

Eel River Cooperative Cyanotoxin Analysis Summary 2013-2017



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Performed for: The Eel River Recovery Project

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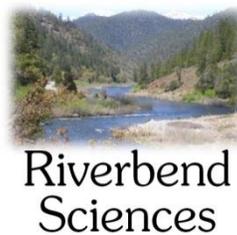
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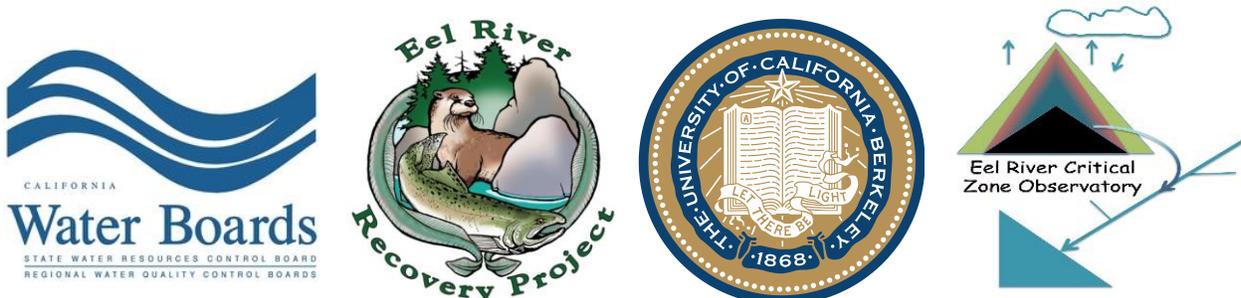
Our greatest thanks goes to Keith Bouma-Gregson who organized us and set the protocols and standards for the project. Keith has now completed his doctoral thesis and has been awarded a PhD from the University of California Berkeley. Dr. Mary Power of UCB has also been supporting ERRP’s efforts by training volunteers at Algal Forays led by algal taxonomists and ecologists Drs. Rex Lowe and Paula Furey at the UC Angelo Reserve. These trainings helped our volunteers develop expertise in cyanobacteria identification, which allowed rapid local response to questions about potential toxic species present. UC involvement and Keith’s studies were enabled by a National Science Foundation Eel River Critical Zone Observatory (CZO) grant (CZP EAR-1331940), Environmental Protection Agency STAR Fellowship (91767101-0), and by the Angelo Coast Range Reserve.

Dr. Raphael Kudela of UC Santa Cruz is a developer of the Solid Phase Adsorption Toxin Tracking (SPATT) devices that are utilized in cyanotoxin studies. ERRP is very grateful to Dr. Kudela and his students who continue to process SPATT samplers for ERRP after direct involvement of UCB ended. This will allow us to do trend monitoring cost-effectively and consistently in the future.

The North Coast Regional Water Quality Control Board (NCRWQCB) is also a valued partner, lending us automated water temperature probes annually, and collecting supplementary samples at other Eel River locations that have led to new discoveries. The State Water Resources Control Board also contributed to Eel River cyanotoxin studies as part of the *Eel River Monitoring and Outreach Project* (ERRP 2016) that promotes cooperative monitoring with local water districts.

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Eli Asarian of Riverbend Sciences led the data analysis for this report, created most of the figures, wrote the first draft of the results and conclusion, and edited. Keith Bouma-Gregson’s dissertation and associated publications provided the foundation for much of this report, and he also provided strategic advice and review of this report. Pat Higgins on ERRP wrote first draft of the executive summary, background, methods, and discussion, and also edited. Diane Higgins formatted and edited this report. Mary Power and Raphael Kudela provided comments on a draft of this report. Jan Derksen compiled the 2016-2017 ERRP temperature data and created analysis tools for temperature and flow.



Executive Summary

Eel River problems with development of toxic cyanobacteria blooms came into focus in 2001 when dogs died as a result of exposure to blooms. The Eel River Recovery Project (ERRP) had recognized this problem as a priority and was highly receptive to overtures from Keith Bouma-Gregson and the University of California to help us organize and carry out watershed-wide cyanobacteria analysis.

Cyanotoxin monitoring began in 2013 with the support of UC Santa Cruz (UCSC), which supplied the solid phase adsorption toxin tracking (SPATT) sampling devices. Keith Bouma-Gregson conducted weekly monitoring in nine sites in 2013 and 11 sites in 2014, and supervised sampling by volunteers. From 2015 on, ERRP took more responsibility in coordinating volunteers, switched to a monthly sampling regime, and began to work directly with UCSC. The NCRWQCB joined in Eel River cyanotoxin sampling in 2016 and together all parties have monitored at 20 sites total and collected 332 total samples. Adjustments in the number of sites and length of SPATT deployments make it difficult to make clean quantitative comparisons across the entire study period; however, general conclusions can still be drawn. This study is one of the first in the Western United States to look at cyanotoxin development in a river system, while blooms in lakes and reservoirs are better studied.

SPATT results indicated widespread occurrence of microcystin and anatoxin-a in the Eel River watershed, but the levels of anatoxin-a were an order of magnitude higher. Microcystin also has a slower acting toxin that works on the liver, while anatoxin-a is a swift acting neurotoxin that kills through paralysis. The organism thought to be responsible for most of the anatoxin-a production in the Eel River watershed is *Anabaena* (Figure 1), a type of cyanobacteria that thrives in warm water, particularly in slow flowing water at channel margins. *Anabaena* often grows on top of non-toxic filamentous macro-algae, such as *Cladophora*. *Phormidium* (Figure 2) is another anatoxin-a producing cyanobacteria that is present in the Eel river watershed. *Phormidium* can tolerate cold water and prefers fast-moving water and riffles. Children, pets, and livestock are at highest risk from cyanotoxins because they are more likely to swallow river water or, in the case of animals, eat toxic algal crusts, mats, and scums which can have cyanotoxin concentration that far exceed those of ambient water.

The years in our study period span a wide range of hydrologic conditions, with summer flows being highest in 2017 and generally lowest in 2014 and 2015. The quantity of anatoxin-a that accumulated on the SPATTs was far higher in 2015 than any other year, which we attribute in part to very low flows and high air temperatures during the spring, which allowed *Cladophora* to start growing earlier than usual, and may have allowed cyanobacteria mats to develop earlier than in other years. Flushing flows did not arrive until late November in 2015.

There appear to be distinct spatial patterns for the anatoxin-a and microcystin within the Eel River watershed, with anatoxin accumulations and detection frequencies generally being higher at sites in the South Fork Eel River (especially the lower and middle reaches) than in other sub-basins (2015 was an exception), while the highest microcystin accumulations were found in the upper Eel River at Trout Creek Campground below Lake Pillsbury. Thermal differences were evident also, with peak anatoxin-a accumulations occurring with water temperatures several degrees warmer than peak microcystin accumulations. However, sites with the hottest peak summer temperatures (Middle Fork Eel and middle mainstem Eel River) generally did not have high accumulations of anatoxin-a, suggesting that other factors in addition to water temperature are contributing to the South Fork Eel River's susceptibility to

Anabaena blooms. We speculate that may be due in part to current and past land use in the watershed, including *Cannabis* farms, timber harvest, and roads, which collectively generate nutrient pollution, sediment, and reduce summer base flows. A warming climate is also increasing water temperatures.

ERRP recommends continuing work with its partners to address the issues contributing to cyanotoxin blooms in the Eel River watershed.



Figure 1. *Anabaena* dark green spires growing up out of decaying *Cladophora* at Phillipsville on the South Fork Eel River in August 2014.



Figure 2. *Phormidium* mat in the Black Butte River.

Background

Historically, the Eel River was a cold water ecosystem with hundreds of miles of low gradient stream channels ideal for Pacific salmon species spawning and rearing (Shapovalov 1939). Although surface temperatures may have been warm during the hottest days in summer, there was likely a substantial surface and groundwater exchange and pools would have been stratified and cold at depth. The 1964 flood buried the stream channel at the convergence of the main Eel and the South Fork at Dyerville with an estimated 40-60 feet of sediment (Brown and Ritter 1971). Pools filled, the channel widened, surface-groundwater connections were disrupted and mainstem Eel River and its largest tributaries became warm water ecosystems. While much of the eastern portion of the Eel River watershed is in recovery from the 1964 flood, sediment over-supply remains a problem in some sub-basins (Higgins 2013, 2014, 2018).

Climate change also seems to be contributing to Eel River warming, as extended droughts in 1976-77, 1986-1992 and 2013-2015 all caused severe prolonged ecological stress. Snow was once a significant source of water in the Middle Fork, North Fork, and upper Eel River watersheds, but is now substantially diminished.

