Several case studies have been used in the creation of this document. The case studies come from different parts of the country and involve different benthic cyanobacteria and cyanotoxins.

Figure 1. Location of benthic HCB case studies.

Source: ITRC Benthic HCB Team

As shown in Figure 1, the case studies presented in this appendix include:

- Appendix B.1: Russian River, Sonoma County, California
- Appendix B.2: Zion National Park, Utah
- Appendix B.3: Lake Travis, central Texas

Appendix B.1 – Russian River

Case Study Name: Russian River Benthic HCB 2015

Author & Affiliation: Keith Bouma-Gregson, California State Water Board

Date: August and September 2015

Location: Russian River watershed, Sonoma County, California

Water Body Type: River

B.1.1 Overview

In 2015, actions taken in response to a dog death attributed to cyanotoxins ground the economy of the Russian River watershed to a halt. The Russian River sits adjacent to the San Francisco Bay Area in Northern California and is a popular recreational destination for residents in the region. Numerous resorts and guiding companies provide beaches and boating trips down the river, which provides a refreshing option to escape the hot summers of Northern California. The river is also a drinking water source for towns and cities in the area.

In late August 2015 a dog died during a float trip down the Russian River. Cyanotoxins were implicated. However, no planktonic bloom was observed, so benthic cyanobacteria were suspected. Benthic cyanobacteria have caused other dog deaths in the region (Puschner, Hoff, and Tor 2008), and benthic cyanobacteria in wadable streams are a documented source of cyanotoxins in Northern California and across California (Bouma-Gregson, Kudela, and Power 2018, Fetscher et al. 2015). Because little was known about benthic cyanobacteria in the Russian River, to prevent other illnesses or deaths, the Sonoma County Department of Public Health ordered a complete closure of the Russian River to recreation. This announcement was made a few days before Labor Day and closed the river during the popular holiday weekend.

Though other cyanotoxin-related illnesses have occurred since 2015 in Sonoma County, no sweeping closure of a water body has been necessary. With response and communication plans in place, and increased agency and private-sector knowledge about cyanotoxins, more tailored communication takes place depending on the context of the cyanobacterial event. Additionally, there is more communication and educational resources provided to the public. Water quality and health agencies continue to develop and improve their response to cyanotoxins in the watershed to ensure safe recreational opportunities in the Russian River and North Coast Region.

B.1.2 Monitoring/Sampling Conducted/Results

No monitoring or response plan was in place in 2015, so minimal data were collected during the event. After the event, agencies came together to develop a monitoring and responses strategy for benthic HCBs in the watershed.

B.1.3 Response Actions

The closure of the river during an economically important holiday weekend led to an outcry from the business community that relies on tourism generated by the river. Businesses lost revenue and income as tourists stayed away from the river. Motivated by the public health and economic impacts of this incident, the North Coast Regional Water Quality Control Board (North Coast Regional Board), the state agency responsible for water quality in the region, began to develop a cyanotoxin monitoring and response plan for the Russian River.

In February 2016, the North Coast Regional Board held a meeting with multiple entities to begin to develop a cyanobacterial monitoring plan for the Russian River to help prevent the shutdown of the river when the next cyanotoxin event occurred. Included at the meeting were the Sonoma County Department of Public Health, the Sonoma Water Agency, the State Water Board, and other representatives from the business and NGO community. These groups began to talk about what would be needed to evaluate and communicate the risks of benthic cyanobacteria to protect public health.

Over the next few months, a framework was built that involved monitoring and communication between the North Coast Regional Board and Sonoma County Department of Public Health. Monitoring involved looking specifically for the development of benthic cyanobacterial mats in the river and the downstream transport of detached mats to recreational beaches. When visual screening identified benthic cyanobacteria, then water samples were collected for cyanotoxin analysis and public health advisories were issued when appropriate. Water sampling occurred at recreational beaches that were also monitored for fecal indicator bacteria by Sonoma County Department of Public Health. In the event of exceeding a cyanotoxin trigger value, advisories were constrained to the recreational beach and did not extend across the entire river.

Additionally, trainings and educational workshops about cyanotoxins and response procedures were conducted by the North Coast Regional Board and State Water Board to train internal and partner staff in how to identify and respond to cyanobacteria and cyanotoxins. River guide businesses were also invited to these trainings to teach river guides how to identify cyanobacterial mats to help keep their clients away from potentially toxic algae.

B.1.4 Lessons Learned

Though other cyanotoxin-related illnesses have occurred since 2015 in Sonoma County, no sweeping closure of a water body has been necessary. With response and communication plans in place, and increased agency and private-sector knowledge about cyanotoxins, more tailored communication takes place, depending on the context of the cyanobacterial event. Additionally, there is more communication and educational resources provided to the public. Water quality and health agencies continue to develop and improve their response to cyanotoxins in the watershed to ensure safe recreational opportunities in the Russian River and North Coast Region (Figures B.1-1 and B.1-2).

Figure B.1- 1. North Coast Regional Board benthic cyanobacteria training workshop in 2016 educating agency and private business staff on how to identify benthic cyanobacteria.

Source: Keith Bouma-Gregson.

