Mat, water, or sediment samples?

- What type of water body will be sampled?

  Lake or river?

Field Method	Type of Sample		Type of Cyanobacteria		Water Body Type			Potential Locations				Water Depth Reached		
	Cyanobacteria Biomass	Cyanotoxins	Planktonic	Benthic	Still Water	Flowing Water	Within Drinking Water Facilities	Shore Line	Mid-stream	Surface	Below Surface	Shallow <1 m	Mid-depth >1 m–8 m	Deep >8 m
Grab Sample	S	S	S	S	S	S	S	S	S	S	S	S	PS	PS
Periphyton Scrape	S	S	NS	S	S	S	S	S	S	S	S	S	PS	PS
Rake Sample	S	S	NS	S	S	PS	NS	PS	S	PS	S	S	S	PS
Ponar Sampler	S	S	NS	S	S	PS	NS	NS	PS	NS	S	S	S	S
Artificial Substrate	S	S	NS	S	S	S	S	S	S	NS	S	PS	S	S
Snorkel & SCUBA	S	S	NS	S	S	PS	NS	S	S	S	S	S	S	S
View Bucket & Bathyscope	PS	NS	NS	S	S	S	PS	S	S	NS	S	S	PS	NS
Sediment Coring	PS	PS	PS	PS	S	PS	NS	NS	PS	NS	S	NS	S	S
Passive Sampler	NS	S	S	S	S	S	S	S	S	S	S	S	S	S
Pigments via field sensor	S	NS	S	S	S	S	S	S	S	S	S	S	S	S

S–Suitable PS–Potentially Suitable NS–Not Suitable

See Section 3.2.4 of HCB-2 Guidance Document

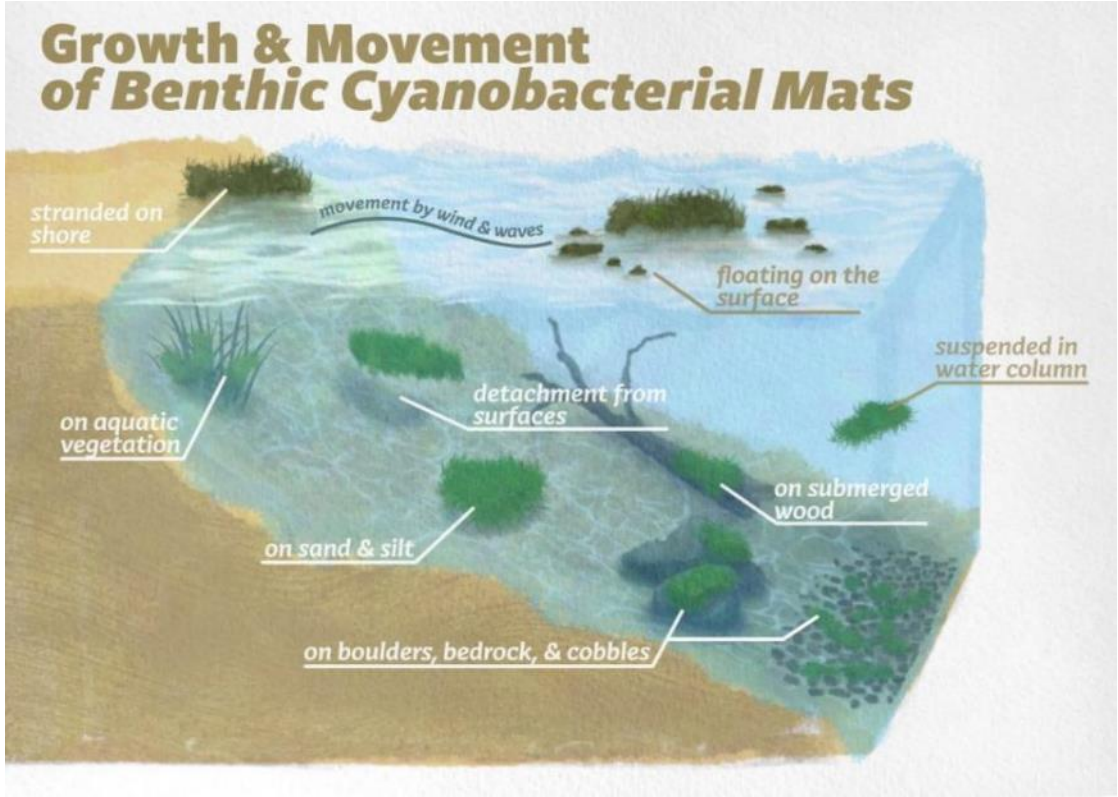
Building A Monitoring Plan

- Consider the location and frequency of monitoring activities

Sampling in high recreation potential areas? Drinking water intakes? Open water?

