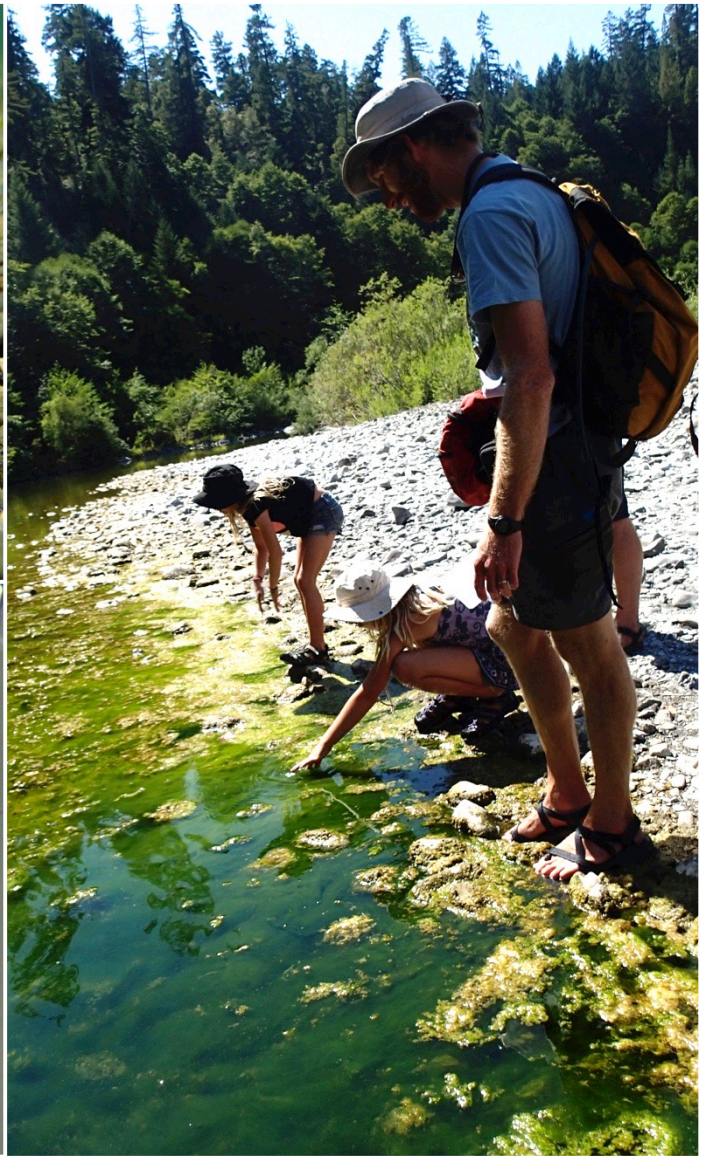
Cyanobacteria and Cyanotoxins in the Eel River, 2013 – 2014



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Summary of Eel River Cyanobacteria (Blue-Green Algae):

- From June – September, 2013 and 2014, sites along the South Fork Eel, Main-stem Eel, and Van Duzen Rivers were monitored for cyanobacterial mats and cyanotoxins (Fig. 1).
- The common cyanotoxin producing cyanobacteria in the Eel are species of *Anabaena* and *Phormidium*, but *Nostoc* also produced cyanotoxins.
- Anabaena grows in slower flowing water, often on top of other types of non-toxic algae. *Phormidium* grows on rocks in fast-flowing riffles and rapids.
- Of the two cyanotoxins monitored, the neurotoxin, anatoxin-a, was detected at higher levels in the Eel than the liver toxin, microcystin.
- Cyanotoxin concentrations were highest in the middle reaches of the South Fork Eel between Meyers Flat and Leggett.
- Cyanotoxin concentrations peak in late July and early August, but local hot-spots may still be present through September.

Introduction

Algae are a very diverse group simple organisms that can photosynthesize. Algae inhabit freshwater and marine environments worldwide. Cyanobacteria (or blue-green algae) are a unique algae; they the only algae that are bacteria and the only bacteria that can photosynthesize by producing oxygen.

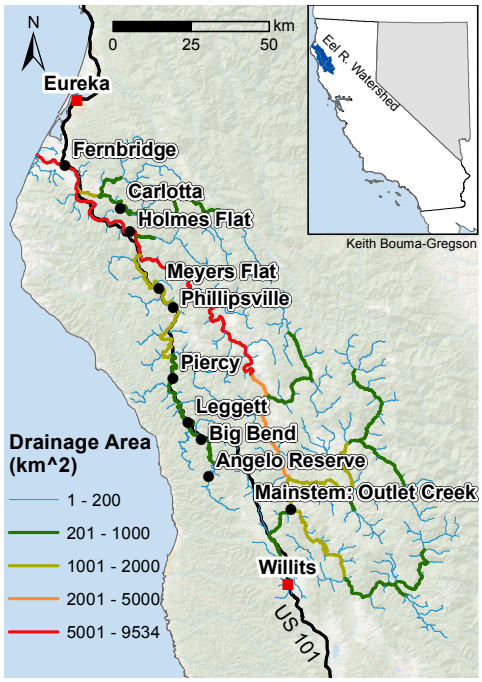

Figure 1. Map of cyanotoxin monitoring locations in the Eel River**.** Cities of Eureka and Willits shown for reference.

In the Eel River (Fig. 1), algae are foundational parts of the summer food web (Power et al. 2013). The warm summer temperatures, clear water, and low flows in the Eel create optimal conditions for algal growth. Many different types of algae grow in the Eel, most of which are non-toxic. These algae are then consumed by fish and insects, which are then consumed by birds, bats, otters, and other predators, creating a productive and complex aquatic food web.

However, in certain environmental conditions cyanobacteria begin to grow and become significant parts of the algal assemblages. Certain cyanobacteria

produce compounds toxic to mammals (Metcalf and Codd 2012). There are numerous types of cyanotoxin molecules with different molecular structures, but cyanotoxins are mainly neurotoxins, liver toxins, tumor promoters, or dermal irritants (Dittmann et al. 2013). Not all cyanobacterial species produce toxins, nor will all cells in a cyanobacterial species produce toxins; therefore, not all cyanobacterial mats are toxic. It is impossible to visually distinguish which cells are producing toxins and which are not. Toxin production can only be determined through chemical or genetic analysis.

Children, pets, and livestock are especially at risk from cyanotoxins because they are more likely to swallow river water or, in the case of animals, eat toxic algal crusts/mats. In the Eel there have been 11 documented dog deaths attributed to cyanobacteria since 2002 (Hill 2010, Backer et al. 2013). Dog deaths occurred on the South Fork Eel River between Piercy and Phillipsville, and in the Van Duzen River near Carlotta.