Emerging scientific evidence from the nearby Mattole River watershed (Stubblefield et al. 2012) suggests that increased evapotranspiration by mid-seral even aged forests may be a significant factor in reducing base flows. Since much of the Eel River watershed has over-stocked 40-60 year mixed conifer and hardwood stands as a result of widespread post WW II logging, it is likely that increased evapotranspiration is a significant factor in diminishing Eel River flows. Logging roads are also known to alter watershed hydrology, increasing peak flood flows and decreasing summer base flows (Jones and Grant 1996). Many road networks built for timber harvest remain, with some now maintained for rural development, and they are also likely a factor in diminished base flows. Extraction of water for *Cannabis* cultivation is significant in some Eel River sub-basins, and is responsible for the complete dewatering of some tributaries in summer (Bauer et al. 2015). The cumulative effects of altered watershed hydrology, increased water diversions, and likely addition of nutrients create conditions that allow cyanobacteria to proliferate seasonally in the Eel River.

Eel River Recovery Project (ERRP) involvement in cyanotoxin monitoring began when Keith Bouma-Gregson asked for our cooperation in late 2012. He was beginning to pursue a doctoral degree from the University of California Berkeley (UCB), studying under Dr. Mary Power in the Department of Integrative Biology. Keith had studied in China prior to beginning his work at UCB and had witnessed cyanobacteria and cyanotoxin proliferation there, which made him keenly aware of challenges we might face here in the United States. Keith was also fascinated by the fact that this toxic cyanobacteria problem was occurring in a river system, not a lake or a reservoir, which is more typical. Keith explained to ERRP that the partnership would allow him to cover more locations in the watershed as ERRP volunteers helped maintain and deploy sampling equipment. ERRP got Keith's expertise, a strengthened alliance with UCB, a study design, and cutting edge technology for analyzing cyanotoxins.

Harriet Hill (2010) of the Humboldt County Public Health Department investigated dog mortality in the Eel River watershed and alertly sent samples from deceased pets for testing. Puschner et al. (2008) discovered the toxic agent was anatoxin-a, which works on the nervous system, and that the dogs had died from paralysis. The environment where dogs were apparently encountering cyanotoxins was warm edge-water areas with algal mats and scums. Early on in cyanotoxin monitoring it became apparent that

the problem was associated with the cyanobacterial species *Anabaena*. Preliminary results of 2013-2014 sampling, reported in Bouma-Gregson and Higgins (2015), confirmed this relationship.

Anabaena in the Eel River are often associated with areas of low flow and warm water temperatures (Bouma-Gregson and Higgins 2015). Low flow velocity has a direct effect because it allows delicate mats of *Anabaena* to overgrow beneficial algae. Severely low summer base flows isolate shallow backwaters in mainstems, creating warm and stagnant hot spots where *Anabaena* proliferates, often in floating algal mats (Power et al. 2015), and accumulates via drift (Bouma-Gregson et al. 2017). Eel River baseline water temperature data were collected by Kubicek (1977) after the river had been altered by the 1964 flood. Friedrichsen (1998, 2000, 2001, 2003) measured water temperatures at over 200 locations from 1995-2003 and found few sites where temperatures had decreased in comparison to data collected by Kubicek (1977) in 1973. Water temperature data collected by ERRP since 2012 indicate warming in some tributaries, which could be a result of flow depletion or a warming climate (Higgins 2013, 2014; Asarian et al. 2016).

Algal and cyanobacterial biomass in rivers often increases with excess nutrients. Due to a combination of complexity and lack of data, patterns of Eel River nutrient enrichment are harder to discern. River nutrient cycling is complex, as nutrients are often absorbed by algae and aquatic plants in short reaches immediately below sources (Vadeboncoeur and Power 2017). In the Eel River, nutrients are often caught and used by *Cladophora*, a beneficial filamentous green algae that is an essential part of the aquatic food web because it is often densely colonized by epiphytic diatoms which are highly nutritious food for grazing macroinvertebrates. In the presence of excess nutrients, however, *Cladophora* growth can impair water quality because the dense mats can drive pH to high levels during diurnal photosynthesis and deplete waters of dissolved oxygen nocturnally as algae respire. Nutrient “spiraling” occurs when *Cladophora* segments detach as they senesce and drift downstream. As clumps of dead algae build up in warm, quiet water eddies, they create a nutrient rich soup that provides the perfect medium for *Anabaena* growth.

Anabaena may not always produce toxins and the greatest risk of toxin release is when the cells lyse or if a human or dog directly ingests cyanobacterial cells (Backer et al. 2013). Bouma-Gregson et al. (2017) found that photosynthesis by *Anabaena* produces oxygen bubbles which cause the colony to rise (Figure 1), break away from the *Cladophora* mats that they are growing on, and float away in the afternoon. These segments form scums downstream from the colonies of dark green spires, which may pose equal or greater risk to public health risk as the cells may be breaking down and are more likely to release toxins.

Bouma-Gregson et al. (2018a) also studied Eel River *Phormidium* (Figure 2) mats to detect cyanotoxin production and to explore relationships with other commensal organisms like bacteria that grow within mats. *Phormidium* is common in some of the more nutrient poor streams in the Eel River basin, where its growth is moderate. The species has an extremely efficient mechanism for phosphorous uptake. It is, therefore, nitrogen limited, so addition of nitrogen to streams may stimulate nuisance blooms (McAllister et al. 2016). In 2016, the NCRWQCB found evidence of dog mortality in the cold water reach of the upper Eel River within the Potter Valley Project (PVP). *Phormidium* is implicated as the source of the toxicant because its growth in this reach is luxuriant and it may be shedding segments and causing edgewater scums (Rich Fadness, Personal Communication), but further study of this newly identified Eel River problem is needed.

Although microcystin is widespread at sites throughout the Eel River basin at low levels (Bouma-Gregson 2018b), tracing its origin could be very complicated given the diversity of cyanobacteria species that can produce the substance. These include *Anabaena* (Figure 1), *Fischerella*, *Gloeotrichia*, *Nodularia*, *Nostoc* (

Figure 3 and Figure 4), *Oscillatoria*, members of *Microcystis*, and *Planktothrix* (U.S. EPA 2018). Since quantities of microcystin are low in the Eel River, studying this question is of low priority relative to anatoxin-a.

Power et al. (2015) highlighted the relationship of flow and cyanobacteria blooms, and postulated that severely low summer baseflows were tipping the Eel River ecosystem from a cold-water salmon producing stream to one dominated by cyanobacteria. Studies conducted on the South Fork Eel River at the Angelo Reserve (Power et al. 2008, 2013; Vadeboncoeur and Power 2017) indicate that in high flow years, grazing aquatic insects wash away, resulting in more lush *Cladophora* growth during the early summer. These growths provide substrate that lifts *Anabaena* into the light, as well as nutrients as the beneficial algae rot under the blanketing *Anabaena*. While shorter during years without scouring winter floods, attached *Cladophora* growths can still support subsequent cyanobacteria blooms further downstream in the South Fork Eel River.



Figure 3. *Nostoc* is small dark spherical or ear-shaped colonies adhered to bedrock, growing with attached *Cladophora* (light green filaments) – SF Eel at the Hermitage.



Figure 4. Larger detached *Nostoc* colonies in warm edge waters at Phillippsville.

Methods

This study uses three sources of data for analysis, U.S. Geologic Survey (USGS) flow gauge data, water temperature data from automated gauges placed throughout the Eel River watershed from 2013-2017 (Asarian et al. 2016), and data from 332 solid phase adsorption toxin tracking (SPATT) samplers placed at 20 locations that were cooperatively deployed by ERRP volunteers and contractors, UCB scientists, and NCRWQCB staff (Figure 6). The cyanotoxin monitoring methods discussion below is taken in part from Bouma-Gregson and Higgins (2015) and Bouma-Gregson (2018b).

Flow Data

We used data from seven¹ U.S. Geological Survey (USGS) flow gauging stations within the Eel River watershed (Figure 1), including one on the Middle Fork, one on the Van Duzen, three on the main Eel River, and two on the South Fork Eel River. Gauges measure flow in cubic feet per second (cfs) and average daily flow data are available on the internet². Gauge accuracy may change as river bed profiles shift, so the USGS offers recent data as provisional, then they check gauge accuracy and make final revisions of estimates, if necessary.

Water Temperature Data

Water temperature data were collected using Onset Instrument Optic Pro and Tidbit automated gauges that are set for intervals of 30 minutes and deployed throughout the Eel River watershed by ERRP and others (Asarian et al. 2016). Probes were placed in the shade in flowing water representative of ambient water temperature, according to Lewis et al. (2000) protocols. Temperature gauges were placed immediately adjacent to cyanotoxin samplers so that relationships could be explored.