- What to do if monitoring results exceed threshold levels

What action will you or can your organization take?



Source: D'yani Wood and Morgan Tarbell

Response Plans

- Response plans tell you:
  - How to respond to HCB event (fish kill, dog death, bloom)
  - What level of cyanobacteria/cyanotoxins will lead to action
  - What action you will take (area closure, signs posted, nutrient management)

Source: Ann St. Amand

Monitoring: Key Things To Remember

- What are your goals?
- Use the selection tool to evaluate options and choose methods that will give you the results you need
- Devise a response plan, before you need it
- Be realistic about monitoring and response capabilities

Source: Eric Evensen

Advancing Environmental Solutions

Introduction to Benthic Cyanobacteria

Cyanotoxins

Monitoring

★ **Management**

Communication

See Section 4 of the HCB-2 Guidance Document

Management Criteria Tool – Interactive Selection

- HCB-2 Section 4 is recast from Section 6 from HCB-1

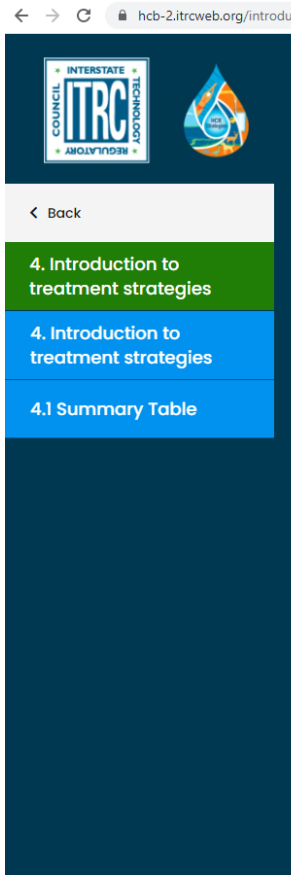


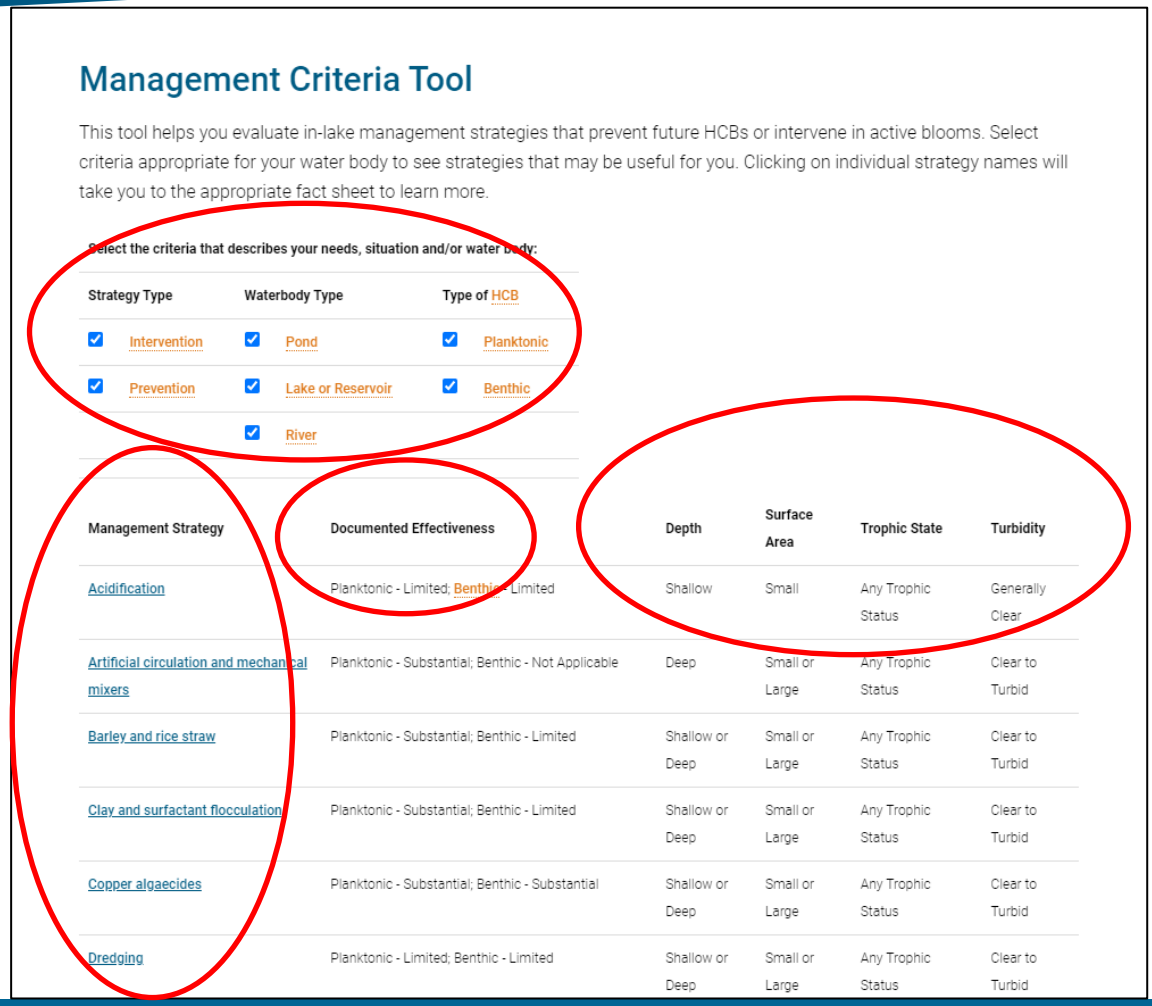
Table 4-1. In-water prevention and direct intervention strategies with typical cost-effective applications

