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Reservoir and Riverine Sources of Cyanotoxins in Oregon's Cascade Range Rivers Tapped for Drinking Water Supply

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Abstract: Reservoirs and downstream rivers draining Oregon's Cascade Range provide critical water supplies for over 1.5 million residents in dozens of communities. These waters also support planktonic and benthic cyanobacteria that produce cyanotoxins that may degrade water quality for drinking, recreation, aquatic life, and other beneficial uses. This 2016–2020 survey examined the sources and transport of four cyanotoxins—microcystins, cylindrospermopsins, anatoxins, and saxitoxins—in six river systems feeding 18 drinking water treatment plants (DWTPs) in northwestern Oregon. Benthic cyanobacteria, plankton net tows, and (or) Solid-Phase Adsorption Toxin Tracking (SPATT) samples were collected from 65 sites, including tributaries, reservoirs, main stems, and sites at or upstream from DWTPs. Concentrated extracts (320 samples) were analyzed with enzyme-linked immunosorbent assays (ELISA), resulting in >90% detection. Benthic cyanobacteria (n = 80) mostly *Nostoc*, *Phormidium*, *Microcoleus*, and *Oscillatoria*, yielded microcystins (76% detection), cylindrospermopsins (41%), anatoxins (45%), and saxitoxins (39%). Plankton net tow samples from tributaries and main stems (n = 94) contained saxitoxins (84%), microcystins (77%), anatoxins (25%), and cylindrospermopsins (22%), revealing their transport in seston. SPATT sampler extracts (n = 146) yielded anatoxins (81%), microcystins (66%), saxitoxins (37%), and cylindrospermopsins (32%), indicating their presence dissolved in the water. Reservoir plankton net tow samples (n = 15), most often containing *Dolichospermum*, yielded microcystins (87%), cylindrospermopsins (73%), and anatoxins (47%), but no saxitoxins. The high detection frequencies of cyanotoxins at sites upstream from DWTP intakes, and at sites popular for recreation, where salmon and steelhead continue to exist, highlight the need for additional study on these cyanobacteria and the factors that promote production of cyanotoxins to minimize effects on humans, aquatic ecosystems, and economies.

Keywords: drinking water; cyanotoxins; harmful cyanobacteria algal blooms (CyanoHABs); benthic cyanobacteria; Cascade Range rivers

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1. Introduction

Harmful cyanobacteria, including planktonic and benthic organisms in lakes, reservoirs, streams, and rivers (Figure 1), are a threat to human and aquatic health [1–3], and may impact fisheries, local, and regional economies [4]. The cyanotoxins they produce include potent neurotoxins and compounds that affect critical cellular functions, affecting liver and kidney functioning as well as being involved in other ailments [5–7]. Illnesses caused by harmful cyanobacteria (CyanoHABs) are not uncommon: the most recent One Health Harmful Algal Bloom System (OHHABS) report cited 372 events in 2021 alone, with 95 human and 102,071 animal illnesses [8]. Large-scale human exposures have also occurred. In 1997, 76 people died in Caruaru, Brazil, after being exposed to cyanotoxins during renal dialysis. Monitoring discovered a CyanoHAB in the drinking water reservoir produced 19.7 μg/L microcystins, causing liver failure in 100 patients [9].

The state of Oregon (USA) has developed guidelines for four cyanotoxins: microcystins, cylindrospermopsins, anatoxins, and saxitoxins [10] (Table S1). The U.S. Environmental Protection Agency (USEPA) has 10-day drinking water guidelines for microcystins and cylindrospermopsins [11] and included all four cyanotoxins in its Fifth Contaminant Candidate List [12].

While blooms of cyanobacteria in lakes and floating blue–green scums are often easily visible to the naked eye, benthic cyanobacteria are less obvious and present a potential danger to the unsuspecting public who may not notice dark mats of cyanobacteria on the bottom or edge of a waterbody or know about their toxicity [6]. *Phormidium*, *Microcoleus*, and *Oscillatoria* mats develop from colonizing filaments, forming "skeins" (Figure 1C,D). Mats may produce and release cyanotoxins during active growth phases [13] and (or) be retained intracellularly and released upon cell lysis [14]. Cycles of accrual and loss occur during the low flow periods, with silts beneath mats [7] and nucleating oxygen bubbles facilitating their periodic detachment.

Cyanotoxins include microcystins and cylindrospermopsins, which are, respectively, inhibitors of protein phosphatases and protein synthesis, that affect the liver, kidneys, and cause other cellular dysfunctions [5,14,15]. A second class are fast-acting neurotoxic cyanotoxins—anatoxins and saxitoxins—which interfere with synapse function and motor control, and can paralyze diaphragm muscles, leading to asphyxiation. Anatoxin-*a* is a small, water-soluble molecule that acts as an agonist of nicotinic acetylcholine receptors [16]. It is degraded by sunlight [17]. Anatoxin-*a* is distinct from anatoxin-*a*-S, now called guanitoxin, which is potentially much more toxic [18]. Multiple studies in New Zealand, Germany, California, and the southwestern United States have found anatoxins associated with benthic mat-forming *Phormidium/Microcoleus* [6,19–21] that are often associated with dog poisonings and deaths [22,23].

Saxitoxins, or paralytic shellfish toxins (PSTs), are produced by dinoflagellates and cyanobacteria; with 57 variations, they are among the most toxic neurotoxins [24]. These carbamate alkaloid toxins block sodium-gated ion channels in nerve synapses, leading to paralysis and respiratory failure [25]. Cyanotoxins released from decaying mats and sloughed biomass [26] can be transported downstream and collect along river margins where dogs and people swim and recreate.

Incidents of cyanotoxin poisonings reveal that benthic *Nostoc*, *Phormidium*, *Microcoleus*, *Oscillatoria*, *Anabaena*, and *Microseira* are often involved [3,6,27,28]. Multiple dog deaths in 2009–2010 at Lawson Bar, South Umpqua River, Oregon, suspected to be due to anatoxins, prompts a year-round advisory [29]. A dog died in 2021 at Zion National Park [1,30], and other fatalities along the Columbia River, Washington [31] were attributed to anatoxins from benthic cyanobacteria [32]. Similar incidents have occurred in New Zealand and 18 other countries [6]. These events highlight that cyanobacteria intoxication can be difficult to avoid when there is no obvious sign of a bloom, and the onset of symptoms can occur rapidly.

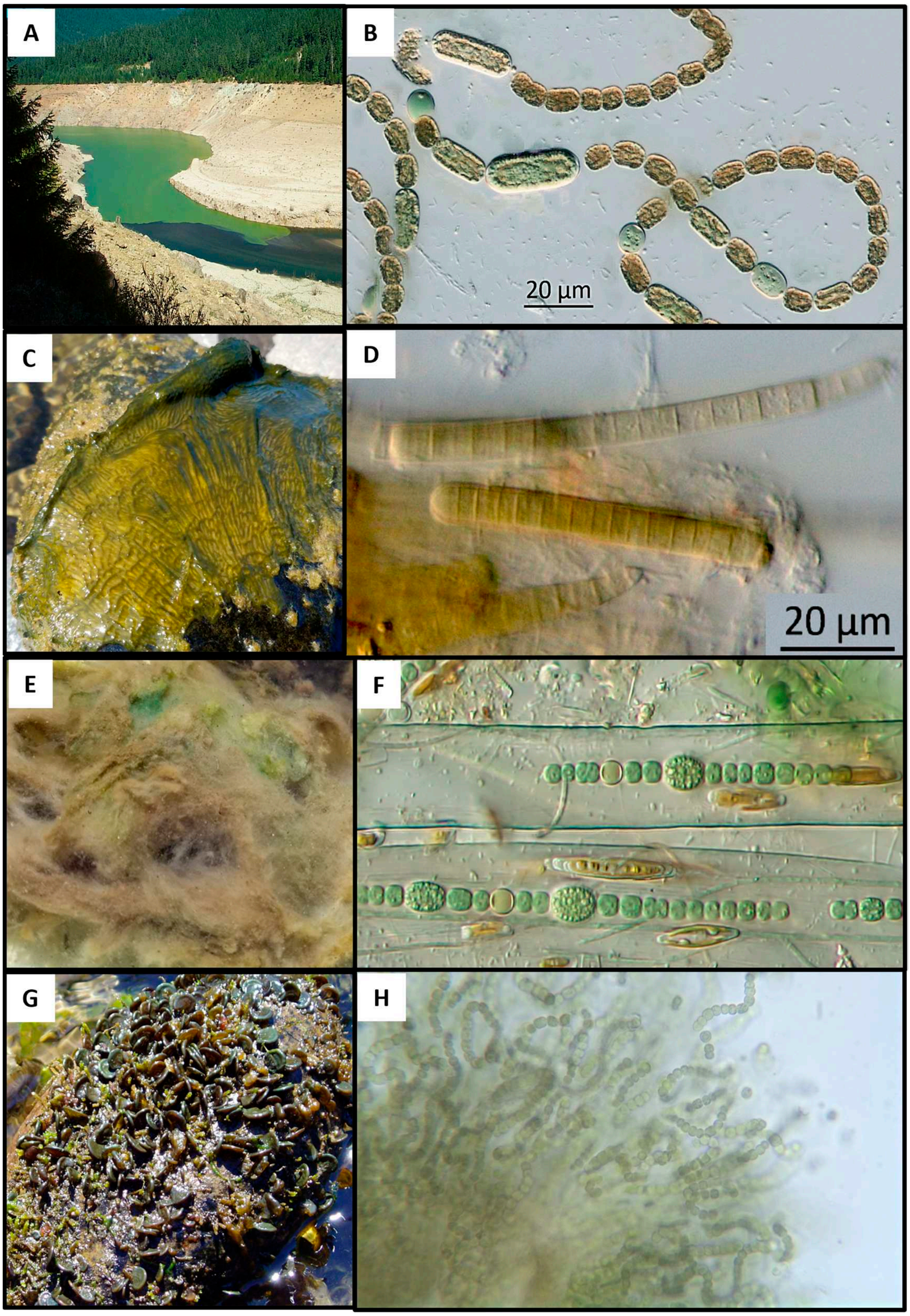

Figure 1. Potentially toxigenic cyanobacteria include (**A**) planktonic blooms in reservoirs such as Cougar Reservoir, Oregon; these are often caused by *Dolichospermum* (**B**). Benthic mats of *Phormidium*/*Microcoleus* (**C**,**D**), cyanobacteria in masses of stalked diatoms (**E**) that harbor filaments of *Anabaena* and *Wollea* (**F**), and colonies of *Nostoc parmelioides* (**G**,**H**) also may produce cyanotoxins. [Photo credits: (**A**) Chauncey Anderson, USGS; (**B**,**D**,**F**,**H**) Barry Rosen, FGCU; (**C**,**E**,**G**) Kurt Carpenter, USGS].

Benthic cyanobacteria were found to produce cyanotoxins in streams and rivers across the U.S. including in the Sierra Nevada [33], the Eel River [34], Russian River [20,35,36], and Klamath River [21]. Microcystins detected in headwater streams of the southeastern U.S. in drainages without reservoirs also point to benthic sources [37]. Given the large number of stream miles, the detection of cyanotoxins in flowing surface waters suggests that benthic cyanobacteria may pose a risk to recreation and drinking water through producing cyanotoxins and taste and odor compounds [38], comparable to planktonic blooms in lakes and reservoirs.

2. Study Background

Oregon's western Cascade Range is drained by several rivers that are part of the National Wild and Scenic River System, including the Clackamas, North Santiam, McKenzie, Middle Fork Willamette, and North Umpqua rivers (Figure 2). These waterways are prized for boating, fishing, and swimming, and are critical sources of drinking water for over half a million Oregonians. They also support populations of endangered salmon and steelhead in their main stems and many tributaries [39].

Most of these rivers are fed by upstream reservoirs (and/or lakes) that develop blooms of planktonic *Dolichospermum* (Figure 1A,B) [40–44]. This cyanobacteria genus has potential to produce multiple cyanotoxins [45,46]. Reservoirs discharge from surface and (or) deep outlets, often for temperature control, and may transport *Dolichospermum* and cyanotoxins downstream to drinking water treatment plants (DWTPs). The downstream outfalls from the reservoirs often harbor populations of filamentous green algae (*Cladophora*), stalked diatoms (*Cymbella* and *Gomphoneis*), along with cyanobacteria (*Nostoc parmelioides* and *N. spongiiforme*), *Phormidium*, and *Oscillatoria* [42,47], which are scoured off the bottom and transported to DWTPs, particularly during sloughing events.