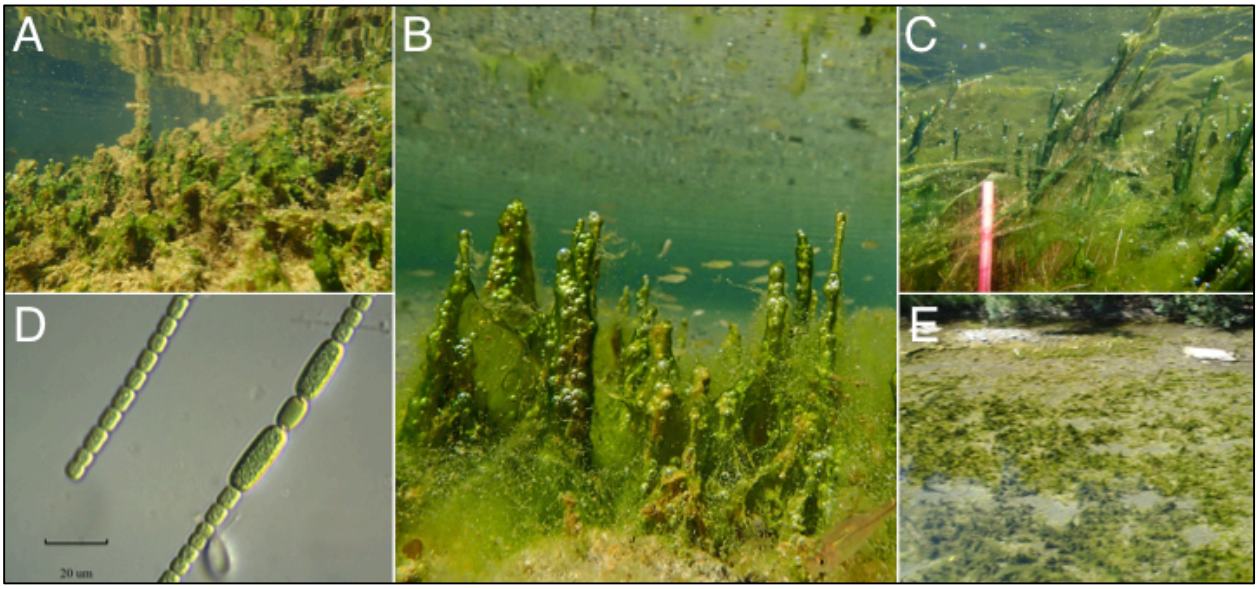

Figure 2. *Anabaena* in the Eel River: A) Dark-green *Anabaena* patches growing on top of senescing macro-algae *Cladophora glomerata*; B and C) *Anabaena* "spires" growing on *Spirogyra* (pink scale bar is 5/8 inch wide); D) Micrograph of *Anabaena* cells (400x); E) *Anabaena* mats covering the riverbed.

This report describes cyanobacterial species present in the Eel River and cyanotoxin concentrations from summers 2013 and 2014. The data come from the on going Ph.D. research of Keith Bouma-Gregson in collaboration with the Eel River Recovery Project (ERRP). In the summers of 2013 and 2014, cyanobacteria and cyanotoxin monitoring was conducted in the Eel River (Fig. 1; Table 2). Sites were concentrated along the

South Fork and lower Eel River. In addition to these monitoring sites, data were also collected from other locations in the watershed during summertime reconnaissance trips. Monitoring began in June and ended in late September, this is the time when river flows are lowest, recreational use of the river is highest, and previous cyanotoxin related dog deaths occurred. This report provides an initial description of the preliminary patterns identified by these monitoring efforts. Monitoring is ongoing, and future reports will include a more complete assessment on the relationship between environmental conditions and the distribution of cyanobacteria.

Cyanobacterial Occurrences and Taxa

Cyanobacterial mats were observed in a variety of environmental conditions throughout our summertime monitoring (June to September). Since cyanobacteria are an ancient and diverse group of organisms, it is likely that different strains are able to exploit various microhabitats that exist throughout the watershed at different times of year. However, based on field observations, cyanobacterial abundance generally increases from late June to mid-August, and declines in September. The first large rain events of the fall scour the riverbed and remove cyanobacterial mats for the rest of the winter. Systematic monitoring during winter and spring did not occur, and so comments on cyanobacterial patterns during these seasons cannot be made.

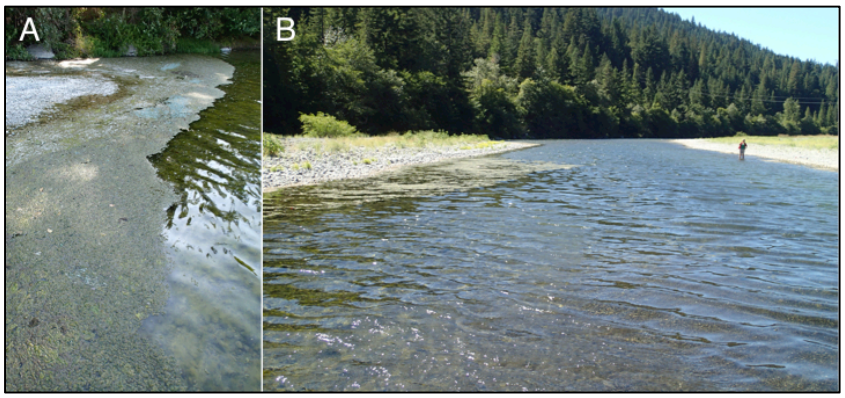

Figure 3. Floating cyanobacterial mats in near Phillipsville: A) in a backwater pool B) along the left riverbank.

Our observations suggest that the three most abundant cyanobacteria in the Eel belong to the genera *Anabaena*, *Phormidium*, and *Nostoc* (most cyanobacteria in the Eel have only been identified to genus) (Table 1). *Anabaena* grows in slow flowing water, often on top of large non-toxic filamentous macro-algae, such as *Cladophora* or *Spirogyra*

(Fig. 2). *Anabaena* mats form distinct "spires" rising from the riverbed. The spires are formed as *Anabaena* secretes mucous, which traps oxygen bubbles produced by photosynthesis causing the filaments to float towards the surface and form the spire. Anabaena is a fragile alga and easily sloughs from the riverbed. Once detached, the oxygen bubbles cause float the spire to the water surface where it then travels downstream. The floating *Anabaena* can get trapped in backwater eddies or along riverbanks, forming conspicuous floating mats in these locations (Fig. 3).