Figure B.1- 2. Recreators travel downstream in a canoe passing a mixed assemblage of benthic algae and cyanobacteria in the Russian River.

Source: Keith Bouma-Gregson.

B.1.5 References and Resources

Bouma-Gregson, Keith, Raphael M. Kudela, and Mary E. Power. 2018. “Widespread anatoxin-a detection in benthic cyanobacterial mats throughout a river network.” *PLOS One* 13 (5):e0197669. doi: https://doi.org/10.1371/journal.pone.0197669.

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Puschner, Birgit, Brent Hoff, and Elizabeth R. Tor. 2008. “Diagnosis of Anatoxin-a Poisoning in Dogs from North America.” *Journal of Veterinary Diagnostic Investigation* 20 (1):89-92. doi: https://doi.org/10.1177/104063870802000119.

Appendix B.2 – Zion National Park, UT

Case Study Name: Zion National Park Benthic HCB

Author & Affiliation: Robyn Henderek, Physical Scientist, National Park Service

Date: September 31, 2021

Location: Zion National Park, Utah

Water Body Type: River and streams

B.2.1 Overview

A suspicious dog death on July 4, 2020, alerted Zion National Park (Zion NP) and Utah Department of Environmental Quality (UDEQ) officials of a suspected HCB event. Park officials collected cyanobacteria and cyanotoxin samples in the North Fork of the Virgin River and found *Microcoleus anatoxia* in abundance and high levels of anatoxin-a (over 550 µg/L) in some of the benthic mats. Subsequent sampling found the toxigenic species present throughout the Virgin River and its tributaries within Zion NP. There are a wide range of potential exposure scenarios, including backcountry drinking water, primary and secondary contact recreation, as well as potential pet exposures.

Furthermore, managing for a benthic HCB event posed a new challenge for the National Park Service and UDEQ, as there do not exist any state or national standards for human recreational advisories during benthic HCB events. Recreational advisories have been developed only for anatoxin-a under planktonic HCB exposure scenarios where cyanotoxins are suspended in the water column. In Zion NP’s benthic HCB event, cyanotoxins were found only in extremely low levels within the water column (under 1 µg/L), whereas high toxin concentrations (over 550 µg/L) were found in the mat material. This signifies the greatest risk to human and pet illness comes from incidental ingestion of the mat material. Given the high level of recreation occurring in Zion NP, these benthic mats are easily disturbed by recreators walking or playing along the river edge (where the benthic mats are most abundant).

Therefore, park and UDEQ staff found it appropriate to develop a new sampling methodology called “benthic disturbance.”

This method simulates the type of recreation occurring in Zion NP, which may detach these mats through typical recreation and make them readily available for human exposure as they are suspended in the water column and floating on the river surface. Zion NP has adopted an interim policy to use this sampling methodology to apply existing recreational advisory thresholds developed for anatoxin-a. Zion NP also applies the presence or absence of cyanotoxins in the water column samples using solid phase adsorption toxin tracking (SPATT) Section 3.2.4.9 samplers as well as visual surveys for the toxigenic species to their decision criteria on recreational advisory issuance. Given the uncertainties behind the ability for backcountry recreational water filtration systems to effectively reduce cyanotoxin levels to those safe for drinking water, Zion NP advises all recreators not to drink from any stream water and to filter drinking water only directly from a spring orifice.

B.2.2 Monitoring/Sampling Conducted/Results

Zion NP has been sampling for cyanotoxins on a monthly basis since September 2020 in three of its major tributaries to the Virgin River: North Fork of the Virgin River, North Creek, and La Verkin Creek. All three of these tributaries had both cyanotoxins and toxigenic cyanobacteria present through late fall 2020, winter 2020/2021, and spring 2021; however, cyanotoxin levels decreased significantly from September 2020 levels to October 2020. One sample taken in March 2021 showed very high levels in the benthic disturbance sample (390 μg/L), which necessitated a Danger Advisory to be issued on the North Fork of the Virgin River. Cyanotoxin levels decreased in the spring, likely due to higher turbidity and slightly higher flows from snowmelt. Cyanotoxin levels have remained low through summer 2021 as the monsoon has brought in regular high-flow events, scouring out growth in the benthos and bringing in sustained high turbidity, which prevents sunlight from reaching the benthos. Results from summer 2021 support the hypothesis that *Microcoleus* prefers clear conditions and low flows as was the case in summer 2020 (where cyanotoxin levels were extremely high and toxigenic species abundant). Summer 2020 was the second driest monsoon on a record dating back to 1928.

Zion NP has taken a multiple-lines-of-evidence approach to monitoring HCB events and issuing recreational water body advisories, considering visual inspections for toxigenic cyanobacteria species, benthic disturbance samples, and SPATT.