The only site that was not deployed in flowing water was a side channel on the Van Duzen River floodplain, monitored in 2015 only, which received subsurface flow but had no surface flow connection to the river in summer.

Cyanotoxin Monitoring

SPATTs were adapted to monitor cyanotoxins in Monterey Bay and nearby waterbodies (Kudela 2011, Lane et al. 2010) after initial development and application in New Zealand (MacKenzie et al. 2004). These devices are comprised of a resin substance (HP20 DIAION®) that is placed within two 4 inch squares of 118 µm Nitex mesh and then attached to a 2.5 inch diameter embroidery hoop ring (Westex/Caron Flex Hoop rings) (Figure 5). The resin is activated and cleaned with 100% HPLC grade methanol (MeOH; Fisher A456) for 20-28 hours. The methanol is then rinsed off for 30 to 60 seconds three times in 500 mL of ultrapure (Milli-Q) water, and SPATTs are placed in plastic bags with 100mL of the same water and refrigerated in the dark at 4°C until deployed.

SPATTs were deployed by attaching them to a metal pipe that is driven into the stream bed in an area with flowing water (except one site on the Van Duzen River floodplain, see Water Temperature Data section above for details) midway up in the water column, and with a temperature probe attached at the base. Since it is undesirable for floating algae to catch on and cover the SPATT, one or two pipes were driven into the river bed upstream of the device to block or lessen such accretions in reaches with high algal drift. The length of time that SPATTs were deployed has varied. UCB retrieved them weekly in 2013 and 2014 (Bouma-Gregson 2017), but ERRP has used mostly monthly sampling intervals since. Under the ERRP regime, SPATTs are cleaned weekly by rubbing the Nitex mesh to remove diatoms that might be growing on them and also to remove any accumulating algae from the pipes. When removed from the river, SPATTs are rinsed with river water, and then frozen (-20°C) until the end of the sampling season when all samplers are returned to UC Santa Cruz (UCSC) for cyanotoxin extractions.

¹ Since this report focuses on the major forks of the Eel River, we do not make use of data from several additional USGS gages on tributaries (Bull Creek, Elder Creek, Cahto Creek) or the mainstem Eel River below Scott Dam (i.e., below Lake Pillsbury), except that we did include a graph of all sites in Appendix A.

² <https://maps.waterdata.usgs.gov/mapper/?state=ca>

To extract toxins, UCSC thaws the SPATT samplers and rinses them with Milli-Q water. Subsequently, the resin is poured into a disposable liquid chromatography tube and placed on a vacuum manifold. Toxins are extracted from the resin with consecutive 10, 20, and 20 mL rinses of a 50% solution of methanol (Fisher A452) and Milli-Q water. Cyanotoxins were measured from the extract with high performance liquid chromatography mass spectrometry (LC-MS) as described in Bouma-Gregson (2018b). The LC-MS measures four microcystin congeners MC-LR, -YR, -RR, and -LA, these values are then summed together as total microcystins. The lab reported concentrations in nanograms of cyanotoxin per milligram resin (ng/g resin). In an attempt to partially standardize the varying deployment lengths and following the convention of Bouma-Gregson (2018b), we present SPATT results in this report as “accumulations” calculated as concentration divided by deployment length, with resulting units of nanograms of toxin per gram of resin per day. Bouma-Gregson (2018b) found that anatoxin-a adsorbs slower than microcystins to SPATTs, but has similar extraction efficiencies to MCY. Both anatoxin-a and microcystins saturate the resin at higher concentrations than those found in the Eel River (Raphael Kudela, personal communication). Consequently, anatoxin-a SPATT values are likely a more conservative estimate of dissolved toxin concentrations than microcystin SPATT values.



Figure 5. SPATT deployed in Middle Fork Eel River with temperature gauge attached to base.



Figure 6. Map showing locations of SPATT samplers, selected U.S. Geological Survey flow gages, major rivers, streams, and towns within the Eel River Basin and adjacent areas.

Results

Flow

After spring rains cease and the dry summer begins, flows at sites in the Eel River Basin gradually decline until the first fall rains arrive in late September or October (Figure 8) and Appendix A. The years in our study period span a wide range of hydrologic conditions, with summer flows being highest in 2017 and generally lowest in 2014 and 2015.

In 2015, there was sufficient flow to scour stream beds in February, but then at most sites the flows in March through early July were lower than in 2014 although there was a small rise in April³. A series of thunderstorms in mid-July caused flows to rise, particularly in the Middle Fork Eel and sites on the main Eel downstream (Figure 8 and Appendix A). Due to lack of rainfall in September and early October 2015, lower Eel River flows remained under 100 cfs at Scotia. The bottom of the river remained carpeted with algae after Chinook salmon entry and many became diseased (Higgins and Stockwell 2016). A summer spate⁴ in late June 2013 kept that year from being the lowest in the 2013-2017 period (Figure 8) and also detached large amounts of *Cladophora*, which moved short distances downstream, then rotted (Power et al. 2015). The absolute minimum average daily flows at all USGS gauges from 2013 to 2017 were during the summer of 2014. Minimum flows for each gauge are labeled in Figure 8.

When flows dropped to critically low levels in mid-August 2014, additional water was released from the Potter Valley Project to increase flows. On August 29, 2014, ERRP used aerial photography to document that the lower main Eel River in Fortuna lost surface flow for the first time on record (Figure 7).



Figure 7. Lower Eel River bed in Fortuna showing loss of surface flow below 12th Street Pool. 8/29/2014.

³ Daily average flow of 3,780 cfs at the Scotia USGS gauge on April 8, 2015.

⁴ Power et al. (2008) defined a spate as “pulses of increased discharge that, while well below bankfull discharge, can potentially detach and export algae and susceptible benthic invertebrates.”

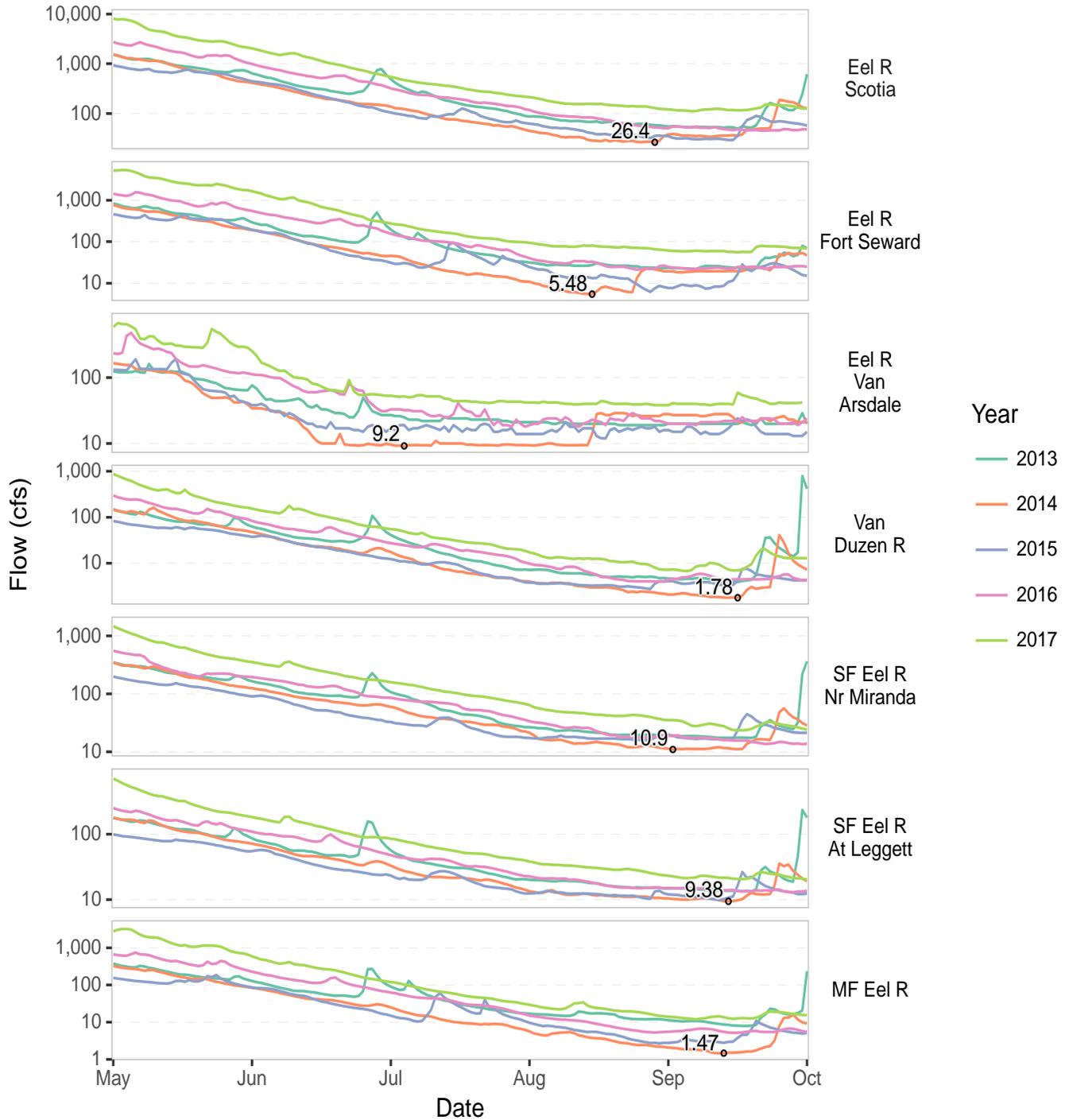


Figure 8. Daily time series of flow for May 1 – September 30 in 2013–2017 at selected USGS gaging stations in the Eel River Basin. Y-axis uses a log scale. The black circles and associated labels indicate the lowest flow observed at each station during the study period. Flow time series for the full calendar year and additional gages are available in Appendix A. Key to abbreviations: SF = South Fork, MF = Middle Fork, R = River.