In July 2015, anatoxin-*a* was detected at the City of Lake Oswego's DWTP in the lower Clackamas River. Although upstream reservoirs were a known source of cyanotoxins [42,48], it was plausible that benthic cyanobacteria—previously reported for the Clackamas River [42]—could have contributed. But no information on benthic cyanobacteria was available.

In 2018, Oregon's first drinking water advisory was issued to drinking water providers on the North Santiam River, which serves the capital city of Salem. Concentrations of microcystins and cylindrospermopsins in treated drinking water exceeded state of Oregon guidelines (Table S1) [49]. This unprecedented advisory lasted 35 days, and the Oregon National Guard was called in to provide potable water to residents [50].

The occurrence of cyanotoxins in source water is a challenge due to resistance to oxidation by chlorine (for anatoxins); even boiling is not effective, serving only to concentrate the toxins [51,52]. As a result, advisories due to cyanotoxins typically state that the water is unpotable, and "do not use". Current guidance for water treatment points to maintaining cellular integrity and avoiding damage that could lead to toxin release in the dissolved phase, which is more difficult to remove [53]. Unlike other cyanotoxins that tend to remain inside cells until death, up to half of the cylindrospermopsins may be extracellular [52]. Methods employed to treat cyanotoxins include coagulation, filtration, powdered activated carbon, which has been effective for removal of disinfection by-products and taste and odors [54], chlorination (for some toxins), and ozone. Ozonation is now in use in Oregon at DWTPs for the cities of Salem, Lake Oswego, and Wilsonville, and has thus far been effective for removing cyanotoxins.

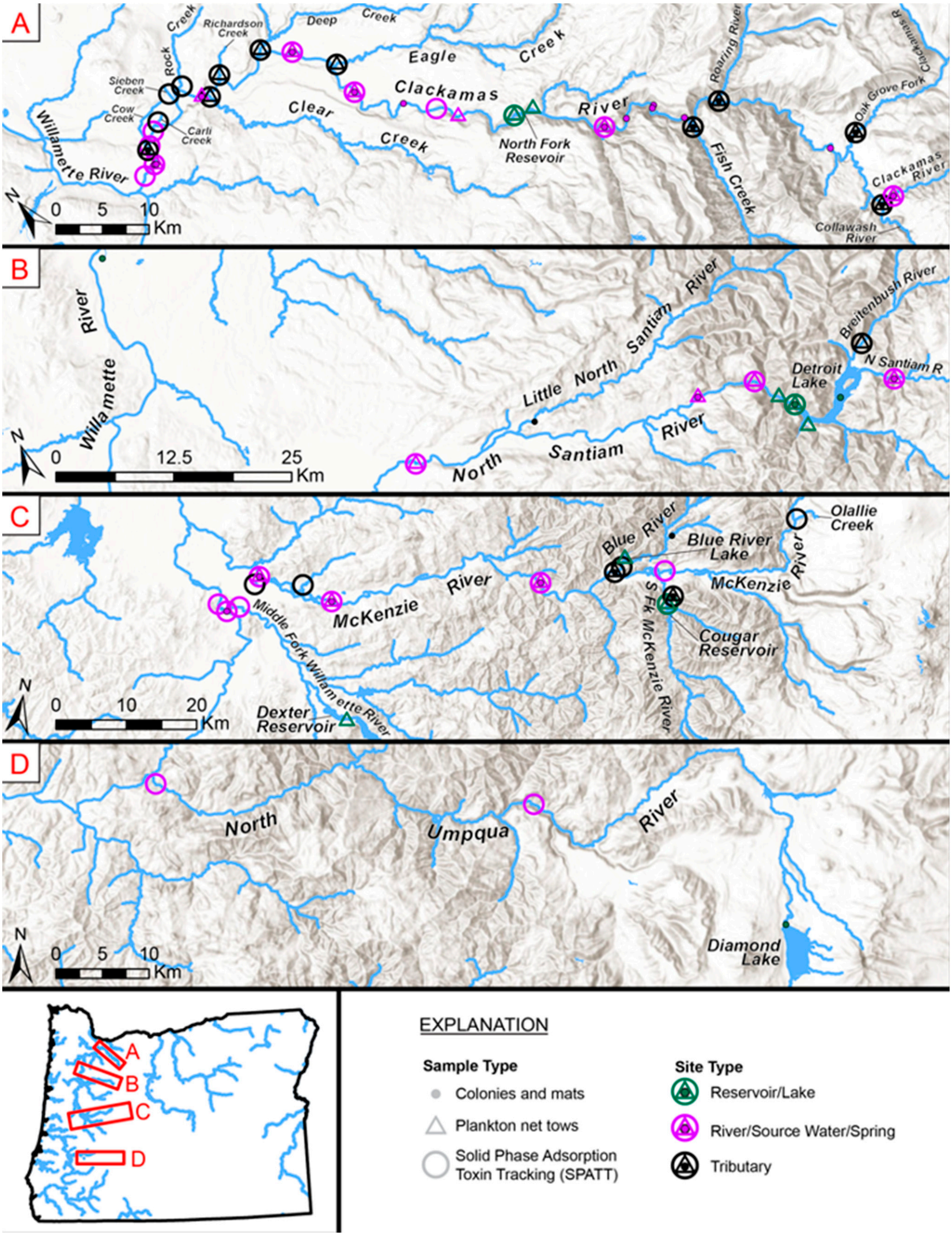

Figure 2. Locations of sampling sites in the (**A**) Clackamas, (**B**) North Santiam, (**C**) McKenzie, Middle Fork Willamette, Coast Fork Willamette, upper Willamette, and (**D**) North Umpqua rivers.

While reservoir CyanoHABs are clearly, at times, a source of cyanotoxins, the sheer amount of habitat suitable for benthic cyanobacteria points to these organisms as potentially more important cyanotoxin producers in the Cascade Range rivers, other Willamette Valley tributaries [55], and perhaps other ecosystems. We provide here the first large-scale assessment of the relative contributions of planktonic, benthic, and the combined sestonic sources of cyanotoxins in transport to downstream DWTPs. We identified cyanobacterial species from multiple habitats using a novel combination of methods—analyzing toxin concentrates from three different media—that goes beyond the conventional strategy of sampling whole water, which often results in sample concentrations below detection. Concentrating extracts increased our ability to detect cyanotoxins and therefore better understand their sources and transport.

This 2016–2020 study of Cascade Range reservoir–river drinking water supplies included sampling of cyanobacteria and testing for cyanotoxins in tributaries, reservoirs, and main stem rivers supplying raw source water to 18 DWTPs. We targeted the late spring, summer, and autumn seasons to understand incidences of CyanoHABs, cycles of growth, and transport of cyanotoxins to DWTPs in seston and dissolved in water.

The study objectives were to:

(1) identify the planktonic and benthic cyanobacteria present and measure concentrations of four cyanotoxins from concentrated "grab" samples of cyanobacteria and plankton net tows;
(2) examine the occurrence of cyanotoxins at or near DWTPs in the dissolved phase using passive samplers; and
(3) characterize the spatiotemporal occurrence and transport of cyanotoxins using multiple lines of evidence from the three methods to identify upstream sources within each of three intensively studied reservoir-river systems.

3. Field and Laboratory Methods

Cyanotoxins were assessed with direct sampling of benthic cyanobacteria colonies and mats, collection of plankton net tow samples, and/or deployment of Solid-Phase Adsorption Toxin Tracking (SPATT) passive samplers at 65 sites in six major river basins (Table S2). Extracts from 320 samples were analyzed for four cyanotoxins using enzyme linked immunosorbent assays (ELISA) [56] on samples collected primarily during the June–August 2016–2020 growing seasons.

Sample Collection, Processing, and Analyses

Benthic Cyanobacteria Colonies and Mats, n = 80. Colonies of *Nostoc parmelioides* and *Nostoc spongiiforme*, and mats of *Phormidium*, *Microcoleus,* and *Oscillatoria* were collected by hand at 32 tributary, main stem, and spring sites (Table S2 and Figure 1). Multiple specimens of each genus—approximately 5 milliliters (mL) of biomass and 5 mL of native water (10 mL per sample)—were composited into 20 mL high-density polyethylene vials. Subsamples were taken for microscopic observation, organisms were identified to genus, photographed, and dominant taxa were determined based on their relative biovolumes.

Plankton Net Tows, n = 90. A 1-ft diameter, 80-µm mesh net was deployed at tributary, reservoir, and main stem river sites near the center thalweg (Figure 3A). Deployments lasted for 1–2 min, adjusting sampling times to not overload the net and make samples from site to site more comparable. Reservoirs were sampled near the dams (Figure 4A) with 10-ft horizontal surface tows, and other locations were sampled by wading out into the current (Figure 4B–D); approximately 10 mL of biomass was collected, evaluated microscopically, and, as with colonies and mats, frozen prior to cyanotoxin analyses.

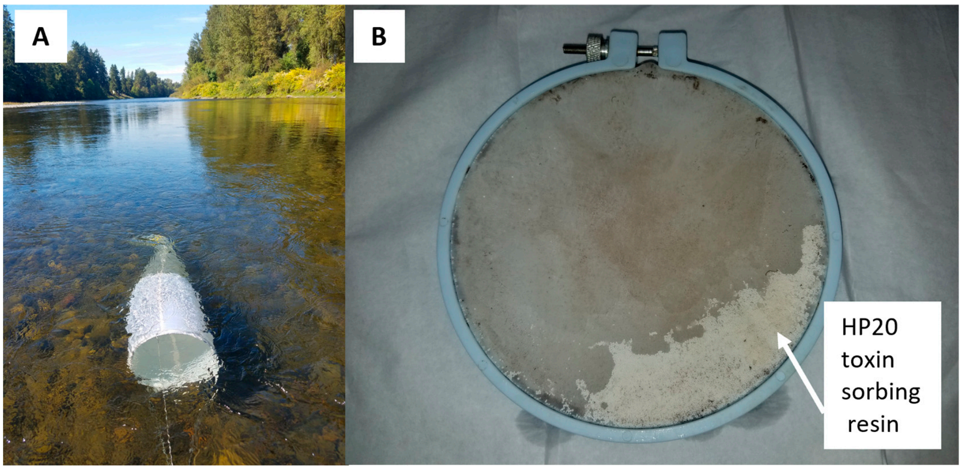

Figure 3. Concentrated extracts from (**A**) plankton net tows, (**B**) Solid-Phase Adsorption Toxin Tracking (SPATT) samplers, and planktonic and benthic colonies and mats of cyanobacteria (Figure 1) were analyzed for four cyanotoxins using ELISA. [Photo credits: Kurt Carpenter, USGS].

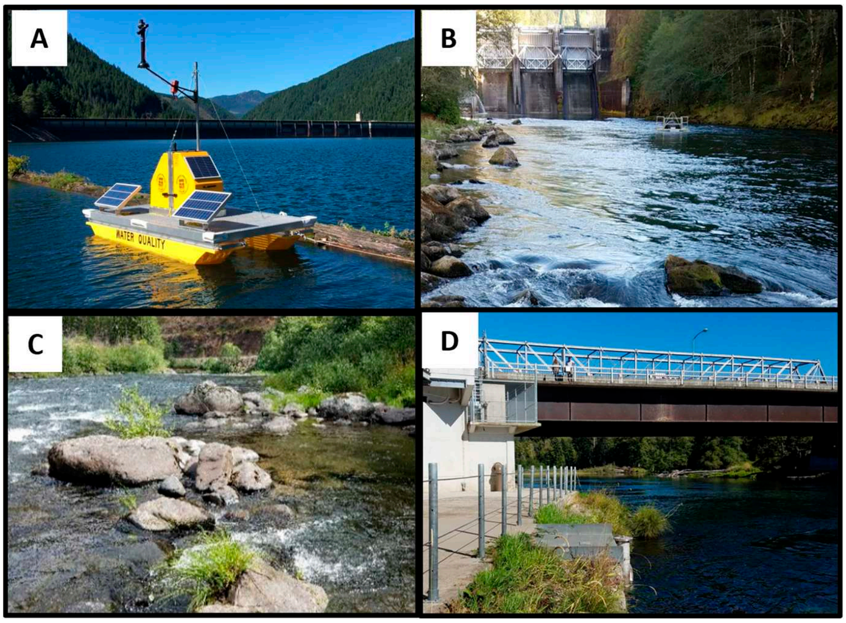

Figure 4. Field sites included reservoirs such as Detroit Lake at the log boom (**A**), reservoir outfalls including the North Santiam River downstream from Big Cliff Dam (**B**), free-flowing main stem sites, such as the Clackamas River (**C**), and sites upstream from drinking water treatment plants, including the Eugene Water & Electric Board (EWEB) intake on the McKenzie River (**D**).