Management Strategy	Management Strategy Type	Relative Cost*	Documented Effectiveness		Water Body Type	Brief Technical Description
			Planktonic	Benthic		
Acidification	Prevention	$$	Limited	Limited	Pond Lake/Reservoir	Lowering the pH out of the optimal growing range for cyanobacteria; changing how well the cell is able to regulate its buoyancy and maintain its cell wall
Artificial Circulation and Mechanical Mixing	Prevention	$$$	Limited	Not Applicable	Pond Lake/Reservoir	Destratifying a water body to reduce limiting nutrient concentrations in the hypolimnion and avoid sudden delivery of nutrient-rich bottom waters into the epilimnion
Barley and Rice Straw	Prevention	$	Substantial	Limited	Pond Lake/Reservoir River	Placing barley straw bales or bags in the shore zone of a water body 1–1.5 months prior to expected bloom
Clay and Surfactant Flocculation	Intervention	$$-$$$	Substantial	Limited	Pond Lake/Reservoir River	Mixing a slightly acidified solution of clay and surfactant and dispersing it over a bloom; sand may be added to cap the settled material
Copper Algaecides	Intervention and Prevention	$	Substantial	Substantial	Pond Lake/Reservoir River	Controlling algae in water bodies (registered by USEPA but prohibited in some states from use); copper algaecides interfere with the ability of algal cells to respire, photosynthesize, and, at some concentrations, maintain cell integrity
Dredging	Prevention	$$$	Limited	Limited	Pond Lake/Reservoir River	Physically removing the upper, nutrient-rich layer of bottom sediments to reduce internal nutrient loads and limit cyanobacterial growth
Floating Wetlands	Prevention	$$$	Limited	Limited	Pond Lake/Reservoir	Planting artificial islands with emergent plants designed to

See Section 4 of the HCB-2 Guidance Document

Management Criteria Tool – Interactive Selection

- Reconfigured tool – ease of use
- Crosslinked with HCB-1
- Now strategy sheets incorporate benthic bloom strategy considerations



Management Criteria Tool

This tool helps you evaluate in-lake management strategies that prevent future HCBs or intervene in active blooms. Select criteria appropriate for your water body to see strategies that may be useful for you. Clicking on individual strategy names will take you to the appropriate fact sheet to learn more.