Water Temperature and Air Temperature

Maximum weekly average water temperature (MWMT) is the average daily maximum temperature during the hottest seven-day period of the year⁵. MWMT values at SPATT sites varied by sub-basin, with hottest temperatures in the Main Eel and the Middle Fork, and lower temperatures in the Lower Eel and the Van Duzen (Figure 9). Downstream of its confluence with the South Fork, the Eel River cools as it flows towards the Pacific Ocean (Figure 9 left panel) due to marine climatic influence (Friedrichsen et al. 1998, Asarian et al. 2016). The South Fork has a complex longitudinal pattern, with cooler water near its headwaters, warming to a peak between Piercy and Phillipsville, and then cooling slightly at Myers Flat upstream of the confluence with the mainstem Eel (Figure 9). The Van Duzen River side channel (see Water Temperature Data section in Methods for details) was cooler than any other SPATT site, likely because it was disconnected from the warm river water and was fed instead by cool subsurface flow. Additional analyses of basin-wide water temperature data will be included in a separate ERRP report at a future date. Appendix C provides additional water temperature information (Figure 25, Table 5a graph of Maximum weekly average water temperature (MWAT) similar to Figure 9 and a table of MWMT and MWAT values for each SPATT site and year.

As expected based on previous analyses (Asarian et al. 2016), water temperatures near the SPATT stations in 2013–2017 are highly variable from week to week, but typically peaked in July or August (Figure 10). The water temperature probes are typically not deployed until sometime in June, so temperatures in earlier months are unknown despite being potentially important factors influencing seasonal progression of algal communities.

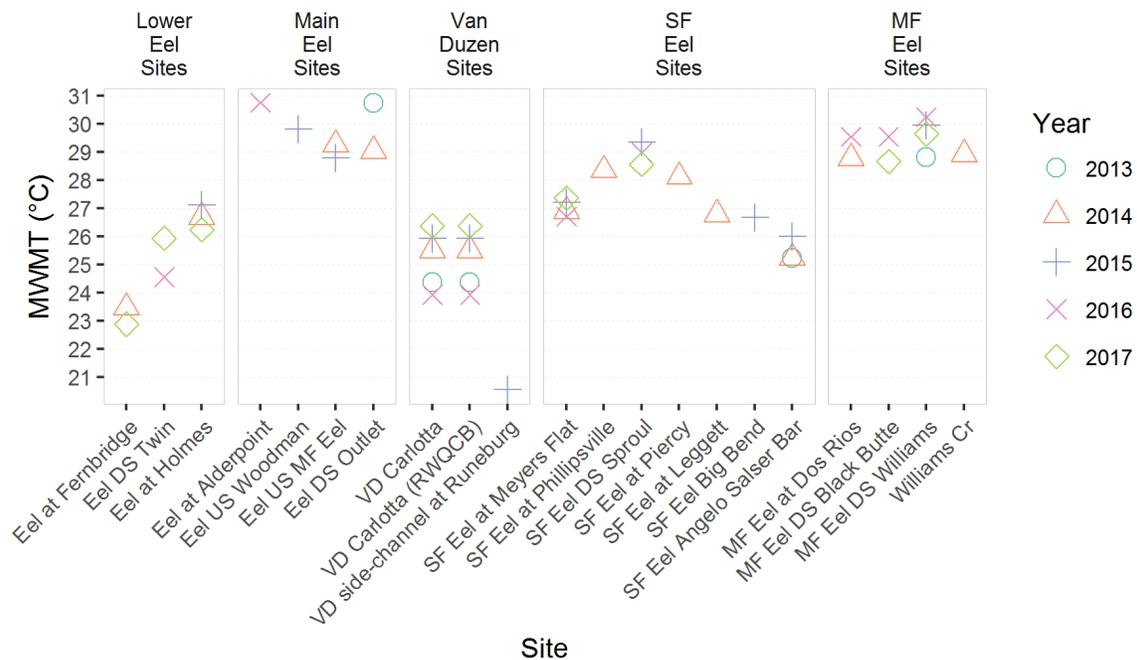


Figure 9. Maximum weekly average water temperature (MWMT) at SPATT sites in the Eel River basin, for each year 2013–2017. Within each sub-basin, sites are sorted from downstream (left side) to upstream (right side). MWMT values were only calculated for site-years in which the temperature logger was deployed for all days in both July and August, so there are some site-years in which there were SPATTs but not MWMT values.

⁵ See Asarian et al. 2016 for an illustrated example.

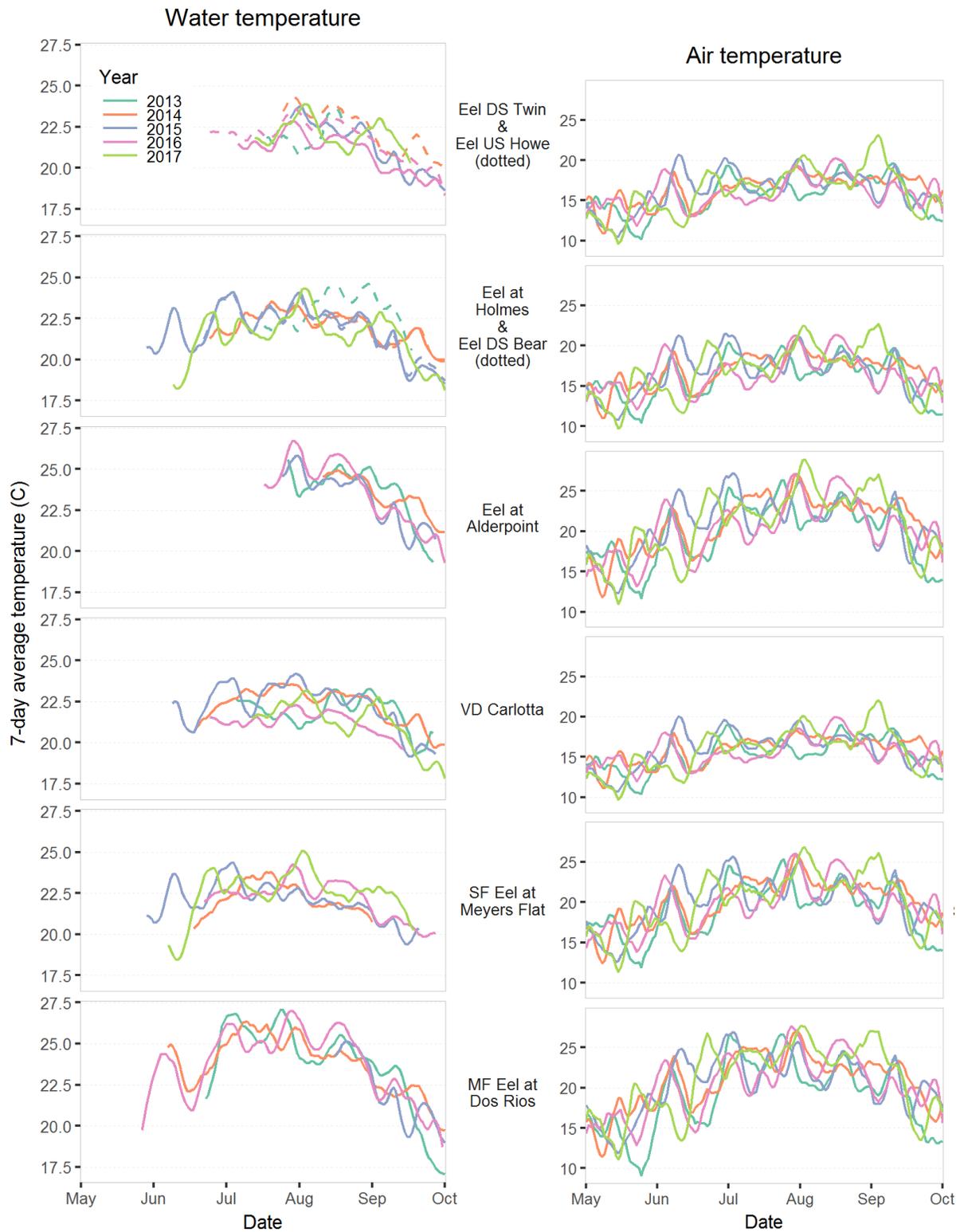


Figure 10. Daily time series of 7-day averages of water temperature (left) and air temperature (right) for May 1 – September 30 in 2013–2017 at selected stations. Sites were selected based on availability of long-term water temperature data and proximity to SPATT samplers and USGS gages. To increase the number of years, the top panels each include two nearby sites. Water temperature data are primarily from ERRP, supplemented by Eel River CZO (UC Berkeley) and PG&E data. Air temperature data are from PRISM Climate Group (2018).