Microscopic Evaluations. Live cyanobacteria were transferred to glass slides and examined using a Leica DM1000 light microscope (Leica, Wetzlar, Germany). Photographs of the dominant organisms were taken with a Zeiss Axiocam Color 208 camera under 400–1000× magnification (Zeiss, Zeiss, Oberkochen, Germany) [56].

Solid-Phase Adsorption Toxin Tracking (SPATT) Samplers, n = 146 (Figure 3B). Passive SPATT samplers were deployed for 5–60 days (average = 25 days) at 43 tributary, main stem, reservoir, and DWTP sites (Table S2) using published methods [57–60]. SPATTs were constructed using 5-in diameter embroidery hoops containing 3 g of Diaion™ HP20® resin enmeshed in 6 in.^{2} sheets of 100 µm Nitex™. The resins were primed with methanol and rinsed in organic-free laboratory grade water and kept hydrated prior to deployment. Diaion™ HP20® been shown to be an effective sorbent for cyanotoxins [61] and extractions from SPATT provide a time-integrated indication of cyanotoxins dissolved in water.

SPATT samplers were deployed submerged under logs, along stream banks, and in deep areas among boulders to sample ambient flow conditions. At DWTPs, SPATT samplers were placed inside water intake structures or suspended in a 3-L glass beaker fed by a raw water tap. SPATT samplers were retrieved and frozen at −18 °C for up to 6 months prior to analysis. Extractions were performed with 50% methanol, and samples evaporated and reconstituted prior to cyanotoxin analyses [56].

Enzyme-Linked Immunosorbent Assays (ELISA) and Data Reporting. Four cyanotoxins were evaluated: total microcystins, cylindrospermopsins, anatoxins, and saxitoxins, at the U.S. Geological Survey (USGS) Oregon Water Science Center laboratory in Portland, Oregon, USA, using ELISA [62–65]. No data quality issues were indicated beyond typical variation for the ELISA method, and there were some replicates that had high variation, as sometimes occurs with ELISA analyses of SPATT sample extracts. There were no detections in field and laboratory blank samples, percent recoveries of quality control checks were within tolerances, and all r-square values for standard curves were >0.99 [56].

Benthic and plankton net tow samples underwent 3 freeze–thaw cycles and filtration prior to analysis. The resulting extract concentrations, reported in micrograms per liter (µg/L), were not normalized to area, weight, or volume, and are not comparable to guidelines for concentrations in water used for recreational purposes or drinking water (Table S1). The samples are comparable among themselves, however, because, as a group they were processed similarly.

SPATT sample extract concentrations represent the average time-weighted toxin concentrations reported in nanograms of toxin per gram of resin per day (ng toxin$\cdot$g^{-1}d^{-1}). This does not account for the potential losses of toxin through degradation or re-equilibrium with the surrounding water. While they are reliable indicators of the presence or absence of cyanotoxins, these reported concentrations are also not comparable to concentrations in water, but they are comparable among themselves because methods were consistent and adjusted for differences in deployment duration, and they may be useful to understand spatial and temporal patterns in cyanotoxin occurrence.

4. Results

Several genera of potentially toxigenic cyanobacteria were identified during this study. The most common were benthic colonies of *Nostoc* (*N. parmelioides* and *N. spongiiforme* Figure 1E,F) and mats of filamentous *Phormidium*, *Microcoleus*, and *Oscillatoria*. Planktonic *Dolichospermum* was found in multiple reservoirs (Figure 1A,B). *Nostoc parmelioides* was most common at upper-basin sites, whereas *Phormidium*, *Microcoleus*, and *Oscillatoria* occurred at sites in the main stem and tributaries draining forests, urban, and mixed land uses. These mats were common on boulders, river cobbles, and soft sediments in undisturbed depositional habitats along river margins.

4.1. Cyanotoxin Occurrence

One or more cyanotoxins were detected in over 90% of the 320 samples [56]. Cyanotoxins were found in plankton net tows (93%), benthic colonies and mats (91%), and SPATT samplers (89%). About two-thirds (68%) of samples yielded more than one cyanotoxin, and all four cyanotoxins were detected in 15% of samples overall, representing all sample types.

Cyanotoxin concentrations varied depending on the sample type and waterbody, but overall, 73% of sample extracts contained microcystins, about half contained anatoxins and/or saxitoxins (47–57%), and 33% contained cylindrospermopsins (Table 1). Granular data for each of the three most studied river basins—the Clackamas, North Santiam, and McKenzie River basins—are shown in Tables S3–S5.

Table 1. Percent detection of four cyanotoxins by sample type and waterbody.

Sample Type/Waterbody	Number of Samples (*n*)	Microcystins	Cylindrospermopsins	Anatoxins	Saxitoxins
All samples	320	73%	33%	57%	47%
Benthic cyanobacteria	80	76%	41%	45%	39%
Plankton net tows	94	79%	30%	29%	70%
SPATT samplers	146	66%	32%	81%	37%
Benthic cyanobacteria (*n* = 80)					
Clackamas River Basin	56	73%	45%	50%	45%
North Santiam River Basin	4	100%	100%	50%	17%
McKenzie River Basin	19	80%	25%	30%	25%
Coast Fork Willamette	1	100%	0%	100%	0%
Plankton net tows (*n* = 94)					
Reservoirs	15	87%	73%	47%	0%
Mainstems and Tributaries	79	77%	22%	25%	84%
Clackamas River Basin	51	78%	6%	10%	73%
North Santiam River Basin	11	56%	78%	33%	56%
McKenzie River Basin	29	86%	54%	57%	86%
Dexter Reservoir	1	100%	0%	0%	0%
Diamond Lake	2	50%	0%	50%	0%
Solid-Phase Adsorption Toxin Tracking (SPATT) samplers (*n* = 146)					
Clackamas River Basin	70	59%	9%	77%	16%
North Santiam River Basin	17	88%	88%	94%	82%
McKenzie River Basin	39	67%	41%	79%	46%
Upper Willamette River	3	33%	0%	67%	0%
Middle Fork Willamette	5	80%	0%	80%	80%
Coast Fork Willamette	3	33%	0%	67%	0%
North Umpqua River	9	100%	100%	100%	100%

Benthic Cyanobacteria Colonies and Mats. Colony and mat-forming cyanobacteria (Figure 1C–H) yielded one or more cyanotoxins in 91% of 80 samples. Samples of *Nostoc*, *Phormidium* and (or) *Microcoleus*, and *Anabaena* (benthic) yielded four cyanotoxins (Figure 5). Extract concentrations from benthic cyanobacteria were highest for microcystins and anatoxins, with lesser cylindrospermopsins and saxitoxins; the highest concentrations came from *Nostoc parmelioides* (Figure 6). Microcystins were detected more frequently (76%), than anatoxins (45%), cylindrospermopsins (41%), and saxitoxins (39%) (Table 1).

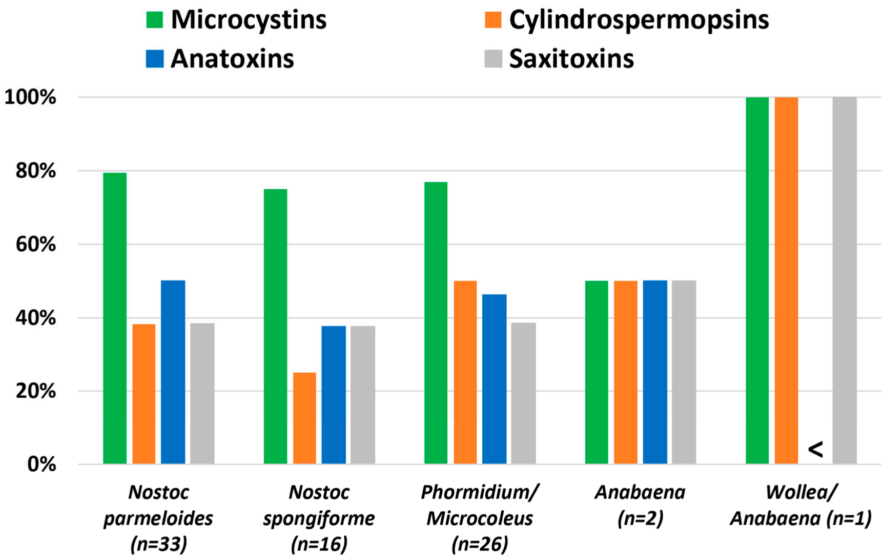

Figure 5. Frequency of detection of four cyanotoxins in extracts from benthic cyanobacteria colonies and mats. (<, anatoxins not detected).

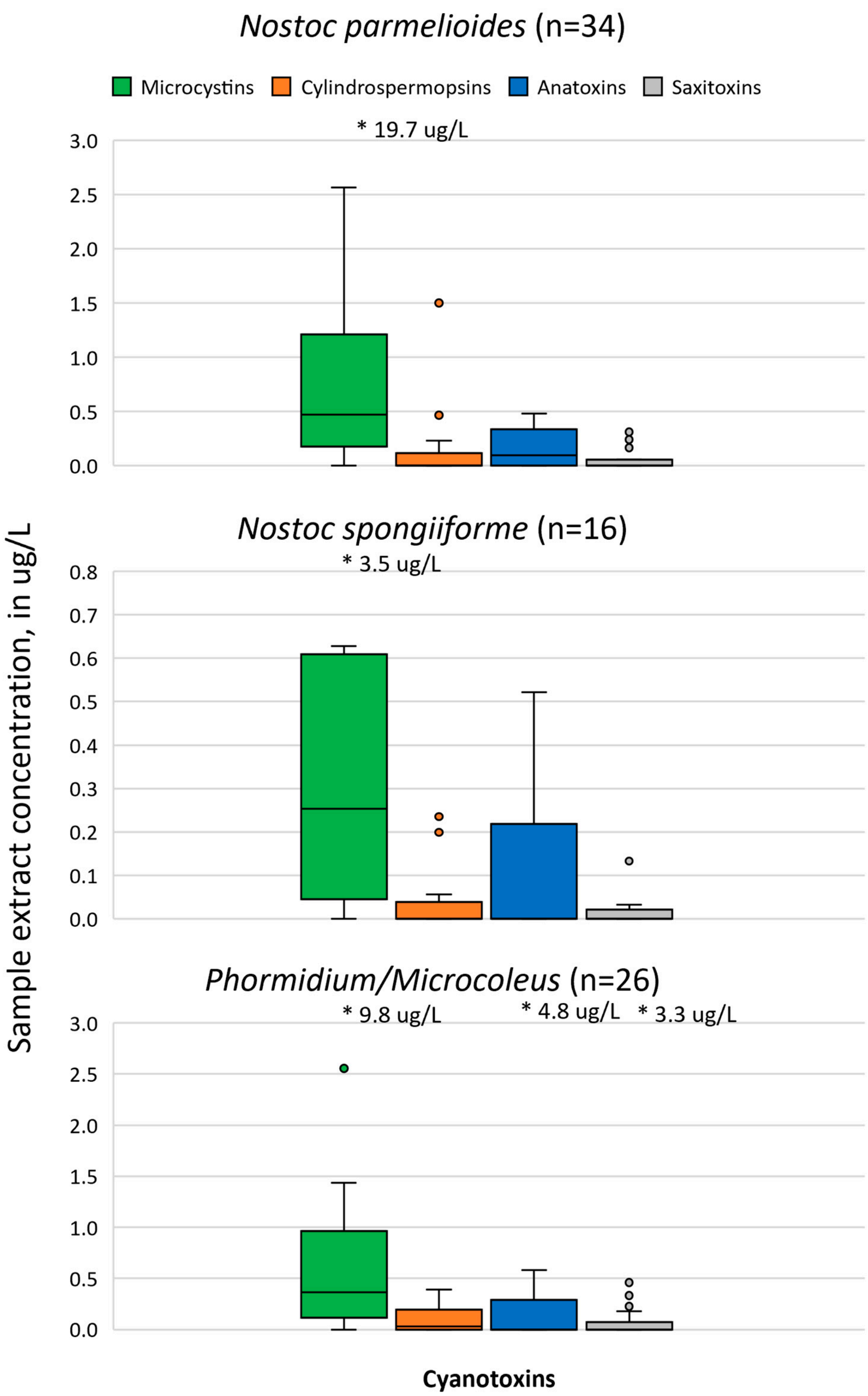

Figure 6. Box plots of cyanotoxin concentrations from three types of benthic cyanobacteria colonies and mats. (Box plots show 10th, 25th, 50th, 75th, and 90th percentiles; outliers shown as individual points; values below laboratory reporting limits were set to zero; * indicates maximum (off-scale) concentration; note variable y-axes).