- **Visual Inspection:** The first criteria is visual inspection for toxigenic cyanobacteria species. Since exposure risk in river systems is not currently well understood, any visual confirmation of a toxigenic cyanobacteria species warrants an advisory to the public. Visual inspection consists of inspecting both sides of the water body 25 meters upstream and downstream of each permanent SPATT monitoring site for one or more toxigenic species. A sample may be collected for further taxonomic analysis. A visual confirmation of one or more toxigenic cyanobacteria species anywhere in the water body during the sampling period may be considered as well.
- **Benthic Disturbance Samples:** The second criteria is benthic disturbance sampling. Benthic disturbance samples aim to capture the reasonable worst-case recreational exposure scenario by artificially disturbing colonies of toxigenic cyanobacteria and creating a reasonable exposure situation for which the cyanotoxin thresholds set by the State of Utah can be applied. Benthic disturbance sampling consists of stepping for five seconds on a roughly 1 square meter area with a toxigenic species present, using a 2.5-gallon bucket to scoop the disturbed bacterial mats and water column, and then subsampling from the bucket. If no toxigenic species are visibly present within 25 meters upstream and downstream of the permanent SPATT monitoring site for the water body, the benthic disturbance samples are taken nearest to the SPATT monitoring site. In some cases, multiple benthic disturbance samples may be taken from one water body. To be protective, the highest concentration detected will be used to issue advisories for the entire connected water body.
- **Solid Phase Adsorption Toxin Tracking (SPATT) Samples:** The third criteria is SPATT. SPATT is an emerging technology that passively collects dissolved cyanotoxins in the water column over a period of time. Zion NP staff chose to deploy the SPATT samplers for a period of 8–10 days as initial SPATT testing in July 2020 detected a higher concentration of toxins per day when bags were deployed for 10 days as opposed to 5 days. Concentrations of cyanotoxins in the SPATT bags may be subject to a variety of factors such as flow and turbidity. Therefore, under guidance from UDEQ, Zion NP staff issue advisories from SPATT cyanotoxin results as detect or nondetect rather than as a concentration per weight of resin beads.

It is appropriate to issue advisories based off all three components because the scientific community does not fully understand under what conditions benthic toxigenic cyanobacteria species produce cyanotoxins (Wood et al. 2020). In some cases in the park, benthic disturbance samples of toxigenic cyanobacteria colonies have come back with less than 15 μg/L of anatoxin-a, but SPATT results from a bag deployed during the same time have detected anatoxin-a. This scenario implies that somewhere in the water body there is toxigenic cyanobacteria that is producing cyanotoxins harmful to human health, but park staff did not happen to catch the toxigenic cyanobacteria while it was producing cyanotoxins through the benthic disturbance sample. Under this scenario, the water body would be issued a Warning Advisory.

Monitoring occurs at each of these sites at least monthly. Monitoring consists of an 8- to 10-day SPATT deployment, benthic disturbance samples, and visual inspections for toxigenic cyanobacteria species. In the North Fork of the Virgin River, the permanent SPATT monitoring site is located at the Zion NP Visitor Center. At least one benthic disturbance sample will be taken at the Zion NP Visitor Center, and additional benthic disturbance samples may be taken at the Narrows, the Temple of Sinawava, and the Flanigan Diversion Dam. In North Creek, the permanent SPATT monitoring site and the site of the benthic disturbance samples and visual inspection are located below the confluence of the Left Fork and Right Fork of North Creek. The permanent SPATT monitoring site, location of benthic disturbance, and visual inspection in La Verkin Creek is located at the first encounter with La Verkin Creek down Lee Pass Trail from Kolob Canyons. Sampling may be increased as field conditions indicate by adding more sites or collecting samples on a biweekly basis, as opposed to a monthly basis, for example. A “sampling event” consists of a visual survey, benthic disturbance sample, and SPATT sample. It may take only one sampling event to issue or upgrade an advisory for a water body, but it must take two consecutive sampling events to downgrade or rescind an advisory for a water body.

Table B.2- 1. Zion National Park benthic HCB recreational advisory decision criteria.

Source: National Park Service.

<table>
<tr><th colspan="6">ZION Benthic HCB Recreational Advisory Decision Criteria</th></tr>
<tr><th rowspan="6">Advisory</th><th rowspan="6">Permitted Activities</th><th rowspan="6">Human Health Risk</th><th colspan="3">Data</th></tr>
<tr><th>Presence of Toxigenic Cyanobacteria Species</th><th rowspan="5">Benthic Disturbance Sample</th><th rowspan="5">8 to 10-day SPATT</th></tr>
<tr><td>1.Visual inspection (25 meters upstream/downstream of the SPATT site)</td></tr>
<tr><td>2.Taxonomic analysis</td></tr>
<tr><td>3.Found anywhere in the waterbody</td></tr>
<tr></tr>
<tr><td rowspan="3">Danger Advisory (avoid all contact with the water, never drink the water)</td><td rowspan="3">Permitted waterbody-related activities allowed; language in the permits indicating Danger</td><td>Potential for acute poisoning</td><td rowspan="3"></td><td rowspan="3">Greater than 90 µg/L of anatoxin-a</td><td rowspan="3"></td></tr>
<tr><td>Potential for long-term illness</td></tr>
<tr><td>Short term effects (e.g. skin and eye irritation, nausea, vomiting, diarrhea)</td></tr>
<tr><td rowspan="2">Warning Advisory (avoid primary contact recreation, never drink the water)</td><td rowspan="2">Permitted waterbody-related activities allowed; language in the permits indicating Warning</td><td>Potential for long-term illness</td><td rowspan="2"></td><td rowspan="2">Less than 90 µg/L but greater than 15 µg/L of anatoxin-a</td><td rowspan="2">Detection anatoxin-a</td></tr>
<tr><td>Short term effects (e.g. skin and eye irritation, nausea, vomiting, diarrhea)</td></tr>
<tr><td>Health Watch (avoid primary contact recreation, never drink the water)</td><td>Permitted waterbody-related activities allowed, permanent language indicating risk</td><td>Unknown</td><td>Toxigenic cyanobacteria present</td><td>Detection of anatoxin-a but less than 15 µg/L</td><td>Non-detect anatoxin-a</td></tr>
</table>