Table 1. Description of common cyanobacteria in the Eel River

Cyanobacterial Genus	Color	Habitat	Growth Form
Anabaena	dark green, blue-green	slow flowing water, on cobbles, on top of other non-toxic algae, or as floating mats	delicate "spires," blue-green coating on macro-algae
Phormidium	brown, orange, amber	boulders and cobbles in rapids and riffles	durable mats, velvety or feathery
Nostoc	brown, beige, blue-green	usually riffles, but sometimes in shallow pools	durable gelatinous balls, ear-shaped, or blades

In contrast to *Anabaena*, *Phormidium* grows on boulders and cobbles in fast flowing riffles and rapids. It forms brown or orange mats on rocks, and when the filaments are long, it has a "feathery" or "velvety" appearance (Fig. 4). *Phormidium* mats are more durable than *Anabaena* mats, so are less likely to be sloughed from the riverbed or be seen floating downstream. *Nostoc* forms distinct brown/amber gelatinous balls on rocks and boulders (Fig. 5).

Beyond *Anabaena*, *Phormidium*, and *Nostoc,* other cyanobacterial taxa also inhabit the Eel. *Cylindrospermum*, *Nodularia*, *Oscillatoria*, *Leptolyngbya*, and other taxa have been identified in samples, but they are rarely the taxa forming the visible mat. One exception is *Cylindrospermum*, which can forming small macroscopic mats in the river. *Cylindrospermum* grows in similar habitats and with a similar growth form to *Anabaena*. However, *Anabaena* was observed more frequently than *Cylindrospermum.* More observations are needed to better describe the preferred habitats of *Cylindrospermum*. Since these taxa are rare, it is unlikely they are significant contributors to observed cyanotoxin levels in the Eel River.

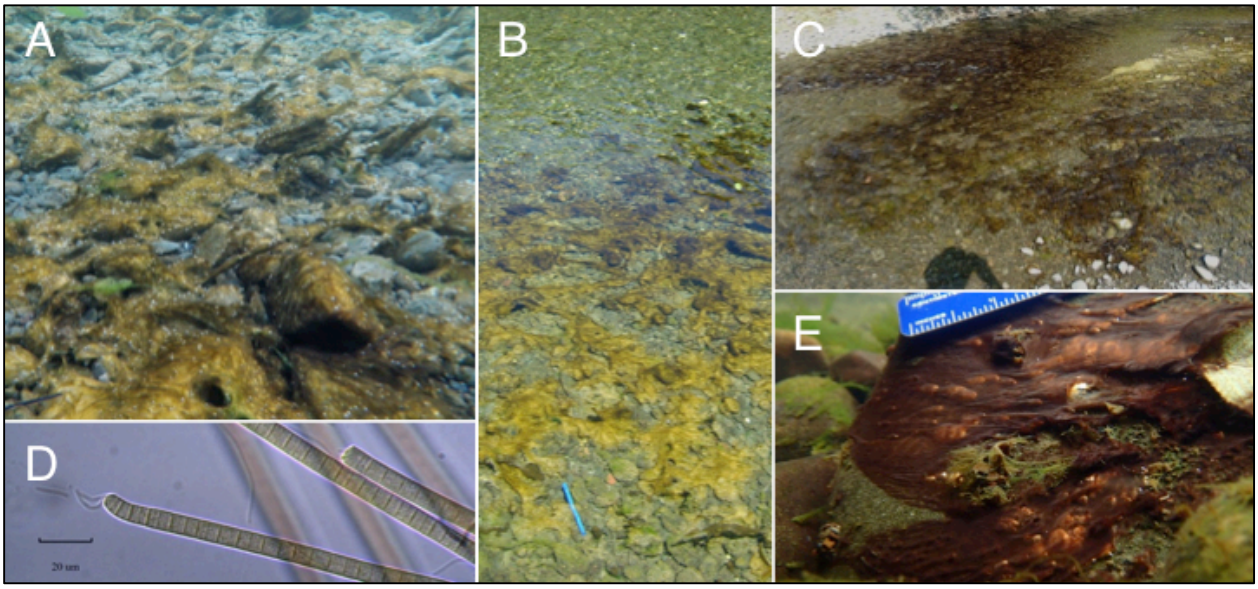

Figure 4. Phormidium in the Eel River: A) *Phormidium* mats growing on cobbles in a riffle, note the orange and brown coloration; B and C) looking down on Phormidium mats (blue thermometer is 15 cm long); D) Micrograph of *Phormidium* cells (400x) E) *Phormidium* growing on a cobble, note the velvety appearance.

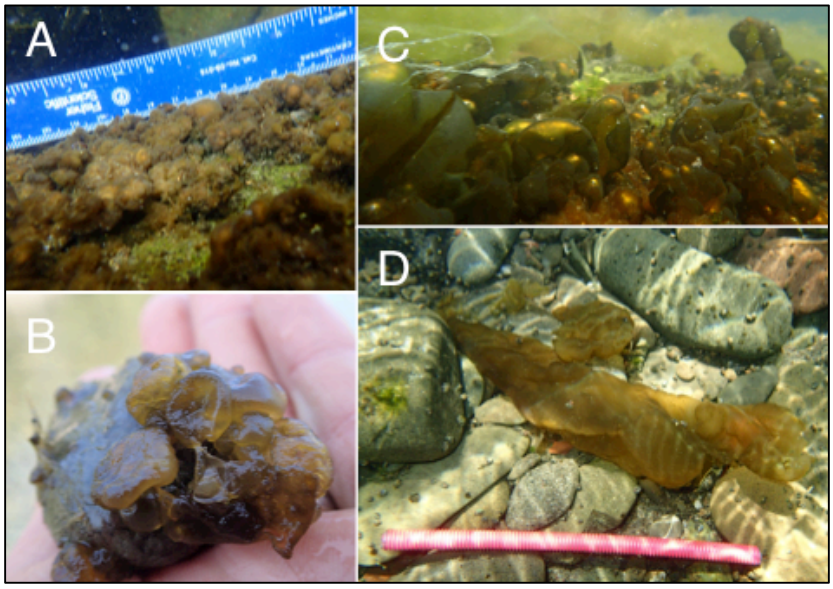

Figure 5. *Nostoc* in the Eel River: A, B, and C) small *Nostoc* balls growing strongly attached to cobbles D); larger *Nostoc* "blades" growing in slower flowing water and loosely attached to the substrate (pink scale bar is 6 inches long).