Benthic *Anabaena* yielded microcystins, cylindrospermopsins, anatoxins, and saxitoxins (Figure 5), while *Anabaena* and *Wollea* found among stalked diatom masses of *Cymbella* and *Gomphoneis* (Figure 1E) yielded three cyanotoxins (Figure 5).

The highest concentration of anatoxins from a mat came from the Coast Fork Willamette River, where 4.8 μg/L was measured in a sample dominated by *Phormidium* and (or) *Microcoleus*. Other locations, including the Clackamas River at Estacada (at Milo McIver State Park), and the McKenzie River upstream of the Eugene Water & Electric Board (EWEB) DWTP intake (Figure 4D), yielded relatively high concentrations of anatoxins in similar benthic sample extracts.

The highest concentrations of cylindrospermopsins came from *N. parmelioides* (Figure 1G,H) and *Wollea saccate* and *Anabaena* (contained within a stalked diatom mat, Figure 1E,F) in the upper Clackamas River at Mile Post 37, and in *Phormidium* and (or) *Microcoleus* mats from the North Santiam River downstream of Detroit Lake, ranging from 0.4 to 1.5 µg/L [56]. Most of the highest saxitoxin concentrations, up to 0.3–3.7 µg/L, came from extracts of *N. parmelioides*, *Phormidium* and (or) *Microcoleus*, and stalked diatom mats containing *Wollea* and *Anabaena*, all from the Clackamas River basin—the upper Clackamas, Collawash, and Roaring rivers.

Plankton Net Tows. Plankton net tow samples from reservoirs contained *Dolichospermum* (*n* = 11), *Gloeotrichia* (*n* = 4), and *Aphanizomenon* (*n* = 2) [56]. These samples yielded microcystins (87%), cylindrospermopsins (73%), and anatoxins (47%); saxitoxins were not detected in reservoirs (Table 1). Extract concentrations were highest for microcystins >> cylindrospermopsins > anatoxins (Figure 7).

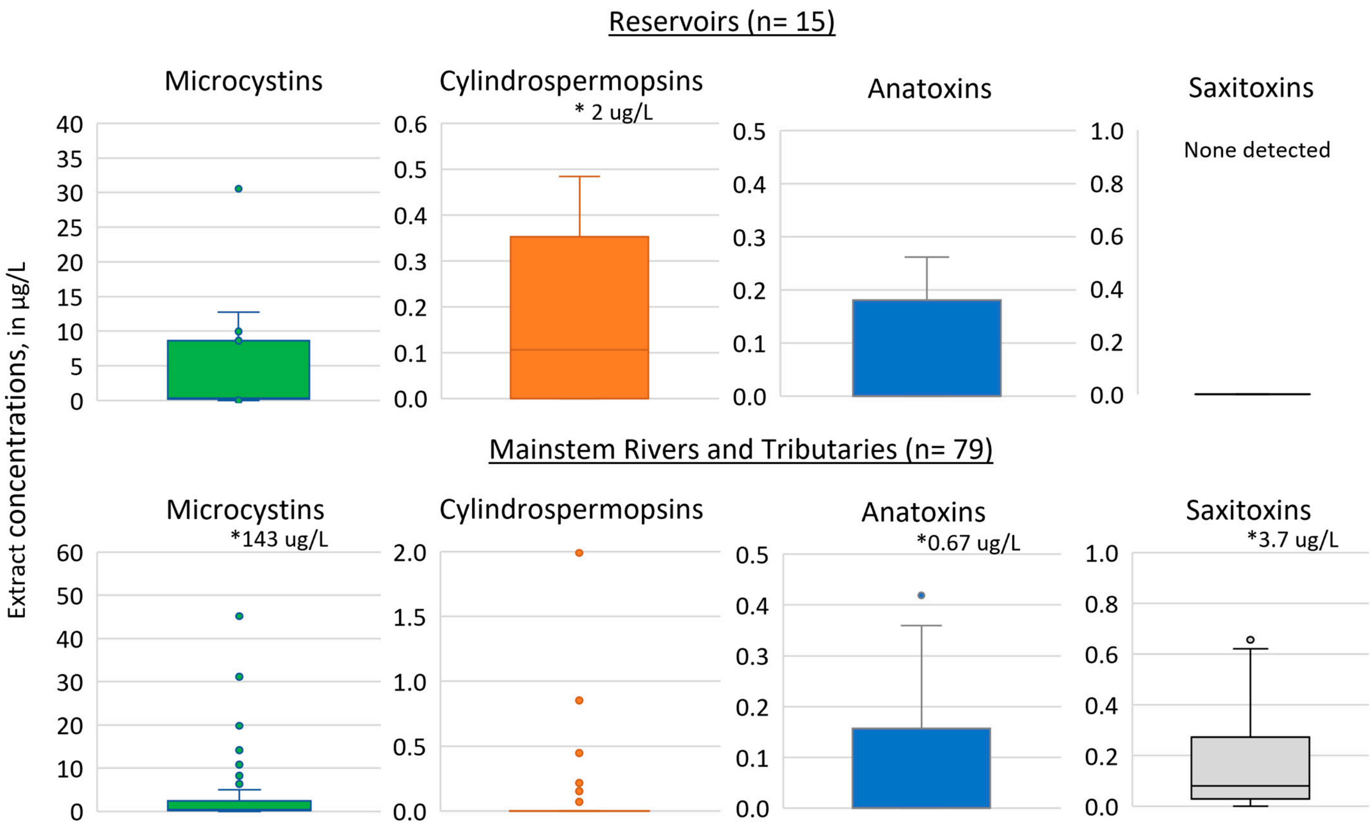

Figure 7. Box plots of cyanotoxin concentrations from plankton net tow sample extracts. (Box plots show 10th, 25th, 50th, 75th, and 90th percentiles; outliers shown as individual points; values below laboratory reporting limits were set to zero; * indicates maximum (off-scale) concentration; note variable y-axes.).

Plankton net tows from tributary and main stem (lotic) sites contained *Tolypothrix* (*n* = 48), *Nostoc* (*n* = 40), *Phormidium* and (or) *Microcoleus* (*n* = 31), *Anabaena* (*n* = 21), *Oscillatoria* (*n* = 20), and *Rivularia* (*n* = 25), all typically benthic taxa. *Nostoc* and *Tolypothrix* (Figure 8) were observed microscopically in about half of these samples. Plankton net tow samples yielded microcystins (77%), saxitoxins (84%), anatoxins (25%), and cylindrospermopsins

(22%) (Table 1). Extract concentrations were highest for microcystins >> cylindrospermopsins > saxitoxins > anatoxins (Figure 7). Saxitoxins were detected in 86% of plankton net tows from the McKenzie River basin (Table 1). The occurrence of saxitoxins (and microcystins) in the plankton net tows was often associated with microscopic observations of *Tolypothrix* and early stages of *Nostoc* (Figure 8).

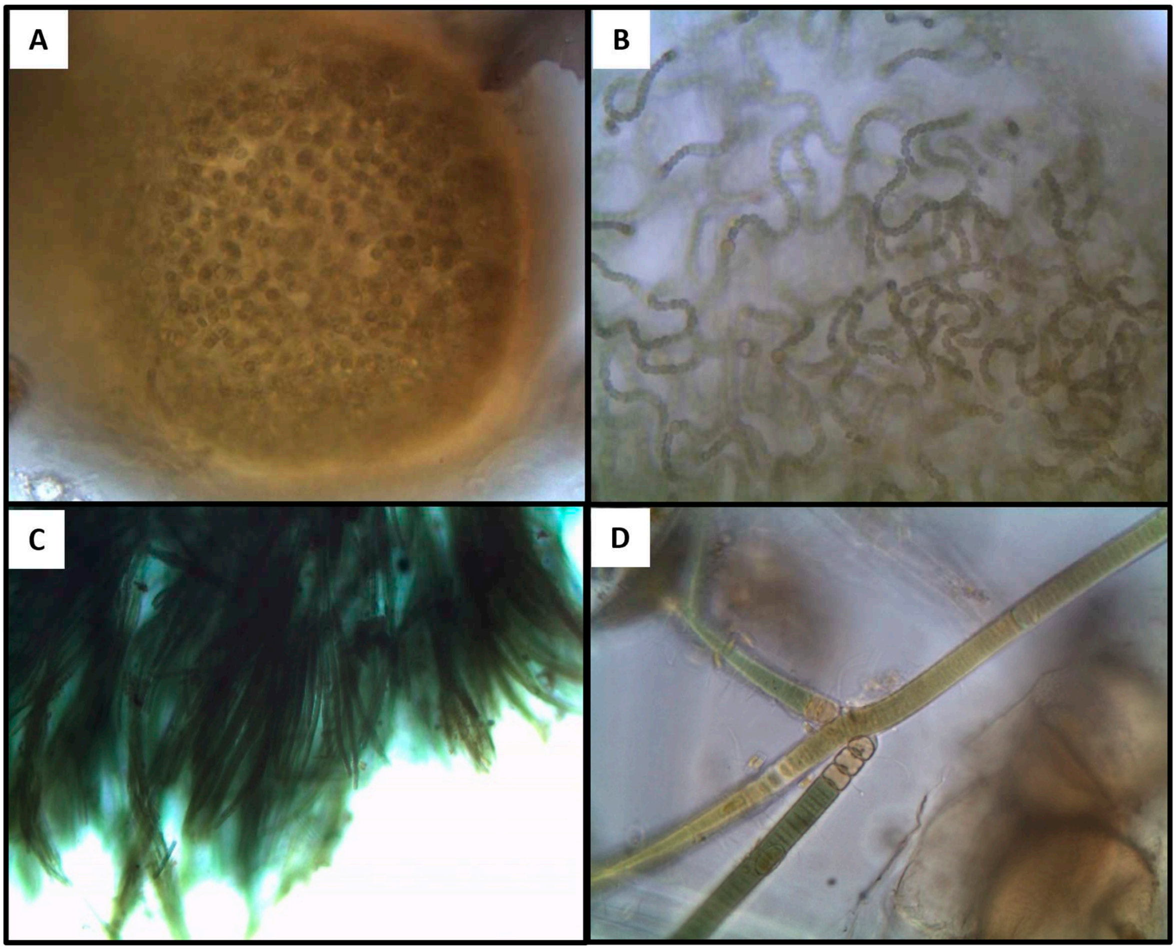

Figure 8. Benthic cyanobacteria common in plankton net tow samples included *Nostoc* (**A**,**B**) and *Tolypothrix* (**C**,**D**).

The longitudinal concentrations of cyanotoxins in plankton net tow samples from the North Santiam and McKenzie Rivers from four dates in 2018 are shown in Figures S1 and S3. Export of cyanobacteria and cyanotoxins from Detroit Lake, Cougar Reservoir, and Blue River Lake was apparent, and multiple cyanotoxins were found downstream at DWTP intakes in extracts from SPATT samplers, plankton net tows, and sometimes in whole-water samples collected by the drinking water utilities (Tables S4 and S5).

Solid-Phase Adsorption Toxin Tracking (SPATT) Samplers. Cyanotoxins were detected in SPATT sampler extracts in 91% of deployments (n = 146) at all 43 sites (Table S2). SPATT samplers at or upstream of DWTPs (n = 56) (Table 2) yielded anatoxins (79%), microcystins (64%), saxitoxins (32%), and cylindrospermopsins (23%), mirroring toxin occurrence in these watersheds based on the other two methods.

SPATT samplers produced the highest detection frequency for anatoxins (81%) (Table 1), which were detected in 50–100% of SPATT samples from these rivers (Table 2). Locations where concentrations of anatoxins exceeded the 90th percentile included: the North Fork Reservoir and downstream DWTPs on the Clackamas River (at Estacada and Oregon City); the upper North Santiam River and Detroit Lake; the Coast Fork Willamette

River and the upper Willamette River downstream from the confluence of the Coast and Middle Forks [56], where a future drinking water intake is being considered.

Table 2. Cyanotoxin occurrence in extracts from Solid-Phase Adsorption Toxin Tracking (SPATT) samplers deployed at or upstream from DWTP intakes.

Drinking Water Sources	Number of SPATT Samplers	Percent Detection			
		Microcystins	Cylindrospermopsins	Anatoxins	Saxitoxins
Clackamas River	31	52%	10%	77%	13%
North Santiam River	2	100%	100%	50%	100%
McKenzie River	9	78%	22%	78%	33%
Middle Fork Willamette River & Upper Willamette River	8	63%	0%	75%	50%
North Umpqua River	5	100%	100%	100%	100%

Average concentrations of cyanotoxins in SPATT sampler extracts varied by site type and location (Tables 2 and 3) and season (Figure 9). Concentrations of microcystins, anatoxins, and saxitoxins in Detroit Lake increased through the summer, peaking in July and August, whereas cylindrospermopsins peaked earlier in the season (Figures 9 and S2).