Select the criteria that describes your needs, situation and/or water body:

Strategy Type	Waterbody Type	Type of HCB
☑ Intervention	☑ Pond	☑ Planktonic
☑ Prevention	☑ Lake or Reservoir	☑ Benthic
	☑ River	

Management Strategy	Documented Effectiveness	Depth	Surface Area	Trophic State	Turbidity
Acidification	Planktonic - Limited; Benthic - Limited	Shallow	Small	Any Trophic Status	Generally Clear
Artificial circulation and mechanical mixers	Planktonic - Substantial; Benthic - Not Applicable	Deep	Small or Large	Any Trophic Status	Clear to Turbid
Barley and rice straw	Planktonic - Substantial; Benthic - Limited	Shallow or Deep	Small or Large	Any Trophic Status	Clear to Turbid
Clay and surfactant flocculation	Planktonic - Substantial; Benthic - Limited	Shallow or Deep	Small or Large	Any Trophic Status	Clear to Turbid
Copper algaecides	Planktonic - Substantial; Benthic - Substantial	Shallow or Deep	Small or Large	Any Trophic Status	Clear to Turbid
Dredging	Planktonic - Limited; Benthic - Limited	Shallow or Deep	Small or Large	Any Trophic Status	Clear to Turbid

See Section 4 of the HCB-2 Guidance Document

Advancing Environmental Solutions

Introduction to Benthic Cyanobacteria

Cyanotoxins

Monitoring

Management

★ **Communication**

See Section 5 of the HCB-2 Guidance Document

HCB Communication And Response Training Objectives

- HCB-1 vs HCB-2
- HCB-2 contains new information specifically in these categories:
  - Reporting, notification, and coordination
  - Visual observation and identification
  - Drinking water coordination planning
  - Advisories, outreach, and signage

Source: Utah Department of Environmental Quality

Reporting, Notification, And Coordination

- Consider new water bodies that may be susceptible to benthic blooms
- Evaluate potential new partners, stakeholders, and agencies that may be impacted by benthic blooms
- Include language provided by the introduction section to emphasize benthic bloom characteristics

Source: Utah Department of Environmental Quality

Bloom Identification And Confirmation

- Visual observations
  - Visual guide
  - New identification video
- Field sampling techniques
- Laboratory analysis approaches

Source: Keith Bouma-Gregson

Drinking Water Sources

- Renew coordination with public water systems
- Review cyanotoxin management response plans for water systems
- Identify private (nonregulated) drinking water sources and develop outreach materials

Source: US EPA

See Section 5.1.3 of the HCB-2 Guidance Document

Health Advisories

- Advisory Thresholds
  - Due to nature of benthic blooms, difficult to compare existing thresholds
- Identify guidance to issue advisory
- Approaches to post advisories
  - Signs
  - Press release
  - Website
  - Social media

ZION Benthic HCB Recreational Advisory Decision Criteria

Advisory	Permitted Activities	Human Health Risk	Data		
			Presence of Toxigenic Cyanobacteria Species	Benthic Disturbance Sample	8 to 10-day SPATT
			1.Visual inspection (25 meters upstream/downstream of the SPATT site)		
			2.Taxonomic analysis		
			3.Found anywhere in the waterbody		
Danger Advisory (avoid all contact with the water, never drink the water)	Permitted waterbody-related activities allowed; language in the permits indicating Danger	Potential for acute poisoning Potential for long-term illness Short term effects (e.g. skin and eye irritation, nausea, vomiting, diarrhea)		Greater than 90 µg/L of anatoxin-a	
Warning Advisory (avoid primary contact recreation, never drink the water)	Permitted waterbody-related activities allowed; language in the permits indicating Warning	Potential for long-term illness Short term effects (e.g. skin and eye irritation, nausea, vomiting, diarrhea)		**OR** Less than 90 µg/L but greater than 15 µg/L of anatoxin-a	Detection anatoxin-a
Health Watch (avoid primary contact recreation, never drink the water)	Permitted waterbody-related activities allowed, permanent language indicating risk	Unknown	**OR** Toxigenic cyanobacteria present	**AND** Detection of anatoxin-a but less than 15 µg/L	**AND** Non-detect anatoxin-a
No Advisory (never drink the water)	Permitted waterbody-related activities allowed, permanent language indicating risk		**AND** Toxigenic cyanobacteria not present	**AND** Non-detect anatoxin-a	**AND** Non-detect anatoxin-a

Source: Zion National Park

See Section 5.1.4 of the HCB-2 Guidance Document

Signage

- Mats vs. water
- Specific information about risks to pets and livestock
- Temporary signs vs. permanent signs for areas that often experience HCBs