Cyanotoxins

Cyanotoxin monitoring in the Eel measured two cyanotoxins, 1) the liver toxin, microcystin (MCY), and 2) the neurotoxin, anatoxin-a (ATX). Microcystin is one the most common cyanotoxins worldwide and has received significant research attention. Anatoxin-a was investigated because some dog deaths in the Eel River were attributed to this toxin (Puschner et al. 2008). Other cyanotoxins such as nodularin or cylindrospermopsin were not measured in this monitoring program.

Cyanotoxin concentrations were measured with solid phase adsorption toxin tracking (SPATT) samplers (Lane et al. 2010, Kudela 2011) and by sampling cyanobacterial mats. When a cyanobacterial cell is healthy and growing, cyanotoxin molecules remain inside cells, but when cells die and cell membranes lyse cyanotoxin molecules are released into the water column. SPATT samplers measure these dissolved cyanotoxin molecules outside of cells. While, cyanobacterial mats were collected to measure toxin concentrations within cyanobacterial cells (intracellular toxins).

SPATT samplers: SPATT samplers are a synthetic micro-bead resin that are placed in the river, then cyanotoxin molecules in the water adsorb (i.e. adhere) onto the resin (Fig. 6). After one week the sampler is removed from the river, and in the laboratory, toxins are extracted from the resin. The extracted toxin concentrations are measured and the results displayed in nanograms of toxin per milligram of resin (for more details, see Appendix 1). In contrast to collecting a grab sample of water, SPATT samplers provide a time-integrated estimate of dissolved toxin concentrations in the river.

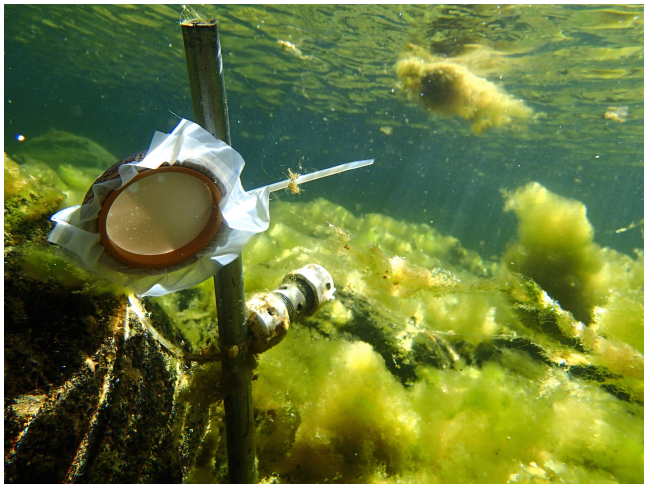

Figure 6. SPATT sampling ring surrounded by *Spirogyra*.

SPATT samplers were deployed at seven sites in 2013 and ten sites in 2014 (Fig. 1; Table 2). Most sites were along the South Fork Eel from headwaters at Angelo downstream to the mouth near Fernbridge. Samplers were retrieved and deployed approximately weekly starting in mid-July (2013) or mid-June (2014) and ending in late September. Deploying and retrieving SPATT samplers in succession creates a time series that approximates toxin concentrations in the river (Fig. 7). SPATT samplers do not provide information about the specific location of cyanotoxin production or what cyanobacteria are producing toxins, rather SPATT provides a sensitive method for detecting cyanotoxin production over large areas through time.

Table 2. Location and drainage area of SPATT monitoring sites.

Site	Drainage Area (km^2)	Fork	Monitoring Years
Fernbridge	9357	Lower Eel	2013 & 2014
Holmes Flat	7908	Lower Eel	2014
Meyers Flat	1571	South Fork	2014
Phillipsville	1426	South Fork	2013 & 2014
Piercy	881	South Fork	2013 & 2014
Leggett	653	South Fork	2013 & 2014
Big Bend	472	South Fork	2014
Angelo	114	South Fork	2013 & 2014
Carlotta	673	Van Duzen	2013 & 2014
MS at Outlet Cr.	1789	Main Stem	2013 & 2014

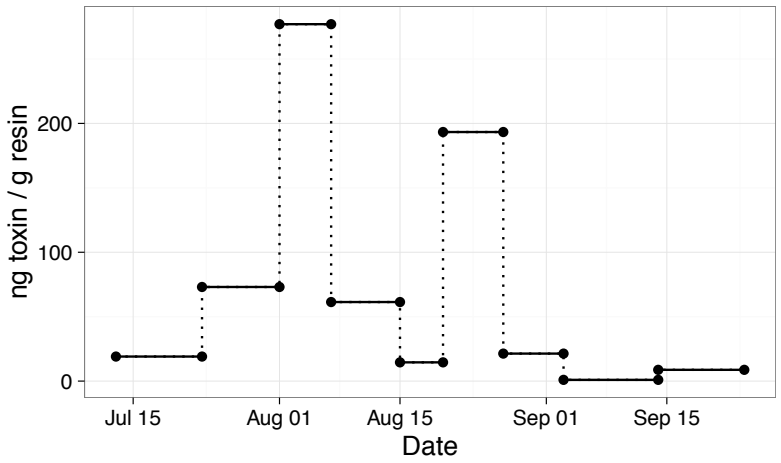

Figure 7. Hypothetical SPATT time series. Each solid black line represents one SPATT sampler that is deployed. Results from each sampler are presented as nanograms of cyanotoxins per gram of SPATT resin.

Cyanobacterial mat samples: To measure toxins inside cyanobacterial cells, 106 mat samples were collected from cyanobacteria growing on macro-algae, cobbles, or floating downstream. These data provide information about the specific location and species producing the cyanotoxins. Samples were collected by placing a 1.5 inch diameter PVC pipe delimiter (Fig. 8) over the cyanobacterial mat and collecting only the algae within the delimiter. For each sample, 3-5 delimiters per cyanobacterial mat were combined in a glass jar and the cyanotoxin concentrations measured (for more details, see Appendix 1).

Figure 8. PVC pipe delimiter about to be placed on a patch of benthic algae. Only algae inside the delimiter were collected.

Cyanotoxin Results: SPATT samplers detected higher concentrations of anatoxin-a than microcystin at our monitoring sites in the Eel River (Fig. 9), but detected microcystin more frequently than anatoxin-a (Table 3). Most of the microcystin values were low, i.e. < 50 ng MCY / g resin, with only the Angelo Reserve SPATT sampler consistently detected microcystin above 50 ng MCY / g resin. In contrast, anatoxin-a was detected more frequently and at higher concentrations than microcystin in the cyanobacterial mat samples (Fig. 10). Both *Anabaena* and *Phormidium* mats produced anatoxin-a or microcystin, though it was rare for a single sample to contain both cyanotoxins concurrently. No microcystin was detected in the ten *Nostoc* samples, though four samples tested positive for anatoxin-a. The widespread and consistent detection of anatoxin-a by the SPATT samplers is consistent with previous reports and publications that described neurotoxic symptoms in dog deaths in the Eel (Puschner et al. 2008, Hill 2010, Backer et al. 2013).