Table 3. Average concentrations of four cyanotoxins in sample extracts from benthic mats and colonies of cyanobacteria, plankton net tows, and Solid-Phase Adsorption Toxin Tracking (SPATT) samplers, by site type. [Cyanotoxin concentrations in benthic mats and plankton net tows derived from sample extracts, in micrograms per liter (µg/L), and from SPATT sampler extracts in ng/g/day; grey shaded cells highlight > 90th percentile concentrations; Abbreviations: *n*, number of samples; ag, agricultural land use; <, not detected] .

Site Types/Dominant Land Use	Microcystins	Cylindrospermopsins	Anatoxins	Saxitoxins
Concentration, in µg/L				
Benthic cyanobacteria				
Forested tributary (n = 13)	1.8	0.05	0.24	0.29
Spring (n = 1)	0.0	0.19	0.00	0.00
Upper mainstem (n = 22)	1.7	0.14	0.13	0.37
Reservoir outflow (n = 5)	0.2	0.11	0.13	<
Mixed ag/private/industrial (n = 2)	3.3	<	1.1	0.01
Lower mainstem/raw water (n = 37)	1.2	0.07	0.24	0.04
Plankton net tows				
Forested tributary (n = 8)	1.4	<	<	0.11
Upper mainstem (n = 24)	5.7	0.12	0.02	0.72
Reservoir (n = 14)	4.6	0.51	0.17	<
Reservoir outflow (n = 10)	0.21	1.11	0.32	0.47
Mixed ag/private/industrial (n = 5)	0.07	<	<	<
Lower mainstem/raw water (n = 32)	6.3	0.23	0.05	0.06
Solid-Phase Adsorption Toxin Tracking (SPATT)				
	Concentration, in ng/g/day			
Forested tributary (n = 15)	0.04	0.001	0.02	0.0005
Upper mainstem (n = 32)	0.02	0.002	0.12	0.0016
Reservoir (n = 14)	137	0.17	0.34	0.0012
Reservoir outflow (n = 12)	0.07	0.03	0.03	0.0014
Mixed ag/private/industrial (n = 14)	0.01	<	0.01	0.0002
Lower mainstem/raw water (n = 59)	0.36	0.01	0.07	0.005

Although extracts from SPATT samplers deployed in the upper reaches of the North Santiam and Clackamas Rivers had detections of all four cyanotoxins, the highest concentrations for all but saxitoxin were from reservoirs (Table 3). The highest concentrations of anatoxins, 2.9 and 2.5 ng/g/d, respectively, were from North Fork Reservoir, in the Clackamas River basin, and concurrently at the Estacada DWTP, located six miles downstream, in September 2016. This occurred during a bloom of *Dolichospermum lemmermannii* and

D. circinale, when extract concentrations were nearly five times higher than in all other samples [56].

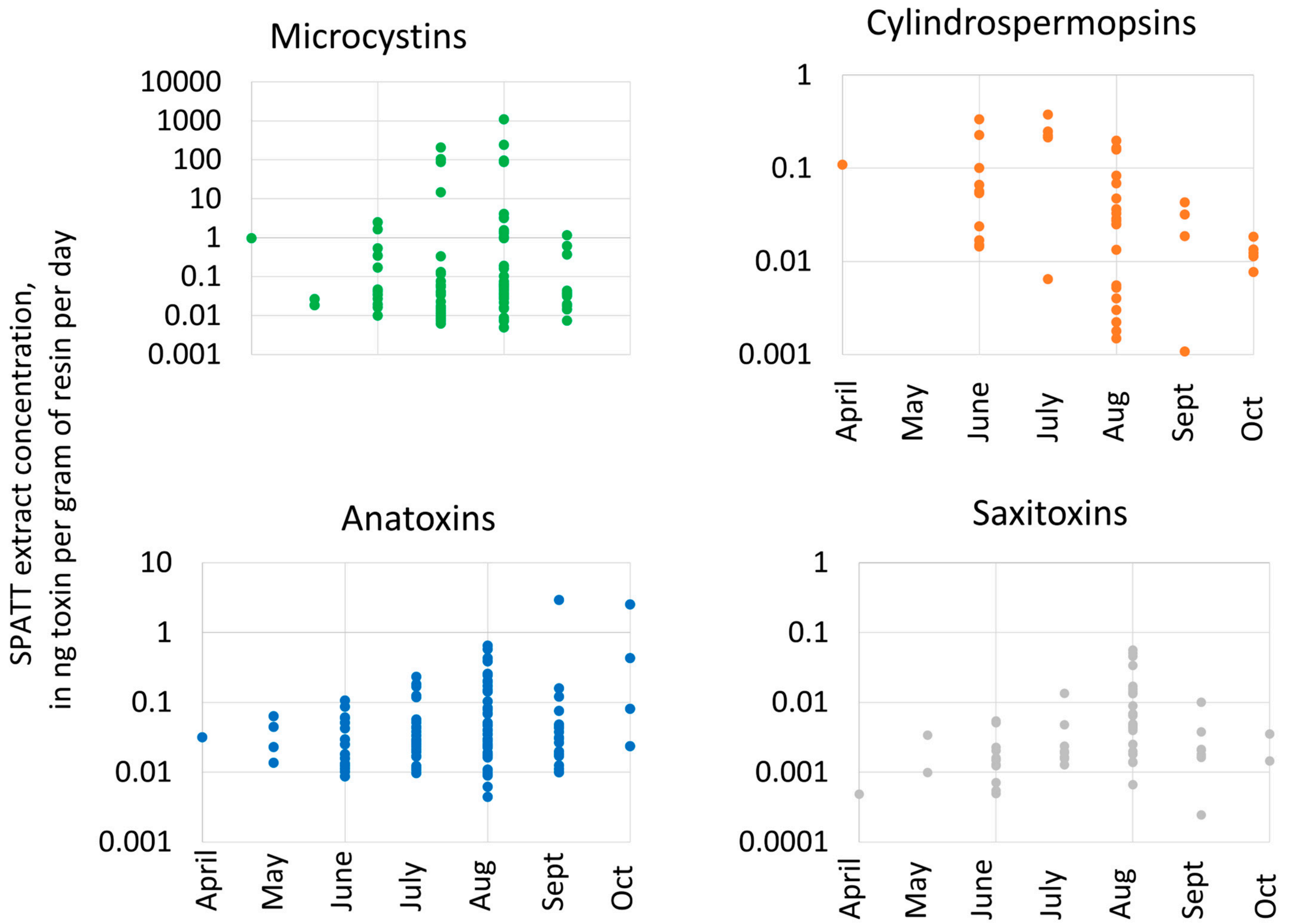

Figure 9. Monthly concentrations of four cyanotoxins in Solid-Phase Adsorption Toxin Tracking (SPATT) sampler extracts.

The highest concentrations of microcystins and cylindrospermopsins in SPATT sampler extracts came from Detroit Lake during a *Dolichospermum* bloom in 2020 (Figure 9). All four cyanotoxins were consistently detected in SPATT sampler extracts from the North Umpqua River (Figure 2 and Table 2), at concentrations exceeding the 90th percentile for microcystins, anatoxins, and saxitoxins [56] at two main stem sites downstream from Soda Springs Reservoir, Lemolo, and Diamond Lakes. Saxitoxins were detected less often in SPATT samplers from the Clackamas (13% of source water samples, Table 2) compared with other basins, despite being sampled more frequently (Table S2). This contrasted with our finding relatively high concentrations of saxitoxin in benthic samples of *Phormidium/Microcoleus* and *Nostoc parmelioides* in the Clackamas [56].

4.2. Sources of Cyanotoxins at Three Water Supply Intakes

All sampling sites were upstream of existing or future planned DWTPs, so any cyanotoxins detected represents a potential source to drinking water supplies. To assign toxin production to the organisms identified microscopically, literature reports were queried against our detections (Table 4). These pointed to planktonic *Dolichospermum* in North Fork and Cougar Reservoirs, Detroit, Blue River, Diamond, and Dexter Lakes, and benthic *Nostoc*, *Phormidium* and (or) *Microcoleus*, *Oscillatoria*, *Anabaena*, *Wollea*, and *Tolypothrix* in discrete benthic samples and plankton net tows, as potential cyanotoxin producing organisms.

Table 4. Cyanotoxin detections in benthic mats and colonies of cyanobacteria, in masses of stalked diatoms, and in plankton net tows from reservoirs and rivers compared with literature reports. [x = literature reports of toxin production by cyanobacteria. Grey shaded cells show toxin detection during this study by the cyanobacteria indicated].

Cyanobacteria	Number of Samples	Microcystins	Cylindrospermopsins	Anatoxins	Saxitoxins
Reservoir plankton net tows					
Aphanizomenon	2	x [1]	x [1]	x [1]	x [1]
Dolichospermum	33	x [1,2]	x [1,2]	x [1,2]	x [1,2]
Gloeotrichia	7	x [2,3]	--	--	x [1,2]
Benthic cyanobacteria					
Cylindrospermum	1	x [2]		x [1,2]	x [1]
Nostoc parmelioides	34	x [1,2]	--	--	--
Nostoc spongiiforme	16	x [1,2]	--	--	--
Oscillatoria	1	x [1,2]	x [1,2]	x [1,2]	--
Phormidium/Microcoleus	26	x [1,2]	x [2]	x [1,2]	x [1,2]
Mixed assemblage in riverine plankton net tows					
Nostoc	41	x [1,2]		x [3]	-- [4,5]
Tolypothrix	48	x [1,2]		-	x [5,6]
Mixed assemblage in stalked diatom masses					
Anabaena	4	x [2]	x [2]	x [2]	x [2]
Wollea saccata	1	--	--		--

[1] [66]; [2] [33]; [3] [18]; [4] [67]; [5] The highest 23 of 25 saxitoxin concentrations contained *Tolypothrix* and *Nostoc*; [6] [68].

Cyanotoxin detections at the DWTPs, based on SPATT sampler extracts and water samples collected by the water utilities, were compared with upstream detections (in any/all media) (Tables S3–S5). The deployment of SPATT samplers provided an extensive dataset to evaluate cyanotoxin presence in raw source water entering DWTPs, with detections of one or more toxins in all drinking water sources assessed (Table 2). In all cases where toxins were detected in source water, there were corresponding upstream benthic or reservoir sources, and (or) occurrence in plankton net tow samples, revealing their transport downstream to these locations.

5. Discussion

This 2016–2020 study found widespread occurrence of cyanotoxins associated with planktonic and benthic cyanobacteria, with cyanotoxins detected in all river basins, site types, and land uses assessed, including frequent detection at DTWPs serving over half a million Oregonians. Although sampling was not exhaustive, the most common cyanobacteria associated with cyanotoxins were planktonic *Dolichospermum* and benthic *Nostoc*, *Phormidium* and (or) *Microcoleus*, *Oscillatoria*, *Tolypothrix*, and *Anabaena/Wollea* (Table 4 and Figure 6).

The finding of cyanotoxins in these river basins is partly attributed to the sensitive nature of our sampling methods, with concentrated extracts producing detection of cyanotoxins when analyses of traditional whole-water samples rarely find them [69]. Our combination of benthic surveys and sampling for toxins in colonies and mats and concentrating samples with plankton net tows and deployment of passive SPATT samplers demonstrates their widespread occurrence and transport downstream to drinking water intakes. This study serves as a warning about the presence of these toxins and sheds light on the organisms and sources in these river basins, which include pristine streams and those draining urban, agricultural, and forestry land uses.

5.1. Cyanobacteria and Cyanotoxin Production in the Cascade Range

Detecting cyanotoxins in these reservoir–river system raises important questions about the extent of their occurrence and seasonality, their sources (dominant producers), why they occur, and the risks to humans and other species. Although the reasons why cyanobacteria produce toxins is not well understood, explanations for their success and widespread distribution in these Cascade Range river basins relate to the exceptionally clear water that allows light for high rates of photosynthesis, along with available phosphorus (P), naturally occurring in the region from volcanic soils [70,71]. The tributaries from the higher portions of the Cascade Range are largely spring-fed and naturally enriched with P, and much of the streamflow in summer is derived from groundwater that has interacted with P-rich volcanic rocks [72]. Although these Cascade Range rivers are generally considered pristine, much of the upland forests were logged decades ago [42], which may have enhanced transport of P to downstream rivers and reservoirs.

Nutrient uptake by benthic algae and cyanobacteria can lead to nuisance biomass conditions [42] with associated diel swings in pH and dissolved oxygen from photosynthesis [73]. Such high biomass can reduce inorganic N to limiting concentrations [42,74–76]. One laboratory study [13] found higher anatoxin production in cultures of *Phormidium* grown on N-depleted media, and these low N concentrations may help explain its frequent detection in these Cascade Range rivers. *Phormidium* and (or) *Microcoleus* was found in streams with paradoxically low P concentrations (<0.01 mg/L) [7] similar to some tributaries draining the western Cascade Range [42].