SPATT monitoring

Toxin Detection Frequencies

Anatoxin-a and microcystin were detected in 50% and 61% of the 332 SPATT samplers analyzed, respectively, and were detected in every year (Table 1, Figure 11, and Figure 13). Changes in the sites and duration of deployments makes comparisons between years difficult, but anatoxin-a detection frequencies were lowest in 2016 (17%) and highest in 2015 (77%) (Table 1). Microcystin detection frequencies were highest in 2017 (100%) and 2015 (87%) and lowest in 2014 (39%) and 2016 (58%) (Table 1, Figure 11, Figure 13). At stations that were monitored for more than one season, detection frequencies for anatoxin-a were generally higher at sites in the South Fork Eel River than in other sub-basins (Figure 12).

Two additional cyanotoxins were analyzed during 2016-2017. Cylindrospermopsin was only analyzed in 2016 and 2017 (Figure 11) and was detected in 1% and 40% of samplers in those years, respectively (Table 3 and Figure 11). Nodularin was detected in 75% of the samples in 2016, which was the only year it was analyzed (Table 3 and Figure 11).

Table 1. Frequency of anatoxin-a, microcystin, cylindrospermopsin, or nodularin detection on SPATT samplers, summarized by site across all years. See Table 3 and Table 4 in Appendix B or Figure 11 for site-specific details.

| Year | Anatoxin-a | Total Microcystins | Cylindrospermopsin | Nodularin |
|-------------|-------------------|---------------------------|---------------------------|------------------|
| 2013 | 55% (38/69) | 75% (52/69) | | |
| 2014 | 53% (66/125) | 39% (49/125) | | |
| 2015 | 77% (36/47) | 87% (41/47) | | |
| 2016 | 17% (12/71) | 58% (41/71) | 1% (1/71) | |
| 2017 | 70% (14/20) | 100% (20/20) | 40% (8/20) | 75% (53/71) |
| Totals | 50% (166/332) | 61% (203/332) | 10% (9/91) | 75% (53/71) |

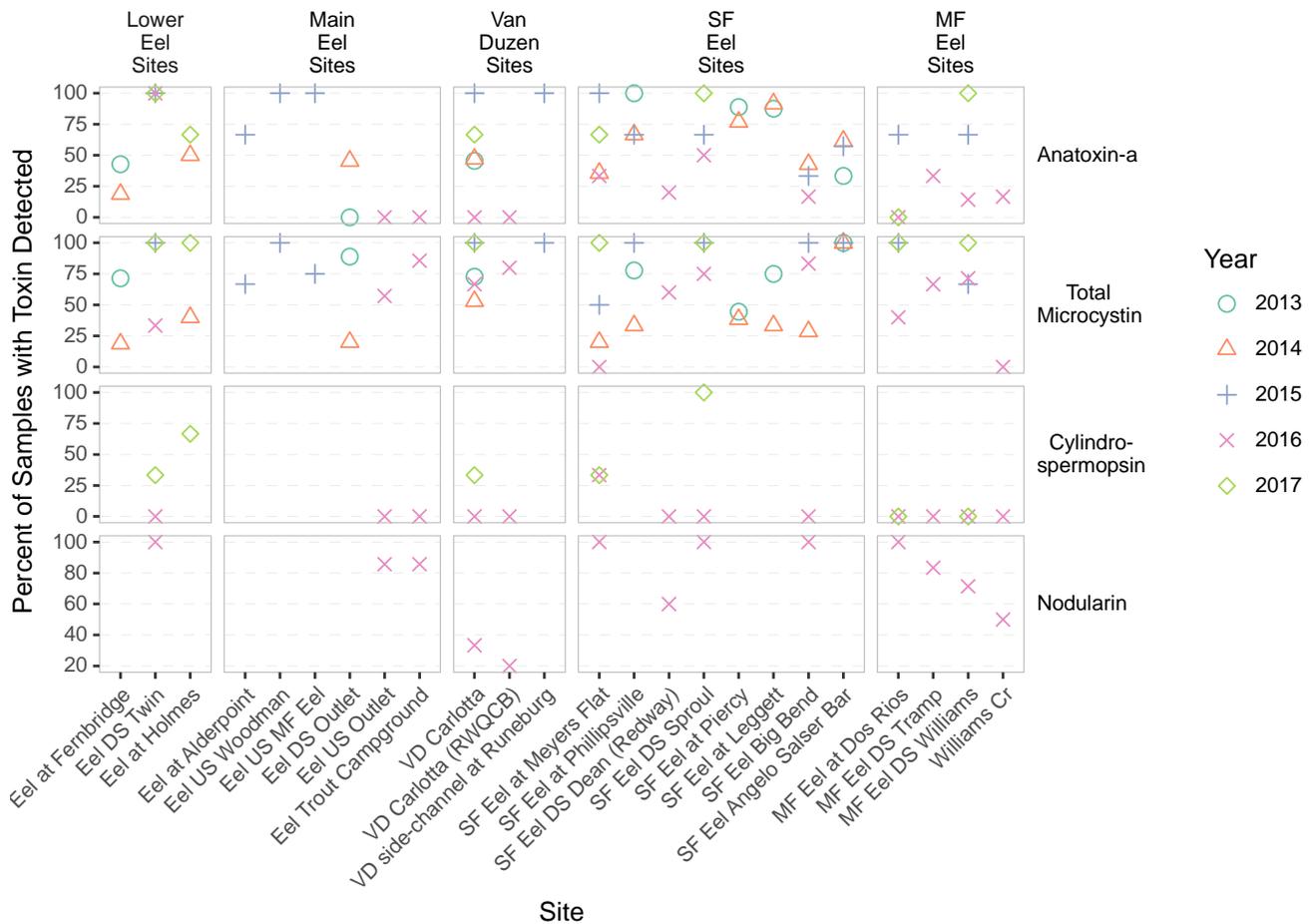


Figure 11. Percent of SPATT samplers with anatoxin-a, microcystin, cylindrospermopsin, or nodularin detected at sites in the Eel River basin, for each year 2013-2017. Sites are grouped by sub-basin. Within each sub-basin, sites are sorted from downstream (left side) to upstream (right side). Only sites with more than one sample per year are shown. Anatoxin-a and microcystin results are also shown in Figure 13. Data are available in tabular form in Table 6 in Appendix B.

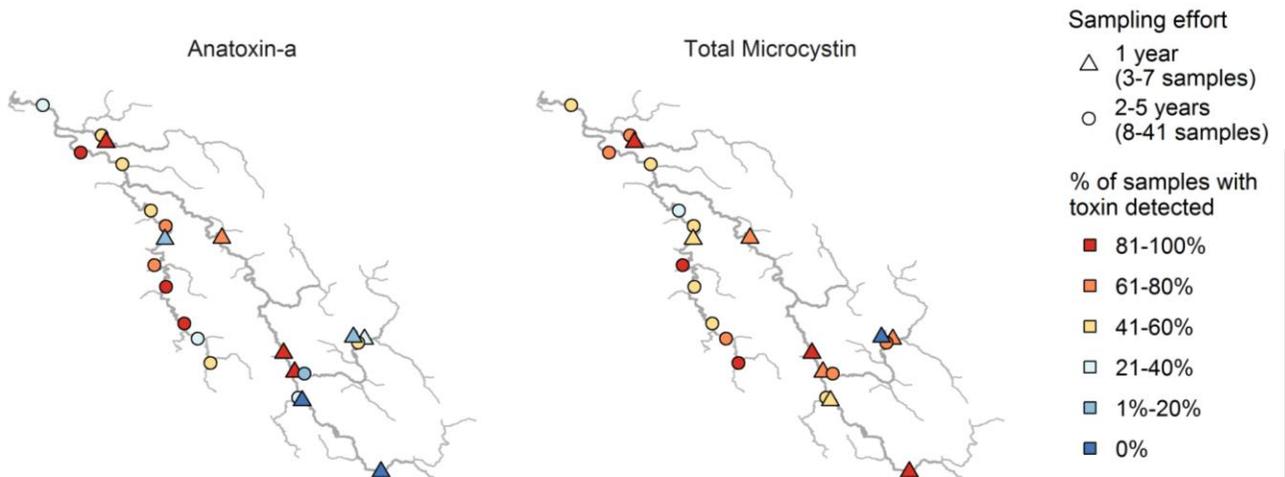


Figure 12. Maps showing the average (lumping samplers from all years together) percent of SPATT samplers with anatoxin-a or microcystin toxin detected at sites in the Eel River Basin in the years 2013–2017. Symbol color shows percent detection while symbol shape indicates whether site was sampled in more than one year. Several sites' coordinates were adjusted slightly to improve legibility.