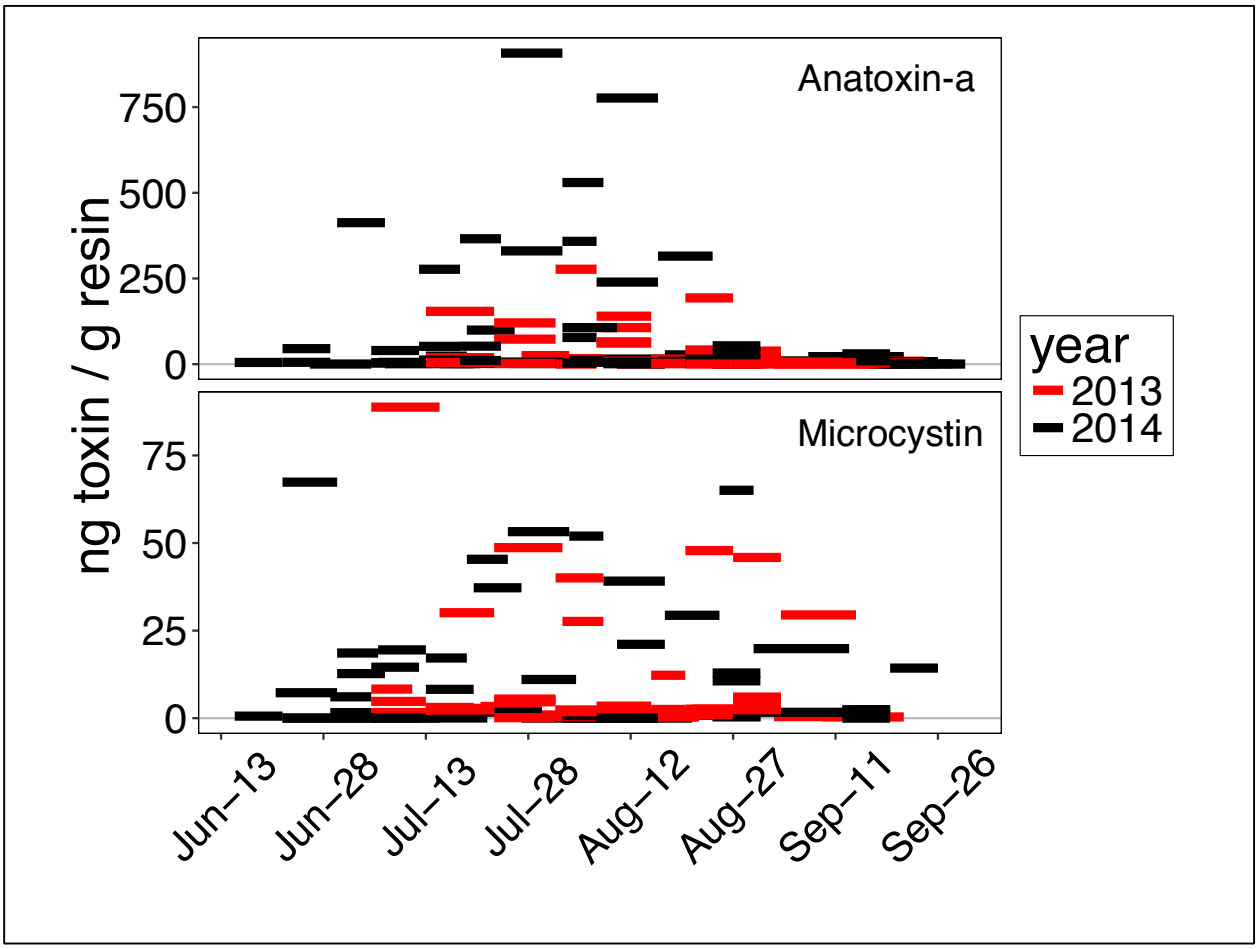

Figure 9. Microcystin and anatoxin-a from SPATT samplers in the Eel River. Note the different scales of the y-axis.

Table 3. The percentage of SPATT samplers testing positive for MCY or ATX. Parentheses show number of samplers testing positive over total number of samplers deployed.

Site	2013 MCY	2014 MCY	2013 ATX	2014 ATX
Fernbridge	77% (10/13)	16% (3/19)	38% (5/13)	19% (3/16)
Holmes Flat	NA	40% (4/10)	NA	44% (4/9)
Meyers Flat	NA	19% (3/16)	NA	33% (5/15)
Phillipsville	78% (7/9)	27% (3/11)	100% (9/9)	64% (7/11)
Piercy	44% (4/9)	36% (4/11)	80% (8/9)	73% (8/11)
Leggett	75% (6/8)	27% (3/11)	88% (7/8)	91% (10/11)
Big Bend	NA	22% (2/9)	NA	50% (4/8)
Angelo	100% (9/9)	100% (13/13)	33% (3/9)	62% (8/13)
Carlotta	73% (8/11)	55% (10/20)	45% (5/11)	57% (8/14)
MS: Outlet Cr.	89% (8/9)	13% (1/8)	0% (0/9)	50% (4/8)

Sites in middle reaches of the South Fork Eel recorded the highest concentrations of anatoxin-a, which peaked in 2014 (Fig. 11). Not all SPATT samplers detected anatoxin-a; the Main-stem Eel at Outlet Creek, Fernbridge, and Big Bend sites never measured high cyanotoxin concentrations (Fig. 11). Weekly reconnaissance of the streambed 100-200 meters upstream of SPATT samplers never resulted in visual observation of cyanobacterial mats at Outlet Creek, Fernbridge, or Big Bend. Anatoxin-a was detected at high levels in a single, early-season SPATT sample from Holmes Flat, but was not detected at high concentrations from subsequent samplers. Additionally, 64 of the 106 cyanobacterial mat samples tested positive for either anatoxin-a or microcystin, indicating that about 40% cyanobacterial mats were not toxic.

The timing of the rise and fall of cyanotoxin concentrations was similar in 2013 and 2014 (Fig. 12). In both years cyanotoxin concentrations start to increase in late July and remain elevated into early August. However, higher concentrations were reached earlier in the season in 2014 than in 2013. By late August concentrations at all sites were close to zero and remained low until the end of September when monitoring ceased. Though there were some cyanobacterial mats still testing positive for cyanotoxins in September.