Planktonic Cyanobacteria in Reservoirs. The highest concentrations of microcystins, cylindrospermopsins, and anatoxins (based on SPATT sampler extracts) were associated with blooms of *Dolichospermum* in North Fork and Cougar Reservoirs, Detroit, Dexter, Diamond, and Blue River Lakes (Figure 2, Tables 1 and 3). CyanoHABs develop in these Cascade Range waterbodies, where they may produce cyanotoxins [41–43], sometimes at levels of concern in Detroit, Dexter, and other lakes [77]. Diamond Lake, in the headwaters of the North Umpqua River basin, has a history of *Dolichospermum* blooms [43,78], which is consistent with our finding microcystins and anatoxins in October 2020 at two sites downstream in the main stem [56], which are located downstream from multiple reservoirs.

Genetic studies are elucidating strain-specific production by *Dolichospermum* [44–46] and determining their phylogenetic relationships. The seasonal pattern in cyanotoxin production (Figure S3) reveals peaks that may correspond to different strains of *Dolichospermum*. Concentrations of cylindrospermopsins tripled from late May to early June 2020, reaching another peak in mid-July. Dreher et al. [47] identified two strains of *Dolichospermum* in Detroit Lake, including one that produces cylindrospermopsins early in the spring season, termed DET69 (*D. lemmermannii*) [79,80], which could have been the producer in our 2020 samples. A second species, termed DET73 (*D. flos-aquae*), has smaller akinetes and often produces none, has a different cellular arrangement from D. *lemmermannii*, and may have produced the peak in microcystins in August 2020 [81]. *Dolichospermum* is also capable of producing anatoxins [51,82] and SPATT sampler extracts revealed multiple peaks of anatoxins in the lake (Figure S3), but specific producers were not confirmed.

The success of *Dolichospermum* in the Cascade Range reservoirs can be partly attributed to its ability to fix N [82]. Planktonic cyanobacteria also have gas vesicles (aerotopes) for buoyancy regulation [82,83], allowing *Dolichospermum* to optimize its position between the well-lit surface and deeper waters where nutrients are often more available. The production and use of carbohydrates determines the size of the aerotopes, and therefore, the buoyancy and ultimate position of a colony or bloom [84,85]. Populations may form distinct layers several meters below the surface as seen in Detroit and Blue River Lakes [86]. Although out of view, they can rise to the surface unexpectedly and form scums that may present a risk to the public through recreation. These blooms also threaten drinking water supplies when discharged downstream.

These blooms have large inter-annual variability spanning orders of magnitude. Spring rains and snowmelt, or extended periods of inclement weather, can delay the growing season with high streamflows that shorten residence times in the reservoirs [87,88]. Conversely, quick spring warm-ups can stimulate early-season blooms, particularly when coupled with reduced streamflows. Earlier, more intense, and longer CyanoHAB seasons have been observed in large lakes across the globe over the past few decades [89], which might also produce larger blooms of toxigenic *Dolichospermum* species in large reservoirs such as Detroit Lake as described above.

Contributing to blooms in these Oregon lakes and reservoirs are long, often cloudless summer days and clear waters that allow deep penetration of sunlight for growth of phytoplankton. The seasonal development of a stratified water column sets the stage for the summer growing season. Surface water temperatures in Detroit Lake at the log boom, for example, can exceed 20 °C during summer, while the bottom hypolimnion temperatures can be 6 °C or less [87]. The resulting thermal stratification favors buoyant cyanobacteria that can take advantage of higher growth rates in the warm water, while other phytoplankton sink [88].

Once a CyanoHAB forms, dam operations may inadvertently release cyanobacteria and cyanotoxins downstream to DWTPs, as occurred during the 2018 drinking water advisory for the City of Salem and other water users (Figure S1), although these data in late July were obtained several weeks after the peak. Plankton net tows from the McKenzie River basin reservoir outflow sites at this same time also contained *Dolichospermum*—along with all four cyanotoxins (Figure S3)—and these were tracked downstream into the main stem McKenzie River and EWEB's source water (Table S6), but no cyanotoxins were detected in the finished (treated) drinking water. In 2024, another *Dolichospermum* bloom occurred in Detroit Lake, leading to another recreational CyanoHAB advisory for the lake and downstream North Santiam River that lasted for over seven weeks [77]. The longitudinal increase in microcystins suggests origins in benthic as well as planktonic cyanobacteria.

CyanoHABs and their toxins discharged from reservoirs may persist for great distances, as documented in the Kansas River following a bloom in Milford Lake; cyanotoxins were transported nearly 175 miles [90]. Wetland discharges also may contain inoculums of CyanoHABs, along with nutrients to fuel downstream blooms, as occurred in 2008 in the Tualatin River following the draining of Wapato Lake, where a bloom had formed [91]. The propagation of blooms in downstream rivers that result from upstream reservoir or lake discharges adds even greater complexity to the dynamics involved in toxin transport, which also may involve benthic cyanobacteria coming off the riverbed.

Benthic Cyanobacteria in Rivers. Multiple genera of benthic cyanobacteria encountered during this study—colonies of *Nostoc parmelioides, N. spongiiforme*, and mat-forming *Phormidium*, *Microcoleus*, and *Oscillatoria* (Figure 1)—yielded multiple cyanotoxins. The finding of these toxigenic cyanobacteria in the benthos is consistent with studies in other American western rivers [20,21,33–35]. *Phormidium* and (or) *Microcoleus* and *Oscillatoria* mats, sometimes described as "skeins", occurred at sites throughout these Cascade Range river basins. *Oscillatoria* was previously found in the Clackamas River basin at eight main stem and 12 tributary sites [42], with similar occurrences in the North Umpqua River [92].

Using qualitative sampling methods, colonies of *Nostoc parmelioides* (Figure 1G,H) were most common in the upper-basin tributaries and main stem sites. Several samples of *N. parme-*

lioides were found to contain all four cyanotoxins (Figures 5 and 6), the highest concentrations coming from the Clackamas and McKenzie River basins. *N. parmelioides* in Oregon occurs in first- and second-order streams draining forests that have been commercially harvested for timber [93], although why this occurs has not been explored. As described above, the naturally occurring P allows the N-fixing *N. parmelioides* to attain high densities, particularly in clear and cold spring-fed rivers such as the upper Clackamas and McKenzie Rivers, Oak Grove Fork Clackamas River, and the North Umpqua River ([48], see photograph 8). This enhanced P availability may contribute to the widespread occurrence of *Nostoc* in these Cascade Range springs, tributaries, and rivers.

Benthic cyanobacteria may also be responsible for the observed seasonal peak in toxin concentrations, inferred from SPATT sampler extracts, that coincided with the late summer senescing period. This timing corresponds well with a study from the Klamath River [21], which hypothesized that older mats of *Microcoleus* release more anatoxins compared with younger mats that are growing quickly, and therefore result in higher toxin concentrations later in the season when cells and filaments within mats decay. Mats in advanced states of decomposition are prone to autogenic sloughing and may release dissolved or cell bound cyanotoxins to the water column. Benthic mats of *Phormidium* and (or) *Microcoleus* and *Oscillatoria* are easily fragmented and susceptible to hydraulic scour from changes in streamflows [94]. Sloughing events can occur once periphyton biomass accumulates on the riverbed in springtime and can be dislodged during storm runoff and reservoir drawdowns, or simply through autogenic processes at the end of the growing season [95]. The mats in soft substrates are sometimes fragile and easily disturbed; they become dislodged, flake off and float up to the surface where ingestion may pose a particularly high risk for dogs, as described above. The Oregon Veterinary Medical Association has additional guidance for water bodies where human recreational CyanoHAB advisories have been issued and then lifted; they recommend that dogs continue to stay out of the water [96] due to their lower thresholds for toxicity (~40–200 times lower than humans) (Table S1).

Because the sloughing of periphyton tends to be episodic, it can be easily missed, but continuous water-quality monitoring can give DWTP operators notice when it occurs. In the Clackamas River, sloughing events have occurred many times over the past 20 years, based on prolonged increases in phycocyanin and total chlorophyll in the water column at the USGS continuous water-quality monitor [73]. A previous study of the Clackamas River documented a springtime sloughing event that produced elevated concentrations of organic carbon, increasing disinfection by-products in treated drinking water [42]. Cyanotoxins were not evaluated, but future studies could characterize toxins during these events using autosamplers, triggered by chlorophyll or phycocyanin thresholds at continuous water quality monitors.

The late summer August-September peaks in SPATT sampler extract concentrations (Figure 9) correspond to periods when sloughing of benthic cyanobacteria may be occurring. This is also a time of year when rivers are approaching their seasonal lows and there is minimal dilution. Seasonal sloughing of periphyton explains the frequent occurrence of benthic cyanobacteria in plankton net tows in these Cascade Range tributaries and rivers [56]. Of the two major types of cyanobacteria encountered, the more loosely attached mats of *Phormidium* and (or) *Microcoleus*, and *Oscillatoria* may be especially prone to detaching and releasing toxins into the water column compared with *Nostoc*, whose thick rubbery colonies are securely attached to substrates and their firm structures are hardier and appear more resistant to decomposition compared with benthic mats (Figure 1).

5.2. Potential Impacts of Cyanotoxins on Recreation and Drinking Water

The occurrence of cyanotoxins in these Cascade Range rivers may have implications for human health, with potential exposures through direct contact with the water, cyanobacteria mats along shorelines, or through drinking water [53,97]. Based on the traditional water sample monitoring data collected to date, most cyanotoxin detections at DWTPs have been low concentrations and almost always below OHA guidelines (Table S1). However, periodic detections above state of Oregon guidelines and USEPA health advisories are cause for some concern. Since 2007, there has been just one drinking water advisory in Oregon (from the 2018 CyanoHAB in Detroit Lake), but 56 recreational advisories due to CyanoHABs lasting, on average, about 5 weeks [77].

About 20 waterbodies in the Cascade Range have had recreational advisories, most often for microcystins. Advisories at Detroit Lake, the most visited lake in Oregon, popular for water skiing and motorized watercraft, raise the potential for illness through exposure to cyanotoxins in aerosols that may be inhaled [98]. We found all four cyanotoxins in SPATT sampler extracts at the surface of Detroit Lake at the log boom during 2020, with discrete water samples from the City of Salem also showing concentrations of microcystins up to 1.3 μg/L (Figure S3). Future research could examine the risks to the public from these and other cyanotoxins. In July–August 2024, concentrations of microcystins in Detroit Lake exceeded 25 μg/L [99] causing a recreational advisory for the lake and downstream North Santiam River. Microcystins were detected in some samples of treated drinking water, at concentrations below guidelines for issuing "do not drink" notices.

Given the propensity for CyanoHABs to be transported from reservoirs, in partnership with the City of Salem, EWEB, and USACE, in 2019 the USGS started operating a seasonal continuous water quality profiler in Detroit Lake (and later, in Blue River Lake) that measures fluorescence of total chlorophyll and phycocyanin (cyanobacterial pigments) and photosynthetic indicators (pH and dissolved oxygen) in the top 25–30 m in real time [86]. These data alert dam operators, DWTP managers, and the public about the presence of a developing bloom, so that management agencies can respond.

The potential implications for widespread occurrence of cyanotoxins are not fully known. The chronic effects of low-level mixtures of cyanotoxins—by themselves or combined with other contaminants—have not been extensively studied, but rivers, including the Clackamas and McKenzie Rivers, can contain contaminants including pesticides, volatile organic compounds, and wastewater compounds [100–103] in addition to cyanotoxins. The potential exists for cyanotoxins, pesticides, metals, and other compounds to produce additive, synergistic, or antagonistic effects on aquatic life and human health [104], and such interactions may be unpredictable [105]. Microcystins and cylindrospermopsins may both impact liver and kidney function and their effects may be compounded; for example, anatoxins and the neonicotinoid insecticide imidacloprid share neurotoxic effects that may not be independent [106].

Co-occurrence rates for binary combinations of microcystins + cylindrospermopsins and anatoxins + saxitoxins in our study were about 27% each, suggesting some potential for additive effects, if they occur. The full effects of cyanotoxins on human and aquatic health are difficult to determine from our data, in part because toxicity depends on the specific congeners present, which were not differentiated by ELISA. This requires liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods that were not used in this study but represent a logical next step in characterizing cyanotoxins from these river basins. The highly toxic nature of cyanotoxins merits careful attention and study to understand the factors that promote CyanoHABs and production of toxins, their fate (degradation, bioaccumulation), and the risks to humans and other life.