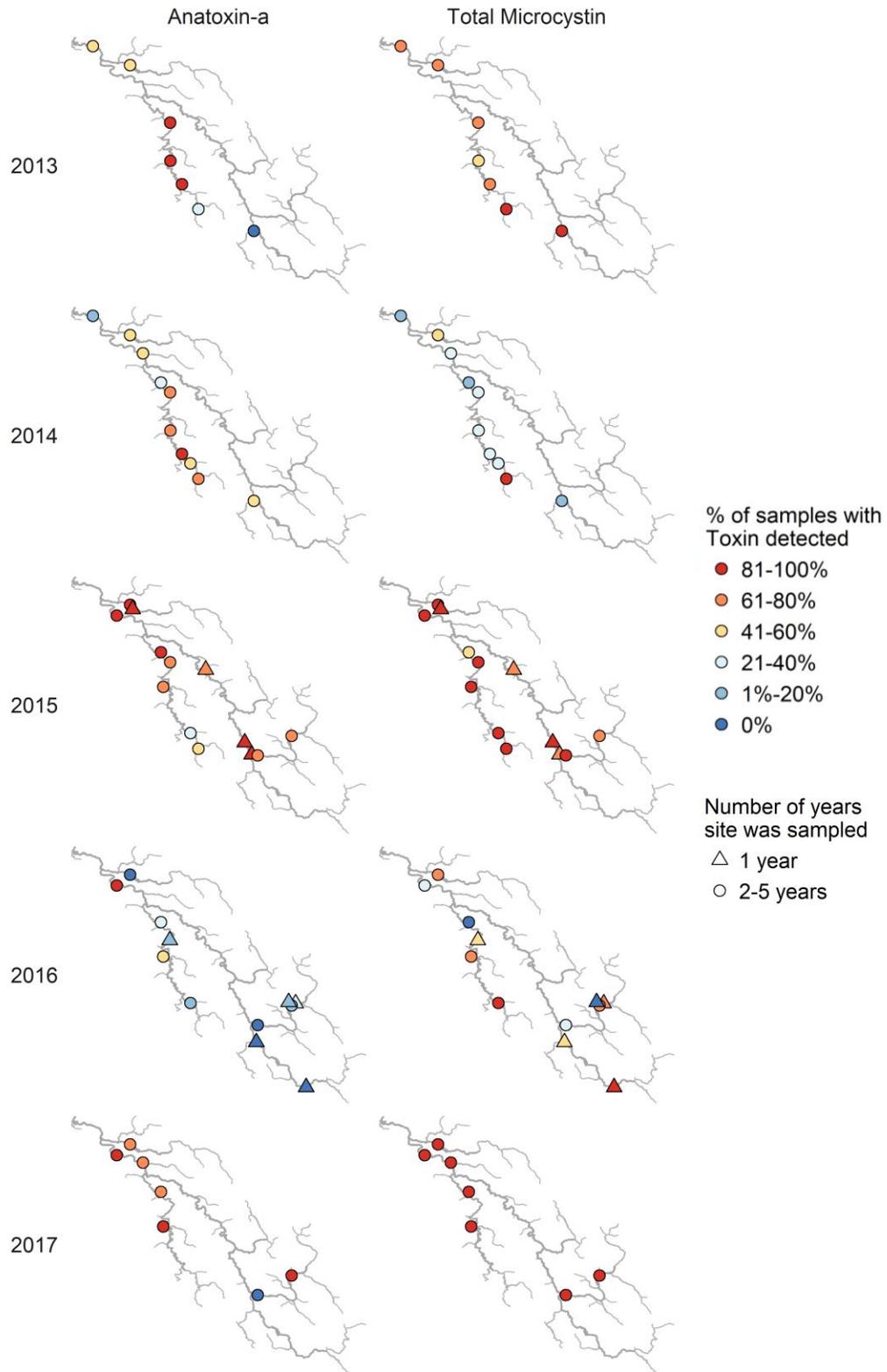


Figure 13. Maps showing the percent of SPATT samplers with anatoxin-a or microcystin toxin detected at sites in the Eel River basin, for each year 2013-2017. Sites are only shown for years with more than one sample per site. Several sites' coordinates were adjusted slightly to improve legibility.

Toxin Accumulations

Although detected less frequently, accumulations of anatoxin-a on the SPATT samplers were generally higher than microcystin accumulations (Figure 14). Some anatoxin accumulations in 2015 were extremely high (e.g., approaching or exceeding 100 ng/g resin/day), including the Eel River downstream of Twin Creek near Scotia, Eel River upstream of the Middle Fork Eel, South Fork Eel River at Myers Flat, South Fork Eel River downstream of Sproul Creek, and Van the Duzen River (Figure 14 and Figure 15). In years other than 2015, anatoxin accumulations appear to be higher at sites in the South Fork Eel River below Big Bend than in other sub-basins (Figure 14). With the exception of 2015, anatoxin accumulations were generally lower on SPATTs retrieved in September and October than earlier months (Figure 15, especially the panel in upper left corner which combines all sites).

The highest accumulations of microcystin were in the Eel River at Trout Creek Campground station (downstream of Lake Pillsbury) which was only monitored in 2016 (Figure 14 and Figure 16). The two most upstream stations on the South Fork Eel River (Big Bend in South Leggett and Salser Bar on the Angelo Coast Range Reserve) also had relatively high microcystin accumulations (Figure 14 and Figure 16). Similar to anatoxin, microcystin accumulations were generally lower on SPATTs retrieved in September and October than earlier months (Figure 16, especially the panel in upper left corner which combines all sites).

A comparison of mean water temperatures and toxin accumulations suggest that peak anatoxin-a accumulations occurred at water temperatures several degrees warmer than peak microcystin accumulations (Figure 17), as found previously by Bouma-Gregson (2017) for the years 2013–2014. Unfortunately, we do not currently have access to an electronic version⁶ of 2016 water temperature data for the Eel River at Trout Creek Campground, which is the site with the highest microcystin concentrations, so we were unable to include data for that site in Figure 17. The scatterplot in Figure 17 has a trend line indicating that anatoxin-a was less prevalent at higher temperatures; however, this may simply reflect the fact that the sites with the highest water temperature had lower susceptibility to developing cyanotoxins due to other factors (see Discussion).

⁶ Pacific Gas and Electric Company monitors water temperatures in that reach, and daily average data were published in their annual monitoring report (PG&E 2017).