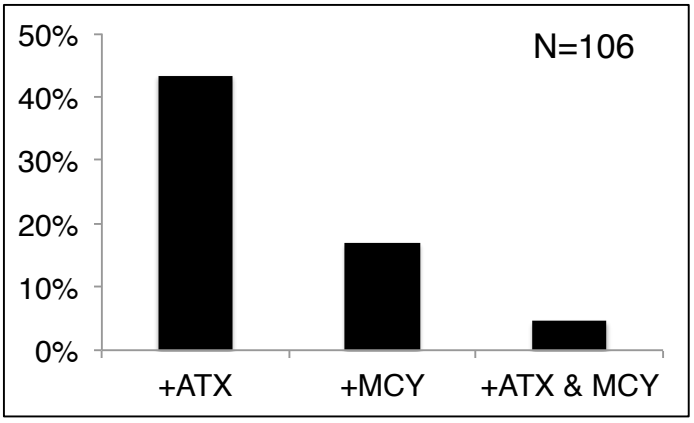

Figure 10. Percentage of cyanobacterial mat samples testing positive for intracellular anatoxin-a (ATX), microcystin (MCY), or both.

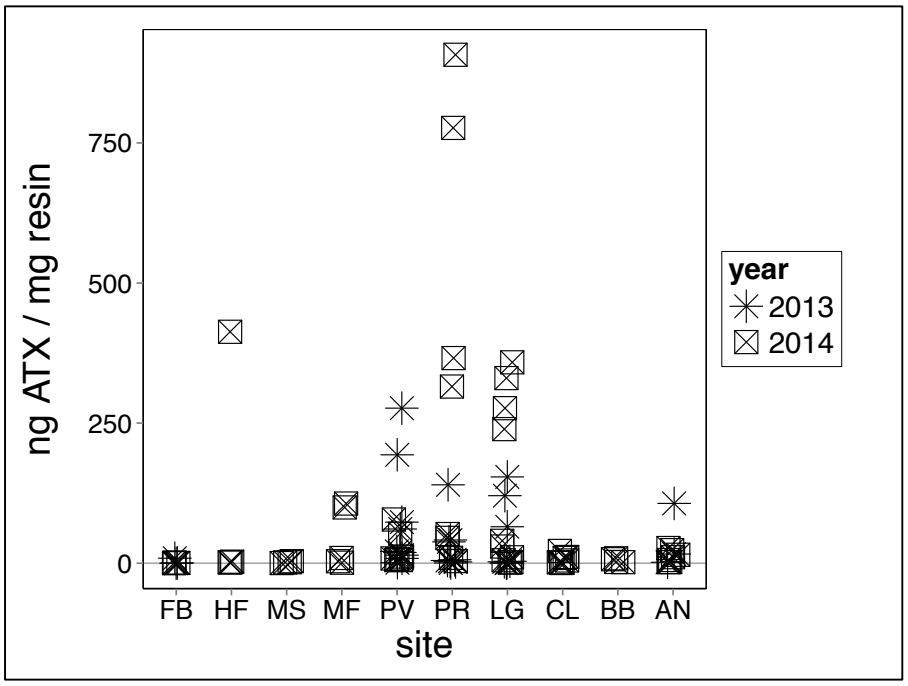

Figure 11. Anatoxin-a values across sites from SPATT samplers that detected anatoxin-a. Samplers with non-detects are not included (see Table 3). (Sites are ordered L-R by watershed size).

The SPATT method is considered semi-quantitative, as SPATT results are not easily converted into cyanotoxin concentrations used to set regulatory guidelines (e.g. µg / L). Since sampling and laboratory protocols are standardized, SPATT values from different sites and days can be compared to one another to produce a time series that approximates cyanotoxin concentrations in the river. Comparisons can be made about the timing of cyanotoxin concentrations between the anatoxin-a and microcystin time series. However, comparisons between the magnitudes of the peaks are less clear. Anatoxin-a has a slower adsorption rate than microcystin; so anatoxin-a SPATT values underestimate actual toxin concentrations more than microcystin SPATT values. Additionally the toxicity of these cyanotoxins is different (Butler et al. 2012). In spite of these methodological cautions, the combination of high anatoxin-a SPATT and cyanobacterial mat values gives evidence for more anatoxin-a production in the Eel River than microcystin production.