Microcystins and Cylindrospermopsins. Microcystins were found in 73% of samples overall (Table 1). Concentrations of microcystins were generally higher than other cyanotoxins (Table 3), which is partly due to the large number of congeners detected with ELISA (>300) [107]. In addition, nine genera of cyanobacteria encountered are known microcystin producers (Table 4). *Nostoc*, which was frequently collected, may also produce nodularins in addition to microcystins, as both have the ADDA moiety targeted by ELISA [108]. *Dolichospermum*, *Gloeotrichia*, *Nostoc*, *Phormidium* and (or) *Microcoleus*, and *Oscillatoria*—all known producers of microcystins [5,51,109,110]—were common in these Cascade Range rivers and tributaries. SPATT sampler extracts yielded 52–100% detection of microcystins in the five drinking water sources surveyed (Table 2). These frequencies are high when compared with raw source water monitoring of cyanotoxins by the state of Oregon and the drinking water utilities (Tables S4 and S5).

As described above, in 2018 a bloom of *Dolichospermum* in Detroit Lake led to the release and breakthrough of cyanotoxins (microcystins and cylindrospermopsins) into the City of Salem's drinking water supply, prompting the first drinking water advisory due to these toxins [49,50]. The SPATT sample extract from the North Santiam River at the DWTP had corresponding detections of microcystins in source water. While the source of the toxins appeared to be the upstream lake, this study showed that benthic cyanobacteria might also have contributed.

Cylindrospermopsins were often detected, occurring in one-third of SPATT samples (Table 1). The OHA first detected cylindrospermopsins at levels exceeding recreational advisory guidelines in 2011 [51], and they continue to be detected in the North Santiam River during *Dolichospermum* blooms in Detroit Lake [46,47]. The highest concentrations were from the McKenzie River basin reservoirs that contained *Dolichospermum.* Benthic cyanobacteria from the Clackamas River basin (*N. parmelioides*, *Phormidium* and (or) *Microcoleus*, and *Wollea saccata*) also yielded relatively high concentrations of cylindrospermopsins.

Anatoxins and Saxitoxins. Anatoxins were widely distributed in the Cascade Range tributaries, main stems, and reservoirs. Their high frequency of detection (Table 1) was associated with planktonic *Dolichospermum* and benthic *Phormidium* and (or) *Microcoleus*, *Oscillatoria*, and *Anabaena* (Table 4), all of which are reportedly producers of anatoxins [23,111,112]. *Dolichospermum flos-aquae* is common in the Cascade Range reservoirs [40,42,47,77] and may also produce anatoxins [81]. Genetic studies are shedding light on the relationships among the Nostocales- and Oscillatoriales-types of cyanobacteria, but their classification and strain-specific toxin production status is still evolving.

Although occasionally detected in water samples at or near DWTPs, the low frequency of detection of cyanotoxins in discrete samples of whole water may be explained by the low concentrations of toxins, the large amount of dilution, and the clear water that may result in photo-oxidation [113]. Recurring alkaline conditions from diel periphyton photosynthesis [73] may also cause degradation of anatoxins [114].

Saxitoxins were frequently detected in benthic cyanobacteria colonies and mats, and in plankton net tows, with concentrations generally declining from the upper-basin main stem and tributaries to downstream main stem locations. Benthic (discrete) samples of *N. parmelioides* and *N. spongiiforme* (n = 19) yielded saxitoxins, and *Nostoc* propagules (and *Tolypothrix* filaments, Figure 8), were found together in 23 of 25 plankton net tow samples having the highest saxitoxins concentrations, suggesting that one or both genera could be the producer(s) (Table 4). *Tolypothrix* was previously suspected of saxitoxin production in Lake Baikal, Russia [68], and tufts of this taxa were relatively common in net tows from these Cascade Range rivers.

6. Detailed Assessment of Three River Basins

Due to their importance as drinking water supplies and value for recreation and aquatic habitat for endangered salmon and steelhead, the Clackamas, Santiam, and McKenzie rivers (Figure 2) are protected under Oregon's Three Basin Rule that prohibits new or increased levels of waste discharge (OAR 340-041-0350). Because of their high value, this study focused on these three river basins. The production and transport of cyanotoxins from upland headwater streams to reservoirs, where additional production by planktonic CyanoHABs may occur, and subsequent transport downstream to DWTPs, was noted during several synoptic sampling events described below. Because of the interconnectivity within each basin, these are presented separately.

6.1. Clackamas River Basin

The Clackamas River has five DWTPs located on the middle- and lower-basin main stem, which provide drinking water to nearly 400,000 people south of Portland. Clackamas River SPATT extracts yielded all four cyanotoxins, at all DWTPs, at one time or another (Table S3). All the cyanotoxin detections can be theoretically tracked upstream to benthic cyanobacteria in the main stem and tributaries, and—for three of four toxins (not saxitoxins)—also to North Fork Reservoir. The lower main stem and tributaries contained benthic mats of *Phormidium* and (or) *Microcoleus* and *Oscillatoria*, which contained multiple cyanotoxins [56]. These mats are prone to sloughing and may directly contribute to cyanotoxins detected in plankton net tows and perhaps indirectly, in SPATT sample extracts, depending on whether toxins are retained intracellularly or released as dissolved toxin (actively) or during decomposition. Anatoxins (and microcystins) were detected most often in samples from upper-basin tributaries and the main stem, and at all five DWTPs [56], indicating that sources are widespread.

In 2015, the year prior to our study, a *Dolichospermum* bloom in North Fork Reservoir resulted in multiple detections of anatoxin-*a* in the reservoir that preceded anatoxin-*a* detections downstream at the City of Lake Oswego DWTP, with maximum concentrations at both locations about 0.8 µg/L (Portland General Electric 2015). In September 2016, anatoxins were detected in SPATT extracts from both North Fork Reservoir and the Estacada DWTP downstream, at concentrations exceeding the 90th percentile (2.5–2.9 ng/g/day) [56]. In 2018, another bloom of *Dolichospermum* occurred in the reservoir, with SPATT sample extracts yielding only microcystins.

While North Fork Reservoir, a run-of-the-river reservoir, has a history of *Dolichospermum* blooms [42] and prior CyanoHAB advisories [77], benthic cyanobacteria including *Nostoc* and *Phormidium* and (or) *Microcoleus* from the Clackamas River basin were found to contain all four cyanotoxins (Table S3). The multiple detections of microcystins at all 5 DWTPs in August 2017 can be theoretically tracked to upper-basin tributaries and main stem sites upstream of North Fork Reservoir. Plankton net tows contained 80 times higher concentrations of microcystins in the upper Clackamas River compared with the lower main stem, while SPATT sample extract concentrations were 4-fold higher in the lower river compared with the upper river. While the dynamics of toxin production, transport, release, and sorption to SPATTs is a complex and dynamic process, these data suggest downstream transformation of microcystins from particulate to dissolved form in the Clackamas River. They reveal transport of cyanobacteria and accompanying toxins—sometimes all four, at various times—from the upper basin to North Fork Reservoir and downstream to DWTPs in the middle and lower basin.

Saxitoxins were detected in cyanobacterial colonies and mats, and in plankton net tows from the upper and lower main stems, often in samples containing *Nostoc* and *Tolypothrix*. Detection of saxitoxins in SPATT sampler extracts from the Clackamas River basin were, however, rare (Table S3), possibly because toxins were contained within cells, they had degraded, or otherwise not available to sorb to SPATT resins. These benthic cyanobacteria are another likely source of cyanotoxins, with multiple species of *Nostoc* and several genera of filamentous cyanobacteria found at main stem and tributary sites draining all types of land uses assessed.

Timothy Lake, in the headwaters of the Oak Grove Fork Clackamas River, also has *Dolichospermum* blooms that produce microcystins, and late season, low-biomass growths of *Microcystis aeruginosa* have been observed [41,42]. Although the lake could be a source of cyanotoxins, the outflow from the lake is at a depth of 70 feet [42], making releases of CyanoHABs to the downstream Oak Grove Fork Clackamas River unlikely until the reservoir is drawn down after Labor Day.

6.2. North Santiam River Basin

In August 2018, SPATT sampler extracts yielded detections of microcystins > anatoxins > cylindrospermopsins > saxitoxins at the City of Salem's DWTP [56]. Each of these cyanotoxins was also detected in whole-water samples (Table S4). The specific sources of these cyanotoxins to the DWTPs downstream from the lake include the upper-basin tributaries—the North Santiam and Breitenbush rivers—and Detroit Lake, as well as the Little North Santiam River, which enters the main stem downstream from the lake (Figure 2).

Anatoxins were detected in SPATT sampler extracts from the upper North Santiam River upstream of Detroit Lake at concentration exceeding the 90th percentile, but their specific producer(s) were not identified. *Nostoc parmelioides* was the most common type of cyanobacteria encountered in this reach, but colonies from September 2016 yielded microcystins and cylindrospermopsins, but no anatoxins. Other possible sources include *Anabaena* and *Wollea*, which, as seen in the Clackamas River, can live among masses of stalked diatoms (Figure 1E,F) that were also observed in the upper North Santiam River.

Detroit Lake has a history of CyanoHABs [49,77] and SPATT sampler extracts from 2020 (Figure S3) revealed all four cyanotoxins, with corresponding detections in water samples from the lake and outflow (Table S4). The dominant cyanobacteria, *Dolichospermum*, has genes for synthesis of at least microcystin and cylindrospermopsins [44–47,79–81] and perhaps other toxins. SPATT samplers from the lake consistently yielded 100 times lower concentrations of saxitoxins, compared with anatoxins, although these neurotoxins shared seasonal peaks suggesting a common source (strain of cyanobacteria). Unexpectedly, none of the 15 net tow samples collected from seven reservoirs contained saxitoxin, so the source could be transient or otherwise missed by the limited sampling.

Cyanotoxins from surface blooms are transported downstream as water is released through the dam's spillway gates, which is an incidental consequence of passing water to aid out-migrating salmon and steelhead smolts, and to manage water temperatures in the downstream main stem river. Water releases from Detroit Dam have sent cyanobacteria and cyanotoxins downstream to DWTP intakes in 2011, 2018, and 2024 [77]. In May 2018, a bloom of *Dolichospermum lemmermanii* in Detroit Lake prompted a recreational CyanoHAB advisory and subsequent detection of microcystins and cylindrospermopsins in the City of Salem's treated drinking water resulted in the State of Oregon's first "do not drink" human-health advisory due to cyanotoxins. The advisory affected preschoolers, elderly, and vulnerable populations for seven weeks. The bloom continued and a second recreational advisory was issued by OHA for microcystins, which lasted until mid-August [77]. Anatoxins were also detected downstream in the North Santiam River and Salem's DWTP in July 2018 (Figure S1). A late-July 2018 plankton net tow sample yielded three of four toxins in Detroit Lake (not saxitoxin), and all four toxins were detected at Packsaddle Park downstream, where concentrations of microcystins exceeded the 90th percentile (45 µg/L) and anatoxins exceeded the 75th percentile (0.23 µg/L, Table S4). SPATT sampler extracts yielded all four cyanotoxins at Salem's DWTP, with three of four also occurring upstream from Detroit Lake in the main stem North Santiam River.

In 2016, SPATT sampler extracts from the North Santiam River downstream of Big Cliff Dam yielded three of four cyanotoxins, but not saxitoxin, with the highest concentrations for microcystins and anatoxins [56]. Possible sources include *Phormidium* and (or) *Microcoleus* (Figures 5 and 6), and *Oscillatoria* mats in the lower Little North Santiam River, which contained anatoxins, microcystins, and cylindrospermopsins. Two benthic samples from the main stem North Santiam River downstream from Detroit Lake contained *Tolypothrix* and planktonic *Dolichospermum*; both contained microcystins and cylindrospermopsins, and one also contained saxitoxin (in 2016). Saxitoxins were detected least often, but in July 2018, plankton net tow and SPATT sampler extracts from the upper North Santiam Rivers contained this neurotoxin (Table S4). At the same time, three sites downstream from Detroit Lake (below Big Cliff Dam, Packsaddle Park, and the City of Salem DWTP middle intake site (Figures 2 and S1) also contained saxitoxins.

6.3. McKenzie River Basin

The combined data from the McKenzie River basin (Table S5) reveal a similar pattern, with the same cyanobacteria—benthic *Nostoc parmelioides* and *Phormidium/Microcoleus* and planktonic *Dolichospermum* in the reservoirs—with all four cyanotoxins detected. Multiple species of *Dolichospermum* have been identified in Blue River Lake, including *D. flos-aquae*, *D. planctonicum*, *D. berezowskii*, *D. circinale*, and *D. lemmermannii*, and some of these were also observed in downstream plankton net tow samples. However, the selective withdrawal structure at Cougar Reservoir allows for water releases from shallower depths compared with Blue River Lake, suggests the former reservoir may be more of a source of cyanobacteria or cyanotoxins for the McKenzie River downstream.