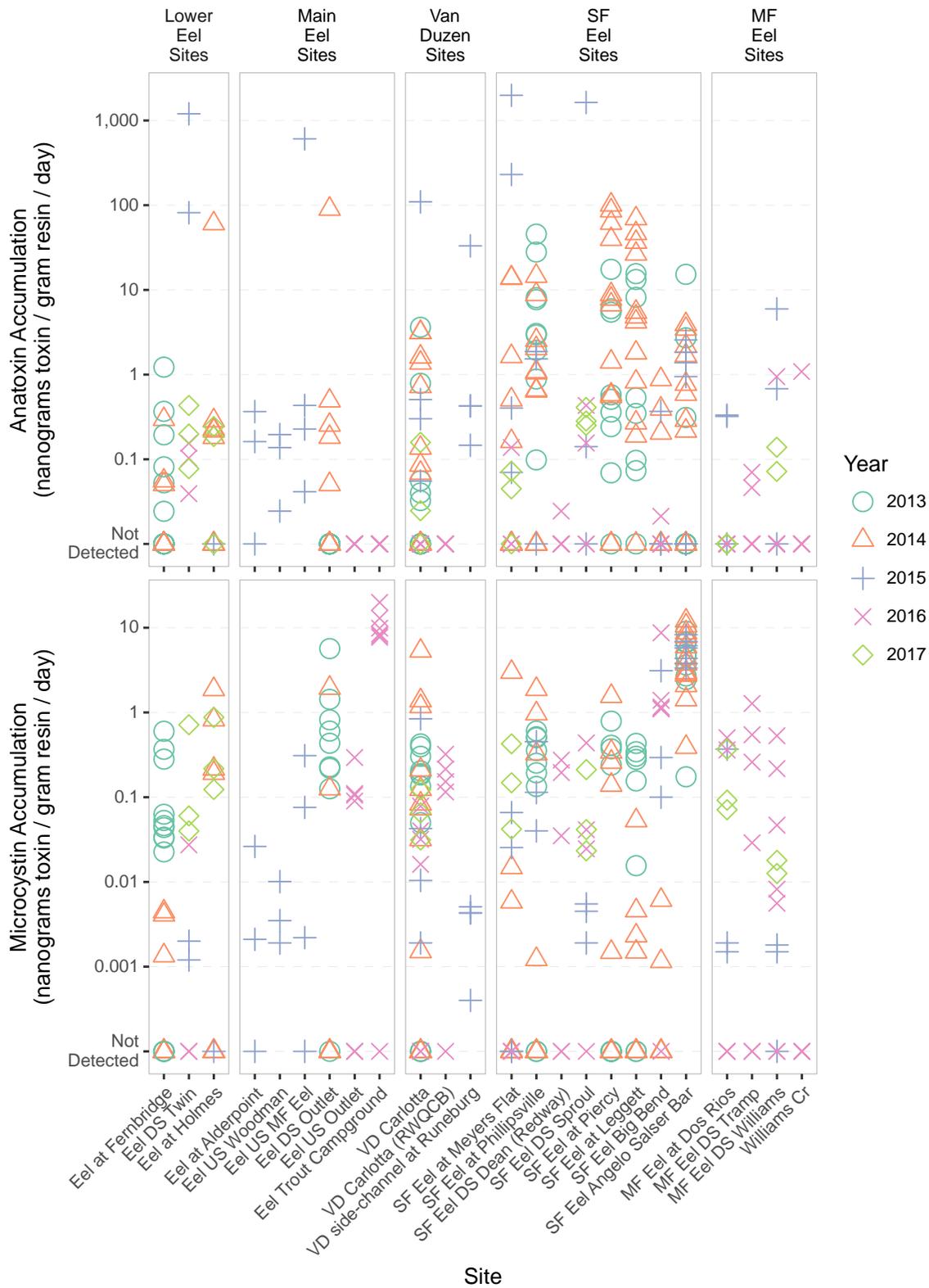


Figure 14. Accumulations of anatoxin-a (top panel) and microcystin (bottom panel) on SPATT samplers deployed at sites in the Eel River basin in the years 2013-2017. Sites are grouped by sub-basin. Within each sub-basin, sites are sorted from downstream (left) to upstream (right).

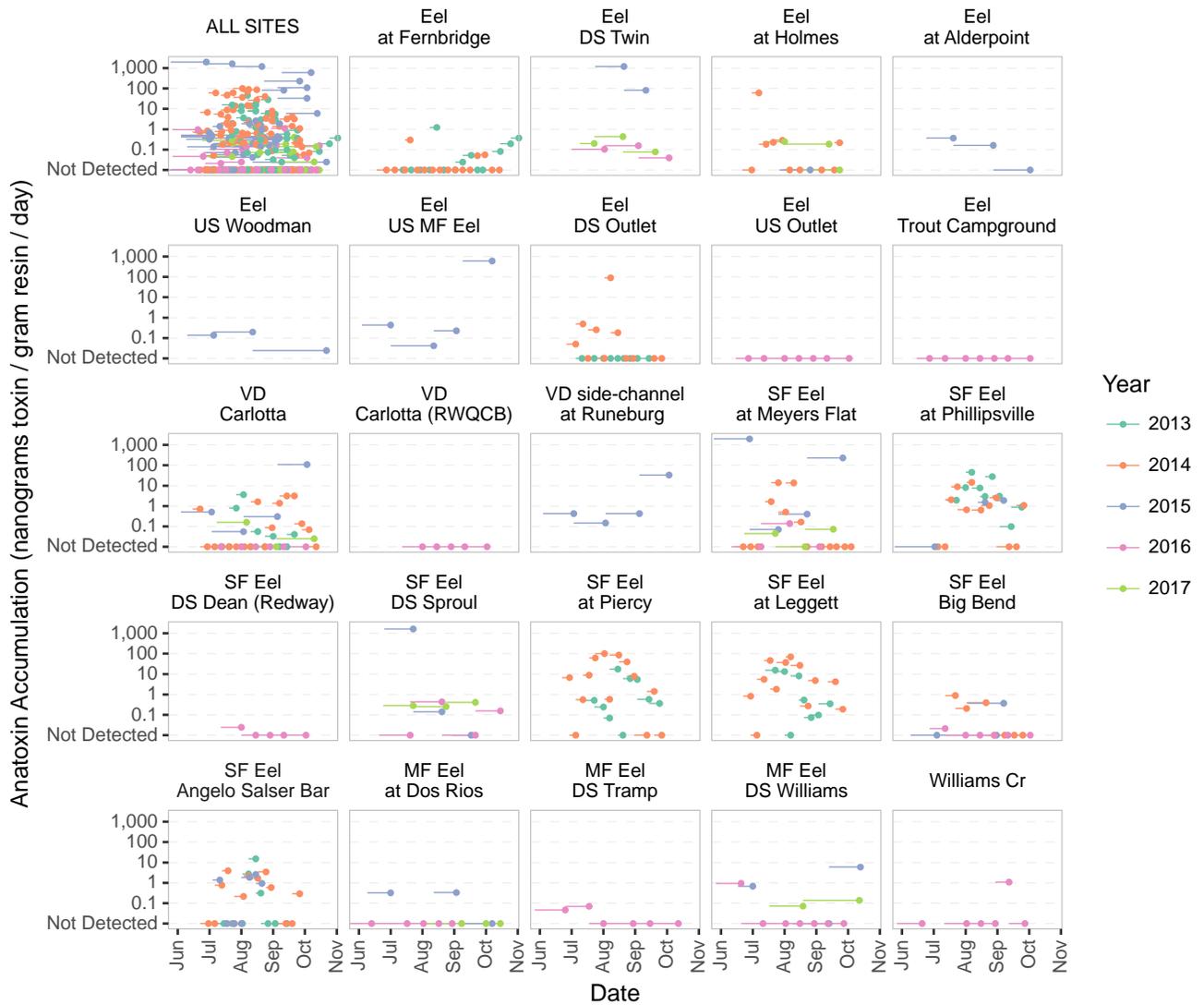


Figure 15. Accumulations of anatoxin-a on SPATT samplers deployed at sites in the Eel River basin in the years 2013-2017. Each point represents the retrieval date of a single sampler, and the line extends back to the day the sampler was deployed.