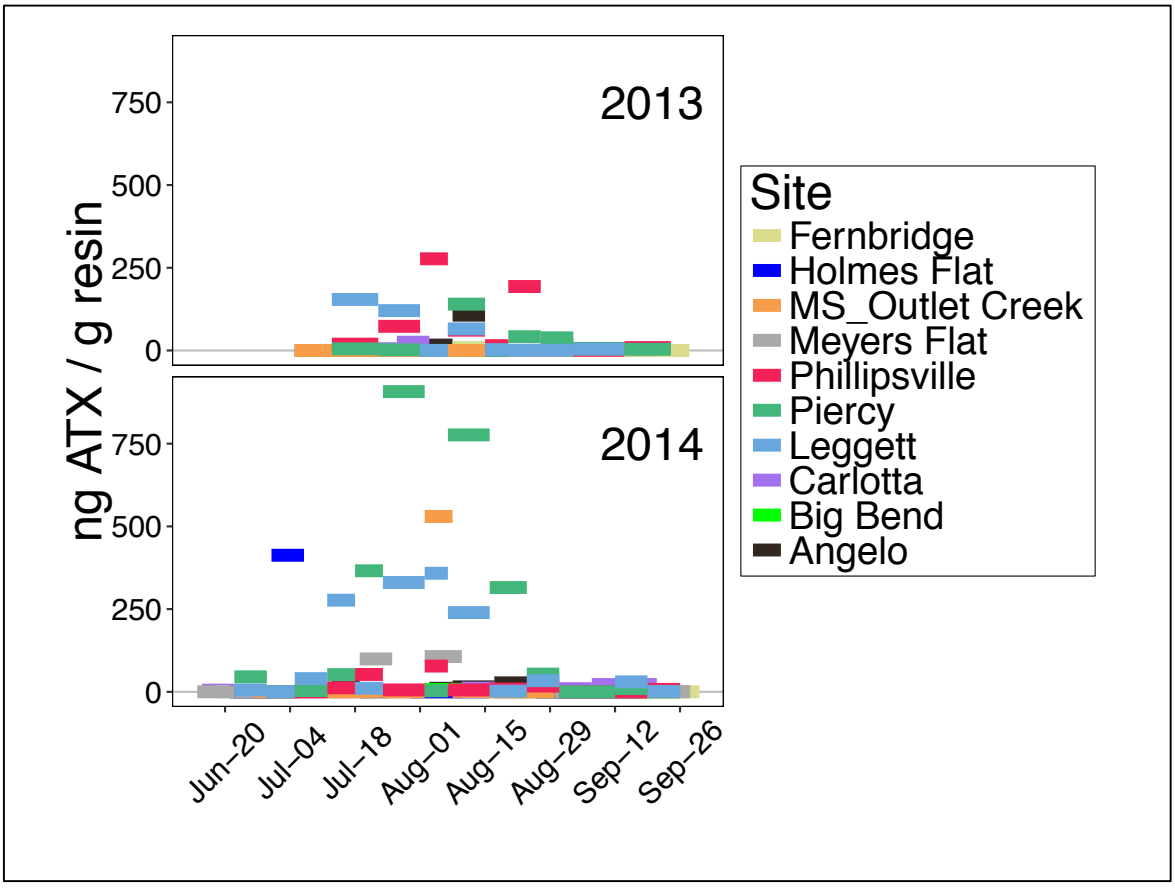

Figure 12. Time series of anatoxin-a concentrations from SPATT samplers in 2013 and 2014 (sites are ordered top to bottom by watershed size).

Conclusions

These SPATT and cyanobacterial mat data show the spatial and temporal patterns of cyanobacteria and cyanotoxins in the Eel River. The Eel is not the only river known to support toxin-producing cyanobacteria; others around the Monterrey Bay have also tested positive for microcystin (Gibble and Kudela 2014). New Zealand also has issues with anatoxin-a producing *Phormidium* mats (Heath et al. 2011, Quiblier et al. 2013).

Cyanotoxins in the Eel River seem to be most abundant in the middle reaches of the South Fork. These are the warmest and sunniest parts of the South Fork Eel. Downstream reaches are increasingly affected by coastal fog, which cools water temperatures and decreases sunlight. One hypothesis for the lack of toxin production in the Lower Eel (Fernbridge and Holmes Flat) is that the coastal fog creates conditions that inhibit cyanobacterial growth. However, a high anatoxin-a concentration was recorded in Holmes Flat in June 2014, and *Anabaena* spires were seen around the

SPATT sampler in June 2014. It is possible that the high Holmes Flat value resulted from a localized cyanobacterial mat die-off, releasing large amounts of cyanotoxins into the environment. It is also possible that cyanobacterial mats around Holmes Flat formed in late spring and early summer, before our monitoring began.

Warm temperatures cannot be the only predictor of cyanobacterial growth, because there were no cyanobacteria in the warmest site, the Main-stem Eel at Outlet Creek. Since it is on a different fork of the river, there could be other environmental factors, apart from temperature, between these two forks affecting cyanobacterial growth. Most sites were located on the South Fork Eel, and more sampling needs to occur on the Main-stem, Middle Fork, and Van Duzen River to describe the presence and absence of cyanobacteria in these watersheds.

In 2014, elevated SPATT concentrations at Phillipsville were expected because the site had the most conspicuous cyanobacterial mats upstream of the SPATT sampler. Nonetheless, in 2014, Piercy and Leggett had the highest anatoxin-a concentrations. At Piercy, four of the thirteen cyanobacterial mat samples tested positive for anatoxin-a, while at Leggett three of the six samples tested positive, indicating that cyanotoxins were being produced around the SPATT samplers at both sites. Therefore, the source of the cyanotoxins were likely a combination of 1) observed *Phormidium* mats upstream of SPATT samplers 2) many small cyanobacterial patches distributed across the river bed, but never forming large conspicuous mats, and/or 3) cyanobacterial mats upstream of our visual observations.

Cyanotoxins move some distance downstream with the river flow before being degraded into non-toxic molecules. It is expected that in a warm, high-light, and biologically active environment like the Eel, toxins would be degraded relatively quickly (Tsuji et al. 1995, Nybom 2013). There was no spatial auto correlation of toxin concentrations among the sites on the South Fork Eel, and so it is unlikely that SPATT samplers at different sites are measuring toxins sourced from the same area and flowing downstream. For example, it appears SPATT samplers in the lower Eel at Holmes Flat or Fernbridge did not adsorb detectable cyanotoxins produced upstream in the South Fork Eel. The exact distance upstream that SPATT samplers are measuring toxins remains unknown. Sampling sites on the South Fork were spaced >10 kilometers apart. Therefore, it's possible that toxins are degraded within several kilometers of being released into the water column, but more research is needed to understand the movement of cyanotoxins downstream.

SPATT and cyanobacterial mat samples detected more amounts of anatoxin-a than microcystin, suggesting that Eel River cyanobacteria produce anatoxin-a over microcystin. Though microcystins were frequently detected, their SPATT values were an order of magnitude lower than the anatoxin-a values. Multiple species of cyanobacteria have been documented producing anatoxin-a (Metcalf and Codd 2012), and *Anabaena, Phormidium,* and *Nostoc* tested positive for anatoxin-a in this study. *Nostoc* is rarely the focus of harmful cyanobacterial bloom research. However, *Nostoc* is abundant in the Eel, and four of the ten *Nostoc* samples tested positive for anatoxin-a, so *Nostoc* may be contributing to cyanotoxin levels in rivers. In the Eel, *Nostoc* does not form large mats and is firmly attached to the riverbed, being rarely entrained into the flow, so it may pose less of a public health threat.

The higher microcystin SPATT values at the Angelo site were unexpected. The Angelo Reserve is in the headwaters of the South Fork and has limited large-scale land development upstream. Additionally, it is located in the transition zone from a cool shaded headwater channel, to a sunnier-warmer mid-river channel; therefore, it is not expected to be an optimal location for cyanobacteria to proliferate. However, cyanobacterial mats were observed at Angelo, and six of the 13 cyanobacterial mat samples collected at Angelo tested positive for microcystin, thus some cyanobacterial taxa inhabit this region. As with anatoxin-a, several genera of cyanobacteria can produce microcystin, and the common microcystin producers around the Angelo Reserve have yet to be identified.

There appear to be two distinct environments likely to support cyanobacterial mats in the Eel River: riffles where *Phormidium* mats are often found, and slow flowing areas where *Anabaena* grows on senescing macro-algae (Table 1). The exact environmental conditions that drive the growth of these cyanobacterial mats in these two different environments are not known. It is likely related to some interaction among temperature, light, water velocity, and nutrients. Current hypotheses to explain the growth of cyanobacterial mats in the Eel remain somewhat speculative and untested. Future work will begin to test hypotheses about the environmental conditions that favor cyanobacterial growth in the Eel River. This information could help improve the environmental management of the Eel River to maintain the public safety and productive aquatic food webs in the river.