Reservoir blooms in 2017, 2018, and 2020 produced microcystins, cylindrospermopsins, anatoxins, and saxitoxins (in 2020), leading to detections in SPATT sampler extracts. Plankton net tow extracts from reservoir outflows sites contained all four cyanotoxins, and benthic colonies in the reach below these dams and in main stem also contribute cyanotoxins upstream of EWEB's DWTP intake (Table S5).

In 2017, *Dolichospermum* blooms in Cougar Reservoir and Blue River Lake coincided with downstream detections of all four cyanotoxins in plankton net tows from the two outfall streams (Figure S3), although benthic sources in dam tailraces or tributaries cannot be ruled out. Similar planktonic blooms occurred in 2018, and plankton net tows revealed transport of *Dolichospermum* colonies, and multiple cyanotoxins, in plankton net tow extracts in May, June, and August from the reservoirs to the main stem down to EWEB's intake (Table S5).

Concentrations of microcystins and saxitoxins in net tow sample extracts increased longitudinally downstream from these reservoirs (Figure S3), consistent with the occurrence of benthic *Nostoc* and *Tolypothrix* in seston that may have been scoured from the riverbed. Saxitoxins were detected less often, in general, and at much lower concentrations compared with the other three cyanotoxins [56], but they occurred nonetheless alongside other toxins in benthic colonies and mats, in SPATT sampler extracts, and plankton net tow samples, especially those that contained *Phormidium/Microcoleus* and (or) *Tolypothrix* and *Nostoc*.

7. Study Limitations

Although information describing the qualitative occurrence of cyanobacteria and their cyanotoxins is presented, this study did not employ the types of quantitative sampling required to understand the dynamic processes of toxin production and release, nor the roles of senescence, partitioning onto sediment, transformation, and transport. These gaps represent future opportunities for additional study toward a better understanding of the potential risks from cyanotoxins to drinking water, recreation, and aquatic life in this region.

Although cyanotoxin extract concentrations collected for this study are not comparable to water concentrations and have no regulatory standards, they provide useful information on the occurrence and sources of these toxins, their relative concentrations spatially, and their seasonality in these river basins.

Also, because of variable, non-isokinetic water velocities, potential degradation, and (or) de-sorption, results from SPATT samplers have some uncertainty, but comparisons longitudinally and over time are reasonable uses of these data. The cyanotoxin concentrations from cyanobacteria colonies and mat extracts are also semi-quantitative, but because samples were handled similarly, these data can be compared to understand spatiotemporal patterns among sites (Tables S3–S5). The variations in sample weights, however, preclude direct comparisons with other studies. Future studies could standardize the sampling biomass and express toxin concentrations per gram of wet weight, for example, or by area, or some other quantitative measure.

The taxonomy of these Oscillatoriales cyanobacteria that were so common in these streams is also uncertain and has included numerous revisions of the *Phormidium/Microcoleus* group based on phylogenetic relationships [115]. Komarek et al. [116] discussed the taxonomy of *Phormidium autumnale* and *Microcoleus*, describing this group as complicated, widespread, and in need of more detailed genetic study. In this study, these organisms were often lumped together, but efforts were made to identify *Oscillatoria* from *Phormidium*/*Microcoleus* based on morphological traits: filament widths, length-to-width ratios, and the presence of a sheath that *Oscillatoria* lacks [117].

This study also did not identify specific congeners or test for all possible cyanotoxins. Each of the four ELISA tests reacts with more than one congener, so it is also uncertain which one(s) were present. There are hundreds of different microcystin congeners, for example, with differing toxicities. In addition, the anatoxin-*a* ELISA reacts with anatoxin-*a* and homoanatoxin-*a*, but not dihydroanatoxin or dihydrohomoanatoxin; the latter made up a substantial portion of the total anatoxins from *Microcoleus* isolates from the Russian River, California, based on LC-MS/MS [118], so this represents a potentially important data gap. In New Zealand, *Phormidium* produces dihydroanatoxin-*a* and dihydrohomoanatoxin-*a*, which can sometimes be more toxic than anatoxin-*a* [119,120]. Similar limitations exist for cylindrospermopsins and saxitoxins; their ELISA tests react with multiple congeners or closely related compounds. More research is therefore needed to elucidate which congeners are present to understand the range of possible effects on human health and aquatic life.

8. Major Findings and Next Steps

This study, with its multiple lines of evidence and ultra-sensitive methods, reveals widespread sources of cyanotoxins within the Cascade Range river basins and provides an early warning about their presence. The extensive network of SPATT sampler deployments and plankton net tows demonstrated the occurrence and transport of cyanobacteria and cyanotoxins from upper-basin reaches into downstream reservoirs, where planktonic CyanoHABs may further enrich waters with cyanotoxins, and on downstream to DWTP intakes and public recreation sites on the lower main stems of these rivers.

Both benthic and planktonic cyanobacteria play a role in determining the blend and amount of cyanotoxins and, therefore, the potential cumulative risk to public health, drinking water, animal health, and aquatic life. A deeper understanding of the annual cycles of cyanobacteria growth, the source(s) of inoculums, the production, accumulation, and release of toxins, are needed to understand the potential risks. Knowledge about where and when toxigenic cyanobacteria grow in these river basins is not extensive, but this and other studies [21,42,47,75,92,121,122] have identified some of the organisms involved, the habitats where they grow and when they occur, and the cyanotoxins they produce. While our study provides some understanding about the occurrence of potentially toxigenic cyanobacteria in these river basins, the risks are still largely unknown.

Continuous water quality monitoring in the Clackamas River over the past 20 years has indicated near-annual cycles of benthic algae (cyanobacteria and other periphyton) that currently result in alkaline pH greater than 8.5 units [86] with the result that water often does not meet state of Oregon water-quality standards, particularly during spring and early summer. No routine or systematic surveys are conducted to understand where and when benthic CyanoHABs occur, or to explain why certain areas or years are more prone to nuisance growths or toxin production than others, so more research is needed.

New methods are being developed to better identify and understand CyanoHABs, which may eventually inform predictive models. One new approach uses hyperspectral reflectance to identify cyanobacteria remotely, with satellites and other sensors [123,124] to generate spectral libraries of target organisms [125]. These methods may allow future tracking of planktonic and benthic cyanobacteria in these Cascade Range river basins with contactless sensors, drones, planes, and satellites to document the cycles of algal growth and senescence. Such tools may reveal how periphyton and benthic CyanoHABs respond to drivers including streamflow, temperature, snowpack, nutrients, light availability, wildfires, or other factor(s) [26].

The watersheds upstream of these Cascade Range reservoirs are largely managed by the U.S. Forest Service, and this has historically included timber harvesting on sometimes steep slopes, which may exacerbate the natural erosive processes and deliver sediment (and P) to streams and downstream reservoirs [42]. The role of sediment-derived nutrients in fueling cyanobacteria in these reservoir–river systems is poorly understood, and the recent 2020 wildfires have increased turbidity and concentrations of suspended sediment in many streams [126]. Internal cycling of P from downstream reservoir sediments is common [88,127] and ripe for future study in this area. The reservoirs are managed by Federal agencies, predominantly the U.S. Army Corps of Engineers [128,129] and private utilities. Regulatory aspects, including development and implementation of a statewide HAB strategy, are largely within the purview of the State (ODEQ and OHA), while drinking water providers are generally City-level. Continued coordination and cooperation among these entities can help minimize potential impacts on water supplies. The collective research and monitoring have contributed to some understanding of the factors that promote CyanoHABs and the organisms responsible, but the specific triggers for toxin production have not been identified.

Mitigation steps to reduce the risks from CyanoHABs in rivers and reservoirs would benefit from a firmer knowledge of the dominant factors promoting their growth, such as nutrients, temperature, and other environmental drivers [130,131]. The relative importance of N versus P, for example, is not known. Studies have found depletion of inorganic N in the water column, suggesting that periphyton are potentially N-limited in these Cascade Range river basins [42,74–76], which may, paradoxically, stimulate production of anatoxin-*a* by some filamentous mat-forming cyanobacteria [13].

The dominant CyanoHAB in the reservoirs—*Dolichospermum*—fixes its own N [46,82], which may result in these blooms being limited by another nutrient, perhaps P, or light, time, or some other factor(s). Future investigations of potential limitation by N or P could include in-lake nutrient-addition mesocosm experiments, such as those conducted in lakes near

Mount St. Helens [132,133]. This information could guide potential future management strategies aimed at reducing the limiting nutrient if one is identified.

Other studies could quantify the importance of upland versus in-reservoir derived nutrients, which could be important for reservoirs that are drawn down 100 or more feet each winter to accommodate precipitation runoff and snowmelt. The shorelines and backwater arms of these reservoirs can collect CyanoHAB biomass that may enrich sediments and later contribute N and (or) P to the water when inundated in the spring. However, for the benthic mats of cyanobacteria, such as *Phormidium/Microcoleus* and *Oscillatoria* that lack heterocytes, both N and P could be important for their growth, in addition to light or some other factor(s). Also, temperature is a driving factor in determining growth rates of cyanobacteria [84], and higher recent temperatures [134] may exacerbate conditions by lengthening the duration of the growing season or increasing biomass, as seen for large lakes over the past 30 years [89].

Since 2018, ODEQ and OHA have monitored cyanotoxins in several water supplies deemed at risk from CyanoHABs [135]. Aside from the 2018 drinking water advisory for the City of Salem, detections in drinking water, or even in raw source water, have been rare [69]. This differs considerably from our finding of persistent, albeit potentially low concentrations of cyanotoxins in these source waters. This is partly explained by the concentrated nature of our samples and use of passive, time-integrating SPATT samplers, which provided an ultra-sensitive means of detecting cyanotoxins compared with traditional analyses of whole water (grab) samples. Filtration of moderate volumes of raw water (250–500 mL) and analyses of the filter, a new approach that we are piloting, may allow detection of cyanotoxins in seston at low parts-per-trillion concentrations. Such methods could reveal cyanotoxin concentrations in water, which may be present, but possibly at orders of magnitude below advisory thresholds, that could be compared with SPATT extract concentrations. While concentrations are often low, detections of cyanotoxins in raw water samples do occur, and some drinking water utilities have added ozone and activated carbon to treat cyanotoxins, and no drinking water advisories have occurred since 2018. Recreation, however, continues to present potential risks to the public at affected water bodies during the bloom season, and aquatic life, including endangered salmon and other animals may be exposed to cyanotoxins in these Cascade Range waters, with unknown effects. New efforts are underway to examine the occurrence of microcystins in out-migrating salmon and their zooplankton foodstuffs, to explore potential effects on their populations.

In conclusion, the high detection rates of cyanotoxins found during this study, along with occasional occurrence of cyanotoxins in treated drinking water, point to the need for further study of CyanoHABs in this region and the factors that promote toxin production. This study found that cyanotoxins occur in a wide range of environments, in streams draining relatively pristine forestland, and mixed urban, agricultural, and rural lands, including watersheds that continue to support salmon and steelhead. Continued research and monitoring of CyanoHABs in this region will ensure clean, healthy, and reliable water supplies for humans and ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/phycology5020016/s1, Figure S1: Downstream occurrence of cyanotoxins in plankton net tow sample extracts from the North Santiam River down to the drinking water intake, 27 July 2018; Figure S2: Seasonal concentrations of four cyanotoxins in Detroit Lake, North Santiam River basin, April to September 2020, based on Solid-Phase Adsorption Toxin Tracking (SPATT) sampler extracts and water sample data; Figure S3: Longitudinal occurrence of cyanotoxins in plankton net tow extracts from the McKenzie River basin reservoirs downstream to the Eugene Water & Electric (EWEB) drinking-water intake in (A) May, (B) June, and (C) August 2018 during blooms of *Dolichospermum* in

Cougar Reservoir and Blue River Lake; Table S1: State of Oregon guidelines for four cyanotoxins in recreational waters and in finished (treated) drinking water [136]; Table S2: Sampling sites and samples analyzed from each river basin; Table S3: Spatiotemporal occurrence of cyanotoxins in the Clackamas River basin during seven time periods, 2016–2018; Table S4: Spatiotemporal occurrence of cyanotoxins in the North Santiam River basin during five time periods, 2016–2020; Table S5: Spatiotemporal occurrence of cyanotoxins in the McKenzie River basin during seven time periods, 2016–2020.

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