No Advisory (never drink the water)	Permitted waterbody-related activities allowed, permanent language indicating risk		Toxigenic cyanobacteria not present	Non-detect anatoxin-a	Non-detect anatoxin-a

B.2.3 Response Actions

Zion NP issues three levels of advisories based on monitoring described in Section B.2.2. These advisories mirror the State of Utah’s HCB advisory levels: Danger, Warning, and Health Watch. A Warning Advisory sign is pictured in Figure B.2- 2. Zion NP uses many aspects of the State of California’s guidance for benthic HCB signage (CCHAB 2020).

Figure B.2- 2. Warning sign used at Zion National Park.

Source: National Park Service.

B.2.4 Lessons Learned

- Benthic HCB events pose a unique communication challenge. There are significant uncertainties with regard to recreational risk and causes of benthic HCB events. Because these uncertainties necessitate further study and standard guidance, they must also be adequately represented to the public as such. Emphasis should be made to the public that despite uncertainties, advisories reflect the park’s current understanding of the HCB using multiple lines of evidence.

- Initially, Zion NP had HCB advisory signage that did not show photos of the benthic HCBs. Therefore, the public became fearful of harmless filamentous green algae or only looked for typical morphologies of planktonic HCBs, such as blue-green pea soup–colored water. It became clear to the park staff that visitors need to be shown what the benthic HCB looks like up close and where they are typically found in the river, because benthic HCBs can be cryptic. Zion NP staff reworked their advisory signage to reflect more closely California's benthic HCB signage showing photos of the benthic HCBs. In addition to Figure B.2-2, Zion NP's current signage can be found in Section 5.
- Signage is important for warning visitors of a danger just before they are about to engage in recreation that may be considered high risk. Social media, websites, and press releases are also used to notify visitors of the risk prior to arrival at the park so they may plan for contingencies.
- It was most helpful in communicating real risk to recreators to share some statistics on potential poisoning cases from exposures in the park. Utah Poison Control regularly sends Zion NP staff a synthesis of all the calls it received from Zion NP that may be a potential poisoning event. Most cases involve a suspected dermal reaction, such as rashes, but some visitors and staff have reported internal illnesses that may be associated with cyanotoxin poisoning.
- Anatoxin-a degrades very quickly and can be difficult to detect even in an autopsy. Anatoxin-a can be detected in the stomach contents as well as throughout the gastrointestinal tract if sampled and frozen within several hours to days of the poisoning incident. As with the case in Zion NP, a suspicious dog death may be an indicator that there is an HCB event. Reach out to local veterinary organizations with guidance on cyanotoxin poisoning symptoms and what samples to collect and how to store those samples in the case of death during an HCB event.

B.2.5 References

CCHAB. 2020. "Benthic mats (toxic algal mats) signs and posting guidelines." California Cyanobacteria and Harmful Algal Bloom Network. https://mywaterquality.ca.gov/habs/resources/benthic_posting_guidance.html.

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B.2.6 Resources

USGS-NPS Nationwide HAB Project Disturbance Grab Sampling SOP for Benthic HABs

Adapted from Zion National Park disturbance sampling methods

Overview:

These protocols replace the "Grab sample" methods in the SPATT and grab sample protocol for parks with benthic HABs (i.e., Zion National Park and Buffalo National River).

Additional supplies needed: 2.5-gallon plastic bucket or collapsible bucket

Disturbance grab samples:

1. Put on nitrile gloves.

2. Only collect grab samples when SPATT samplers are retrieved.

3. Retrieve the SPATT samplers before taking the benthic disturbance grab samples.

4. Wash bucket three times in stream/river water.

5. Take the benthic disturbance sample in area of highest visible cyanobacteria concentration near where the SPATT samplers were deployed. Step in roughly 1 square meter area for 5 seconds. Scoop water from disturbed area with 2.5-gallon bucket, attempting to capture any dislodged mats and disturbed sediments.

6. **Phytoplankton grab sample**: Remove the vial of preservative from the 250-mL amber plastic sample bottle by tipping bottle upside down and dumping contents of bottle onto a flat surface (do not tip bottle upside down into your hand in case the preservative vial opened during shipment – if this happened, use a new sample bottle). Dip the bottle into the bucket and fill to shoulder of the bottle, then follow the "Phytoplankton grab sample" directions in the SPATT protocol.

7. **Water toxin grab sample:** Dip the 500-mL amber glass bottle into the bucket and fill HALF FULL, then follow the "Water toxin grab sample" directions in the SPATT protocol.