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Works Cited

Backer, L. C., J. H. Landsberg, M. Miller, K. Keel, and T. K. Taylor. 2013. Canine cyanotoxin poisonings in the United States (1920s-2012): review of suspected and confirmed cases from three data sources. Toxins 5:1597–1628.

Butler, N., J. Carlisle, and R. Linville. 2012. Toxicological Summary and Suggested Action Levels to Reduce Potential Adverse Health Effects of Six Cyanotoxins. California Department of Environmental Health Hazard Assessment. Sacramento, CA.

Dittmann, E., D. P. Fewer, and B. A. Neilan. 2013. Cyanobacterial toxins: biosynthetic routes and evolutionary roots. FEMS microbiology reviews 37:23–43.

Gibble, C. M., and R. M. Kudela. 2014. Detection of persistent microcystin toxins at the land–sea interface in Monterey Bay, California. Harmful Algae 39:146–153.

Heath, M. W., S. A. Wood, and K. G. Ryan. 2011. Spatial and temporal variability in Phormidium mats and associated anatoxin-a and homoanatoxin-a in two New Zealand rivers. Aquatic Microbial Ecology 64:69–79.

Hill, H. 2010. Blue-green algae (BGA) detailed fact sheet. Humboldt County Department of Health and Human Services. Eureka, CA.

Kudela, R. M. 2011. Characterization and deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. Harmful Algae 11:117–125.

Lane, J. Q., C. M. Roddam, G. W. Langlois, and R. M. Kudela. 2010. Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of the hydrophilic phycotoxins domoic acid and saxitoxin in coastal California. Limnology and Oceanography: Methods 8:645–660.

Metcalf, J. S., and G. A. Codd. 2012. Cyanotoxins. Pages 651–675 *in* B. A. Whitton, editor. Ecology of Cyanobacteria II: Their diversity in space and time. Springer, Dordrecht.

Nybom, S. 2013. Biodegradation of Cyanobacterial Toxins. Pages 147–170 *in* M. Petre, editor. Environmental Biotechnology - New Approaches and Prospective Applications. InTech, Rijeka, Croatia.

Power, M. E., J. R. Holomuzki, and R. L. Lowe. 2013. Food webs in Mediterranean rivers. Hydrobiologia 719:119–136.

Puschner, B., B. Hoff, and E. R. Tor. 2008. Diagnosis of Anatoxin-a Poisoning in Dogs from North America. Journal of Veterinary Diagnostic Investigation 20:89–92.

Quiblier, C., S. Wood, I. Echenique-Subiabre, M. Heath, A. Villeneuve, and J.-F. Humbert. 2013. A review of current knowledge on toxic benthic freshwater cyanobacteria - Ecology, toxin production and risk management. Water Research 47:5464–5479.

Tsuji, K., M. Watanabe, and M. Suzuki. 1995. Stability of Microcystins from Cyanobacteria--II. Effect of UV Light on Decomposition and Isomerization. Toxicon 33:1619–1631.

Appendix 1: Methods

SPATT samplers: The construction and analysis of SPATT samplers was adapted from Lane et al. (2010) and Kudela (2011). To make SPATT samplers, 3 g of HP20 DIAION© resin were sandwiched between two 10 cm squares of 118 μm Nitex mesh and placed in a 2.5 inch diameter embroidery hoop ring (Westex/Caron Flex Hoop rings). To activate and clean the resin, the ring was submerged in 100% HPLC grade methanol (MeOH; Fisher A456) for 20-28 hours. After activation, the methanol was rinsed off by agitating the ring for 30-60 s three times in 500 mL of ultrapure (Milli-Q) water. After rinsing, samplers were placed in plastic bags with 100mL of Milli-Q water and stored in the dark at 4°C.

To deploy the SPATT samplers, samplers were attached to a metal pipe that was hammered into the riverbed in the thalweg. The sampler was attached to the stake at approximately half of the water depth (Fig. 6). Every 5-10 days the samplers were removed from the river and a new sampler placed in the river. Upon removal, samplers were rinsed with river water, and then stored in plastic bags at -10°C until toxin extractions.

To extract toxins, SPATT samplers were thawed and the resin rinsed with Milli-Q water. Then the resin was poured into a disposable liquid chromatography tube and placed on

a vacuum manifold. Toxins were extracted from the resin with consecutive 10, 20, 20 mL rinses of a 50% solution of methanol (Fisher A452) and Milli-Q water.

The three extracts were then analyzed separately using high performance liquid chromatography mass spectrometry (LC-MS). Microcystin and anatoxin-a concentrations were measure on an Agilent 6130 Liquid Chromatography-Mass Spectrometry system with a Cogent Diamond-Hydride column and direct-injection of 20 µL. The LC-MS measured four microcystin congeners MC–LR, –YR, –RR, and –LA, these values were then summed together. The concentrations from the three scintillation vials were then summed to give nanograms of cyanotoxin per milligram resin.

Laboratory experiments were conducted to describe the adsorption and extraction kinetics of anatoxin-a on HP-20 DIAION resin (data not shown), microcystin kinetics are described in Kudela (2011). Anatoxin-a adsorbs slower than microcystins, but has similar extraction efficiencies to MCY. Both anatoxin-a and microcystins saturate the resin at higher concentrations than measured in the Eel River. Anatoxin-a SPATT values are likely a more conservative estimate of dissolved toxin concentrations than microcystin SPATT values.

Algal scrapings: Algae was collected from the riverbed by collecting algae inside a 1.5 inch diameter PVC pipe delimiter. For a given cyanobacterial patch, 3-5 delimiters were collected and combined into a single sample. In the lab, any macroinvertebrates were removed from the sample and stored in ethanol. Then algae were homogenized in a blender and the volume of the homogenate recorded. A 10 mL subsample was collected for cyanotoxin analysis, placed in a 20 mL glass scintillation vial, and frozen at -20°C until toxin analysis.

For intracellular toxin analysis, samples were thawed and 3 mL of sample added to a glass culture tube. Then, 3 mL of 50% MeOH was added and the tube was sonicated for 30 s. For anatoxin-a analysis, a 1 mL subsample was taken and 0.2 µm filtered into an LCMS vial. Microcystin samples were SPE cleaned using a Baker C18 column, and then 1 mL was added to a 2 mL vial. The cyanotoxin concentrations in the vials were measured using LC-MS.