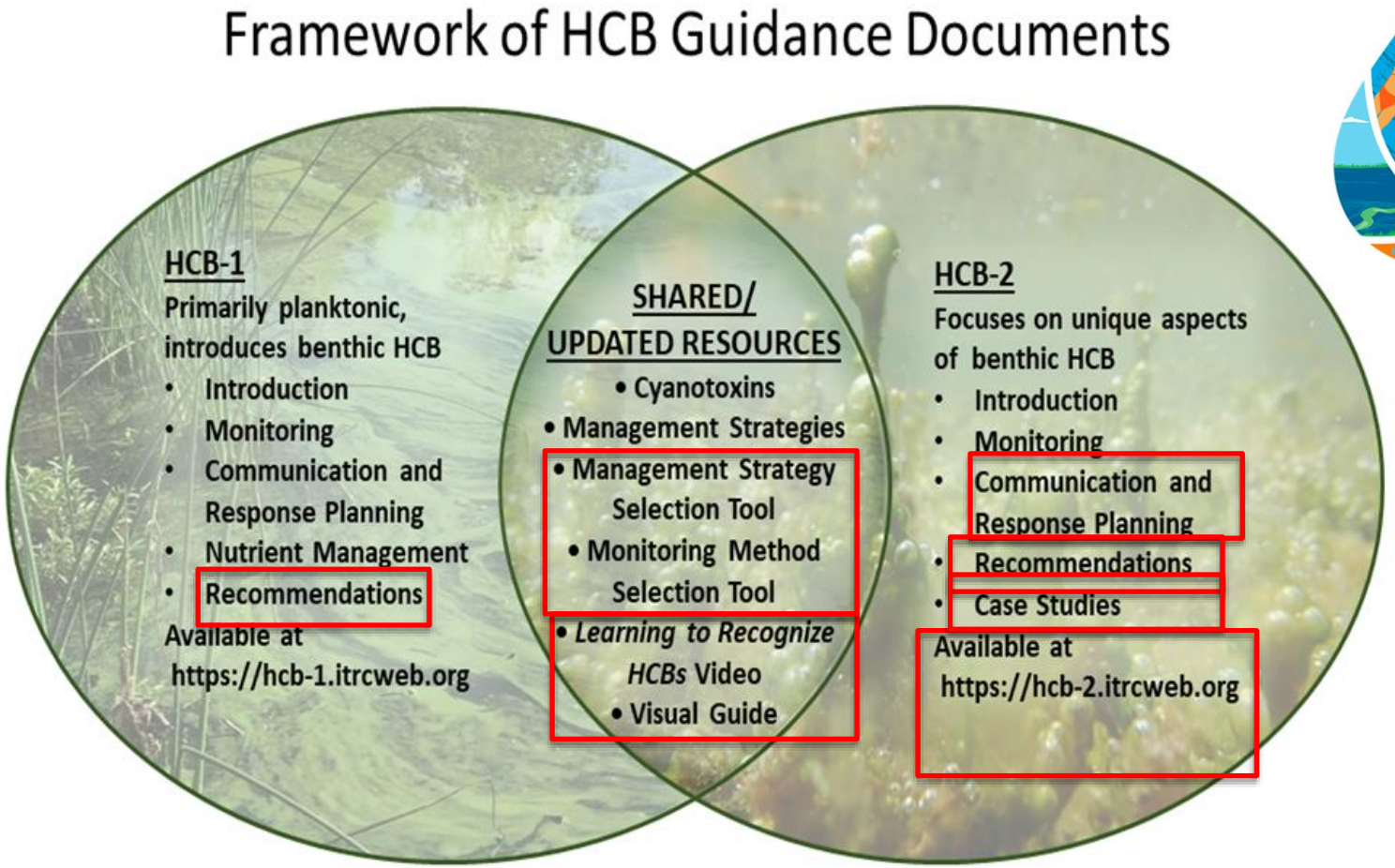
Source: California Cyanobacteria and Harmful Algal Bloom Network

Case Studies

See Appendix B of the HCB-2 Guidance Document

Resource Review

- Visual Guide
- ID Video
- Recommendations
- Case Studies
- Interactive Tools
- Trainings



Advancing Environmental Solutions

★ Questions?

See HCB-2 Guidance Document