8. For simplicity, use the SAME TIME for each grab sample collected concurrently. Use the time that the first sample was collected.

6. Wash off bucket in stream water. Remove and wash off boots/waders. Wash hands using soap and water bottle.

Disturbance Grab Sampling SOP for Parks with Benthic HABs, Page 1 of 1
Version 1, 07/26/2021

Appendix B.3 – Lake Travis, TX

Case Study Name: Lake Travis

Author & Affiliation: Anthea Fredrickson, Lower Colorado River Authority

Date: February 20, 2021

Location: Lake Travis, Texas

Water Body Type: Freshwater Lake

B.3.1 Overview

On February 20, 2021, a dog (referred to as C1) swam in Lake Travis, a reservoir on the Colorado River in central Texas (Figure B.3-1) and died from suspected neurotoxicosis. Symptoms were consistent with ingestion of cyanobacteria (trembling, inability to stand, panting, vomiting, and stumbling). On February 21, 2021, the Lower Colorado River Authority (LCRA) received a report of the dog death, and given the symptoms, promptly investigated the site. Full publication documenting this case can be found here.

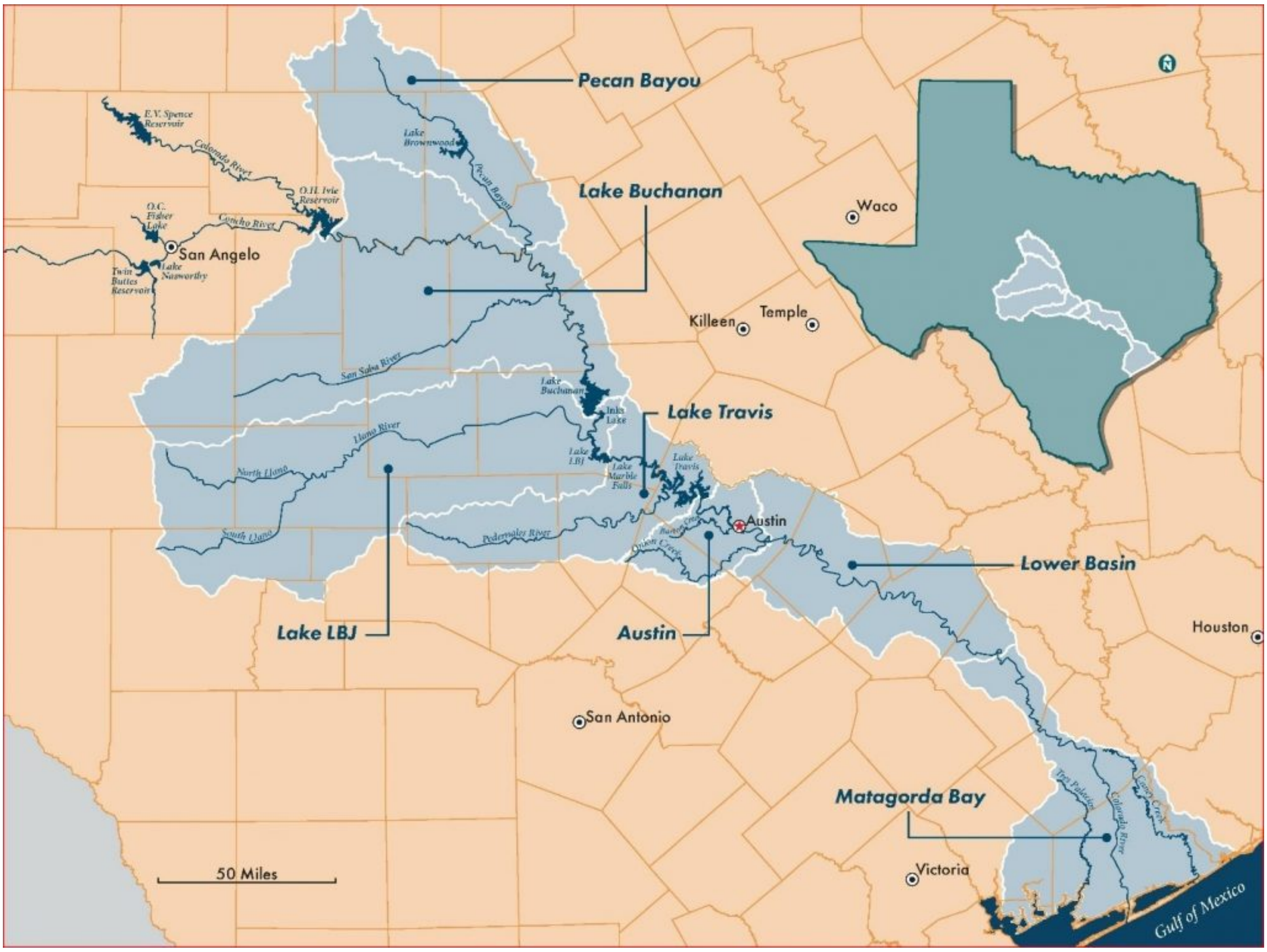

Figure B.3- 1. Map of the Highland Lakes, a series of lakes located along the Lower Colorado River in central Texas.

Source: Lower Colorado River Authority.

LCRA collected a composite sample of algae and water material and sent it to GreenWater Laboratories, located in Palatka, Florida. LCRA's assessment of the site gave no visual indication of a planktonic bloom or cyanobacterial bloom (Figure B.3-2). Beginning February 11, 2021, central Texas experienced a winter event causing temperatures to range from 3 to -17

°C (37 to 0 °F) for 8 days prior to the death of C_1. Due to the time of year (winter), the unusual circumstances of the winter event, and the lack of any visible evidence of a bloom, LCRA and the pet owner cooperated to order a necropsy of C_1. The necropsy was performed by Texas A&M Veterinary Medical Diagnostic Laboratory in College Station, Texas, on February 24, 2021. Several tests were ordered, including botulism A, B, and C; drug screen; and a pesticide screen. Stomach content was tested for saxitoxin, cylindrospermopsin, microcystin, and anatoxin-a (ATX). Additionally, digestive tissues (jejunum, duodenum, and stomach content fluid) were sent to Dr. Schonna Manning's laboratory at the University of Texas at Austin, for HPLC-MS analysis of the anatoxin-a congener dihydroanatoxin-a (dhATX). Results showed that trace amounts of anatoxin-a were present in the stomach of C_1, while high levels of the congener, dhATX, were found in multiple digestive tissues, including duodenum tissues (average of 10.51 ng/g DW, n = 2), jejunum tissue (average of 6.076 ng/g DW, n = 2), and from mixed stomach content (average of 974.88 ng/g DW, n = 2).

Figure B.3- 2. Photos taken on February 22, 2021, from the initial site on Lake Travis where C1 died on February 20, 2021. There were no visual signs, smells, or other evidence of a planktonic or cyanobacterial bloom at the time these photos were taken.

Source: Anthea Fredrickson.

Algae and water composite samples collected from the site and sent to GreenWater Laboratories reported back high levels of dhATX (630 and 640 ng/mL). The World Health Organization (WHO) does not currently have any reference values for dhATX, but a recent study showed that dhATX is roughly 3-4 times more toxic when ingested orally than ATX (Puddick et al.

2021). The WHO provisional recreational water health-based reference value for ATX is 60 ng/mL (Chorus and Welker 2021).

All other necropsy tests ordered by the diagnostic laboratory came back negative, narrowing down the cause of death to dhATX. dhATX was first suspected of causing a dog death in 2019 in Lady Bird Lake, which is also located in central Texas (Manning, Perri, and Bellinger 2020), but no necropsy was performed during that incident. The February 2021 incident was the first confirmed dog fatality associated with dhATX in Texas.

B.3.2 Monitoring/Sampling Conducted/Results

Following the necropsy and composite sample results, LCRA chose to do a one-time survey of several sites on Lake Travis to determine whether dhATX was present throughout the lake. During this survey, nine sites were sampled along Lake Travis. Whole water samples and composite algae samples were collected from each site and sent to GreenWater Laboratories for anatoxin-a suite analysis, which included testing for dhATX, homoanatoxin-a, and dihydrohomoanatoxin-a. The toxin dhATX was detected in every algae sample collected from Lake Travis. dhATX levels in algae samples ranged from 0.06 to 8,270 ng/mL. dhATX was found in three whole water samples with values that ranged from 0.06 to 0.15 ng/mL. Algae samples were mounted on slides and microscopically analyzed for the presence of potentially toxigenic (PTOX) cyanobacteria. The PTOX screen showed that *Phormidium/Microcoleus* was present in every single algae sample taken from the Lake Travis survey. *Phormidium/Microcoleus* is a benthic species, capable of forming mat proliferations that have been known to produce ATX and dhATX (Quiblier et al. 2013).

Based on the results of the Lake Travis survey, LCRA decided to expand the survey and collect whole water and composite algae samples from multiples sites in each of the other Highland Lakes managed by LCRA (Lake Buchanan, Lake Lyndon B. Johnson, Inks Lake, and Lake Marble Falls). dhATX was present in algal samples taken from two of the lakes, Inks Lake and Lake Marble Falls. The PTOX species *Phormidium/Microcoleu*s was present in at least one sample taken from each of the Highland Lakes, indicating that the species was present, but not always producing cyanotoxins at the time of sampling. Following this information, LCRA decided to implement a long-term plan for monitoring cyanobacteria in the Highland Lakes.

For the initial site located in Lake Travis, LCRA continued monitoring efforts by collecting biweekly samples of whole water, and two separate types of algae composites. Monitoring efforts at this site began March 3, 2021, and continued until June 22, 2021, when two consecutive sampling events yielded a nondetect for dhATX. Beginning July 1, 2021, the initial site became part of LCRA’s preliminary study to determine new long-term monitoring sites for cyanotoxins.

B.3.3 Response Actions

In response to the events of February 2021, LCRA began a routine long-term cyanotoxin monitoring program starting July 1, 2021. Before the death of C_1, LCRA did not monitor specifically for cyanotoxins, but has since included them in a year-round monitoring effort.

Routine water quality monitoring was already in place at existing open water sites throughout the Highland Lakes system. LCRA supplemented this current monitoring by adding the collection of whole water samples for one site on each reservoir. These samples are analyzed for cyanotoxins (cylindrospermopsin, microcystin, anatoxin-a, and anatoxin-a congeners) on a monthly to bimonthly basis depending on the size of the lake. New sites were added for long-term routine monitoring based on the results of a preliminary study conducted by LCRA from July to September 2021. The preliminary study involved deploying SPATT Section 3.2.4.9 (solid phase adsorption toxin tracking) bags in several shallow water, high recreation potential sites throughout the Highland Lakes. The resin inside the SPATT bags can accumulate dissolved cyanotoxins over time and can allow the user to generate a time series of relative cyanotoxin levels (Wood, Holland, and MacKenzie 2011). Each SPATT bag was deployed following protocols outlined in Howard et al. (2018) for 1 month at a time and then replaced with a new bag. SPATT bags were attached to available structures, such as fishing docks, piers, or buoys, or installed with a T-post. Every SPATT sample was analyzed using HPLC-MS (see Section 4.3.2.5 in ITRC 2021) and ELISA (See Section 4.3.2.3 in ITRC 2021) for cyanotoxins. Cyanotoxin results from SPATT bags are reported in nanogram cyanotoxin per gram resin (ng_{ATX}/g).

At the end of the preliminary study, the sites with the highest amount of cyanotoxins detected consistently were kept as new long-term study sites. Once the new sites had been established, LCRA continued to deploy SPATT bags monthly and analyze them using HPLC-MS and ELISA. Randomized sampling of algal material at each SPATT bag site was collected and analyzed for cyanotoxins and screened under a microscope for detection of PTOX species. At the beginning of each month when a new SPATT bag is deployed, turbidity samples are collected and a phycocyanin probe is deployed to collect relative fluorescence data. A YSI is used to collect basic water quality parameters (temperature, dissolved oxygen, conductivity, and pH) at each SPATT site, and every SPATT bag that is retrieved is inspected for visible algal growth. LCRA intends to use this approach year-round to evaluate spatial and temporal patterns of cyanotoxins in the Highland Lakes.

B.3.4 Lessons Learned

LCRA learned there was a gap in current monitoring efforts for cyanotoxins in the Highland Lakes. With the addition of cyanotoxin monitoring in routine sites, as well as the addition of new monitoring sites, LCRA hopes to close this gap. The events of February 2021 also highlighted a lack of reporting and documenting of dog illnesses and deaths in the Highland Lakes. Following the death of C_1 and subsequent media coverage of the incident, LCRA received reports of several other dogs that became ill after swimming in Lake Travis between January 20 and February 192021. LCRA also received a report of an additional dog (C_2) that died on January 20, 2021, after swimming in the same region of Lake Travis where C_1 swam. LCRA learned there was a disconnect in suspected dog harmful algae intoxication events and reporting. LCRA created and submitted a questionnaire (see Appendix B.3.6) to all dog owners who reported a canine illness or death to document symptoms and create a timeline of when cyanotoxins may have been present in the system. LCRA then began a collaboration with the U.S. Environmental Protection Agency, the City of Austin, and the National Park Service to create a document for use in Austin, TX, but can be used in other locations to compile dog illness and death information related to cyanotoxins.

During LCRA's event response phase, LCRA provided regular updates on its website about sampling results in the Highland Lakes. In response to the detection of toxic algae in the lakes, LCRA created warning signs for its lakeside parks and made signs available digitally for both public and private landowners to display at popular recreation sites. This experience showed LCRA that the old visual indicators of blooms did not apply to benthic cyanobacteria blooms and neither did the premise of "safer" winter months. In response to the events of February 2021, the City of Austin changed their public messaging away from "safe" winter months or times of typically low occurrence of toxic blooms (Figure B.3-3) and shifted toward danger may always be present messaging because of the cryptic nature of benthic blooms (Figure B.3-4). This shift in messaging will help recreators and dog owners know the risks before swimming in the Highland Lakes. LCRA hopes the addition of new signage and proper cyanotoxin monitoring will prevent future dog deaths and illnesses.

Figure B.3- 3. Sign designed and used by the City of Austin to warn recreators about the dangers of harmful algae.

Source: City of Austin, TX.

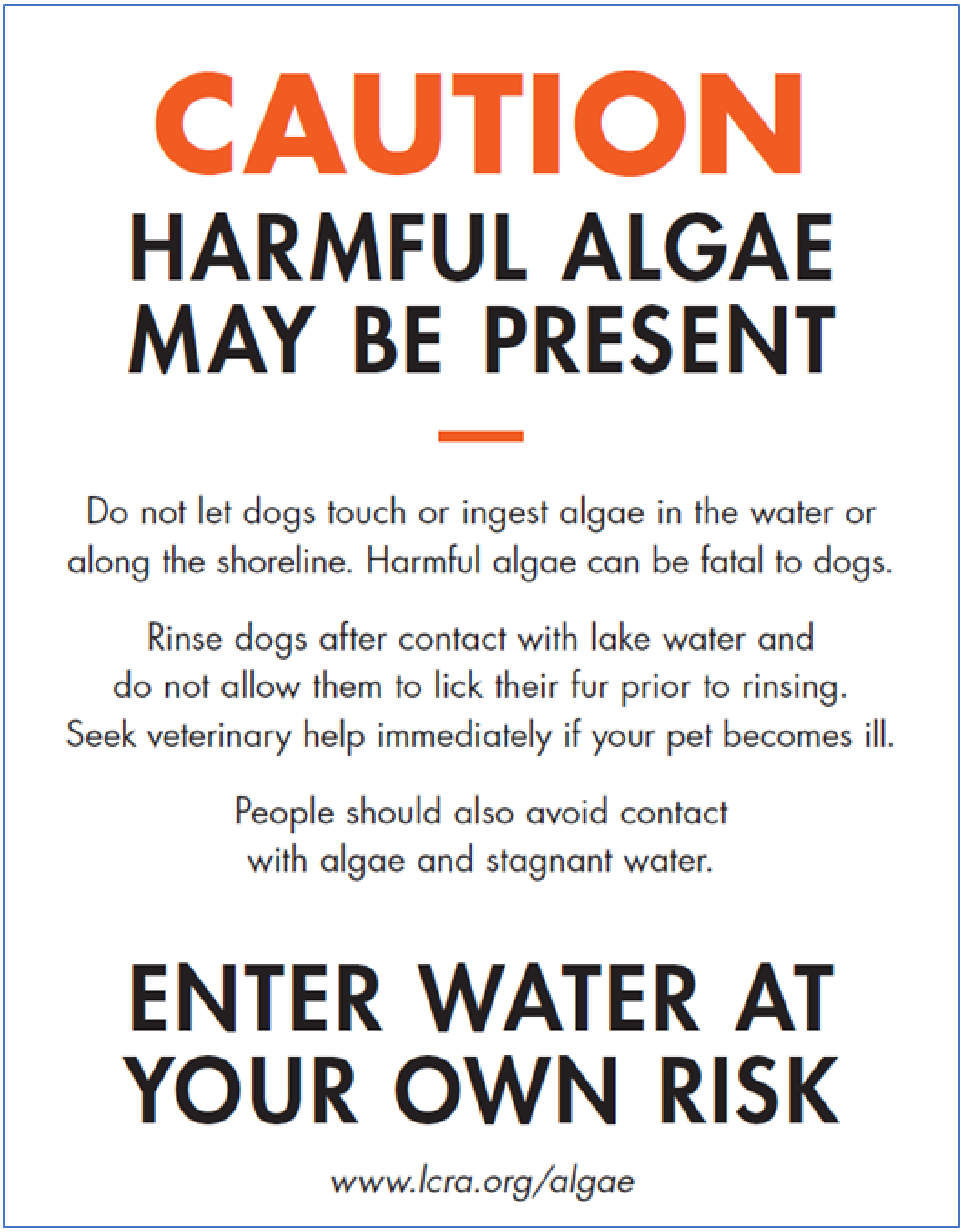

Figure B.3- 4. Sign created and used by the Lower Colorado River Authority after the events of February 2021, cautioning that algae may be present year-round.

Source: Lower Colorado River Authority.

B.3.5 References

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B.3.6 Resources

Questionnaire designed by the City of Austin, U.S. Environmental Protection Agency, Lower Colorado River Authority, and National Park Service following the events of February 20, 2021, to help collect, compile, and track all dog-related illnesses and deaths that occur in Austin, TX.

Dog Questionnaire for potential HAB related dog illnesses or death

(if you have multiple dogs displaying symptoms, please fill out two separate forms)

1. **Your Name:**
2. **Contact Information:**
3. **Dog Name:**
4. **Breed:**
5. **Age:**
6. **Weight:**
7. **Date of Exposure:**
8. **Location (please be as descriptive as possible):**
9. **Approximately how long was the dog exposed to the water and how many minutes/hours after exposure did the symptoms begin?**
10. **Did you see the dog drink any of the water?**
11. **Did your dog swim in the water?**
12. **Did you witness the dog eating any algae, playing with algae, chewing on sticks covered in algae?**
13. **What symptoms did your dog display? Please check all that apply**

Symptoms	**Please check the symptoms your dog displayed:**
Immediate:	
Vomiting	
Diarrhea	
Weakness/Unable to stand	
Disorientation	
Excessive drooling	
Tremors/Involuntary shaking	
Rapid breathing/Excessive panting	

Seizures	
Limp	
Symptoms displayed hours or days later:	
Vomiting	
Diarrhea	
Decreased food or water intake	
Lethargic	
Jaundice (yellowing of skin or eyes)	
Head tilt	
Abnormal gait/Balance problems	

14. **Did your dog display any symptoms not listed above? Please describe.**
15. **Was your dog regularly taking any medication before the onset of symptoms, if so please list:**
16. **Did a vet confirm harmful algae related illness or death?**
17. **If dog passed away from the event, how long after exposure until death?**
18. **If dog survived, how long after exposure did symptoms subside?**
19. **Any other information you would like us to be aware of:**

Thank you for taking the time to complete this questionnaire. Please keep an eye out for a follow-up questionnaire in two weeks. This follow-up questionnaire will help us assess whether your dog possibly suffered any permanent damage as a result of its encounter with harmful algae.