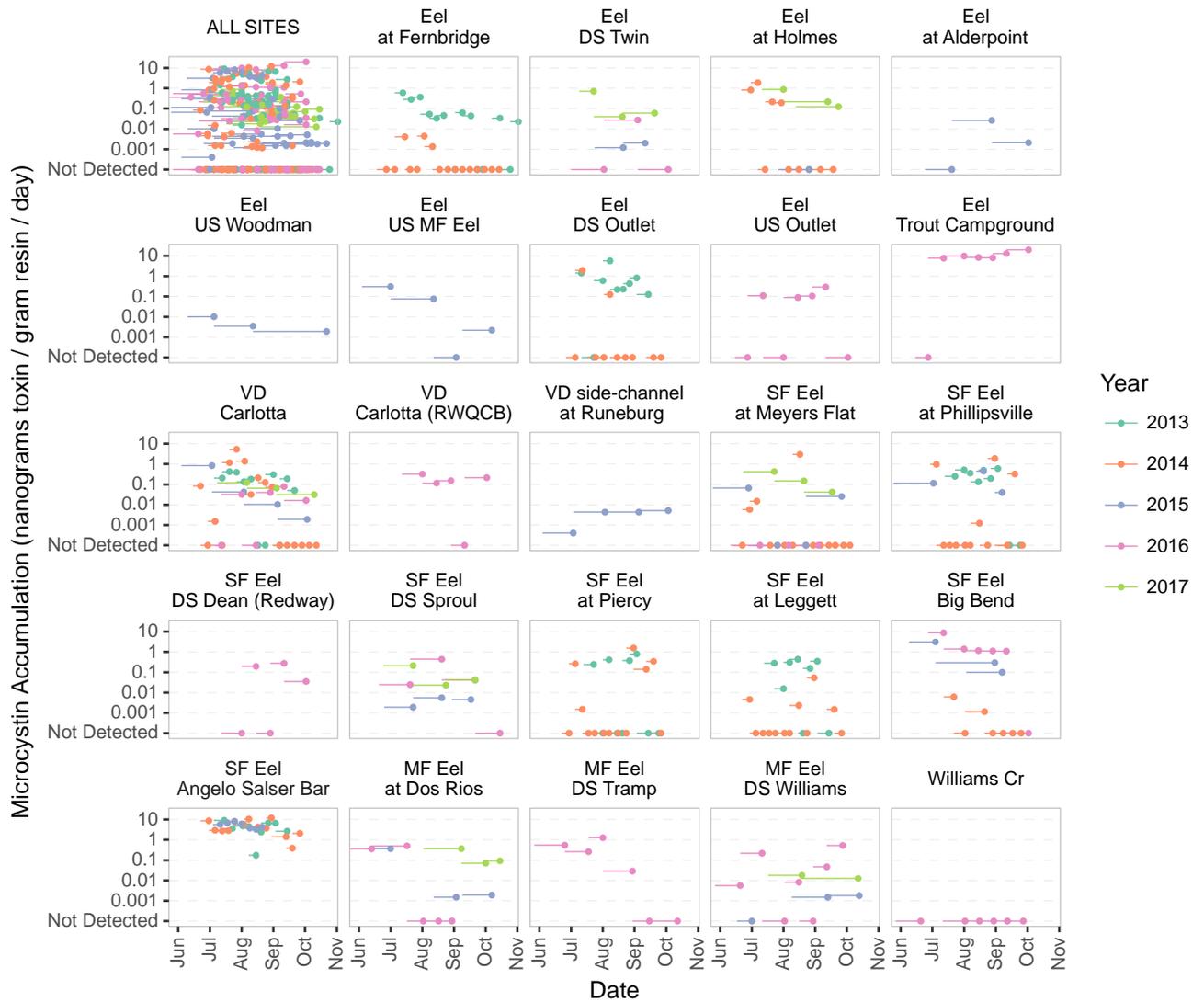


Figure 16. Accumulations of microcystin on SPATT samplers deployed at sites in the Eel River basin in the years 2013-2017. Each point represents the retrieval date of a single sampler, and the line extends back to the day the sampler was deployed.

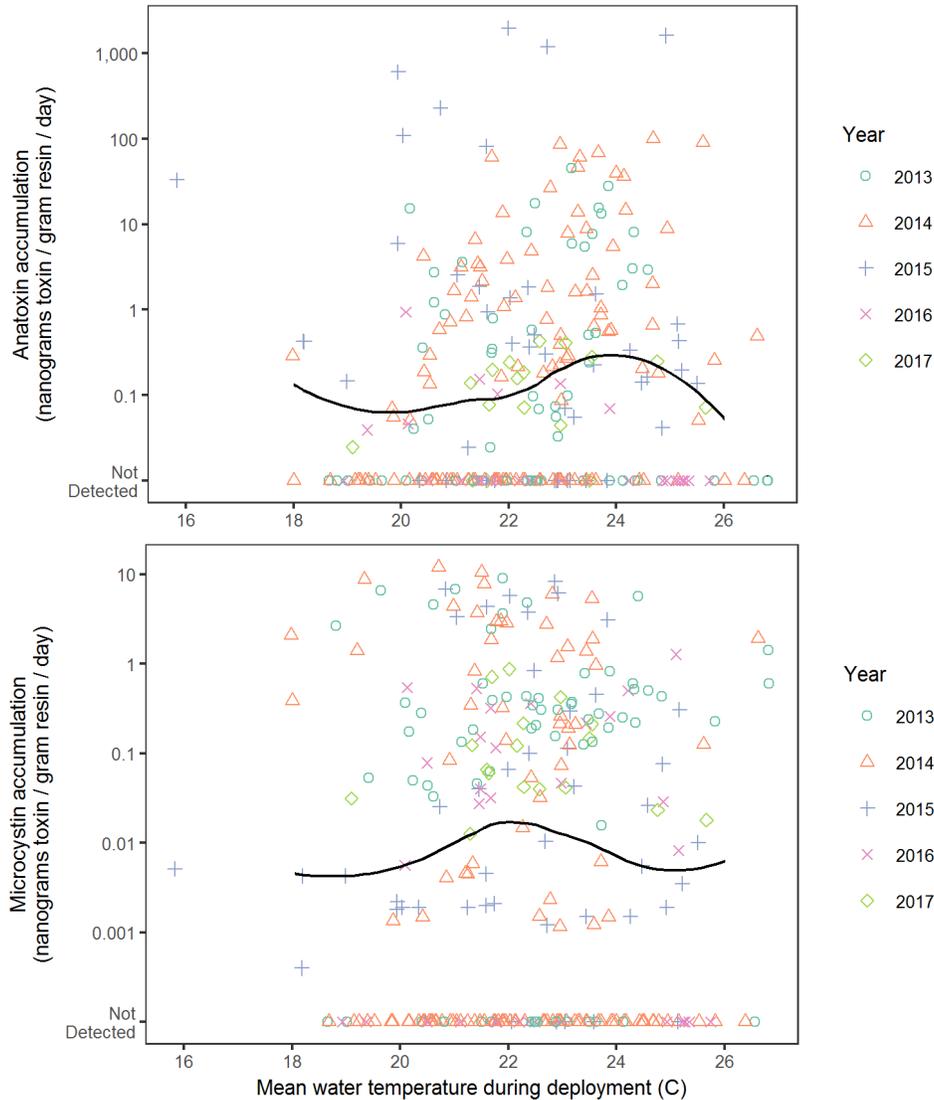


Figure 17. Scatterplot of anatoxin-a (top) and microcystin (bottom) toxin accumulations versus mean water temperature for SPATT samplers deployed at sites in the Eel River basin in the years 2013-2017. Black line is a polynomial smoother provided as a visual aid. Note that some SPATT deployments, especially those from NCRWQCB, did not have any temperature data so are not shown here.

Discussion

Our understanding of cyanobacteria and cyanotoxins in the Eel River watershed has been greatly advanced by this study, particularly the patterns of occurrence of anatoxin-a. However, new observations in the upper Eel River basin suggest that *Phormidium* that can tolerate cooler water temperatures may be an emerging public health issue in the upper Eel River. Relationships between human caused impacts and cyanobacteria blooms are hard to define quantitatively, but we offer hypotheses on what may be driving cyanotoxic blooms and recommendations on what actions are needed to reverse these conditions and tip the ecosystem back towards a food web structure that better supports fish and humans. Our discussion culminates with exploration of interannual variation in anatoxin-a production.

Spatial Variability of Anatoxin-a and Microcystin

The highest anatoxin-a accumulations in our study were on the South Fork Eel River downstream of Big Bend in almost all years, except for 2015 when very high values occurred were also measured in the lower Eel River, Middle Fork, upper Eel River, and Van Duzen. Water temperatures are much higher in the middle main Eel River, upper Eel River, and Middle Fork Eel, and yet stations in these reaches had low anatoxin-a accumulations in most years. These river segments drain mostly wildlands with extensive federal and large ranchland ownership and are in recovery from past flood events. They are no longer over-supplied with sediment, which is allowing Chinook salmon recovery (Higgins 2017), and our assumption is that they are relatively nutrient poor. This suggests that factors in addition to water temperature are contributing to the South Fork Eel River's susceptibility to blooms.

Although quantitative data on nutrient concentrations are lacking, the high density of *Cannabis* farms in the South Fork Eel River basin (Bauer et al. 2015, Butsic and Brenner 2016) indicates greater potential for non-point source pollution, including nutrient-laden runoff from fertilizers and soil amendments. The lower South Fork Eel River below Garberville has a wide valley bottom and low channel gradient and sediment yield from sub-basins is very high (Higgins 2014, Higgins 2018). Sediment moves slowly through this reach or goes into storage, filling pools and causing channel widening. This channel morphology causes increased water temperatures and tends to form shallow edge-water habitat that creates ideal conditions for *Anabaena* blooms, especially in low flow years. High flow years tend to scour and create fewer ideal side-water sites for cyanobacteria and less risk of exposure for pets and humans the following summer.

Sediment and nutrients from the South Fork Eel River are transported to the lower Eel River, which also has a low gradient channel that tends to respond similarly to the lower South Fork. The main Eel River tends to cool below its confluence with the South Fork at Dyerville (Figure 9) due to the marine climatic influence (Friendrichsen et al. 1998, Asarian et al. 2016). The detection of anatoxin-a on the lower main Eel River SPATTs, sometimes at high levels, indicates that anatoxin-a was either transported from upstream or that water temperatures were warm enough for local production.

Although anatoxin-a was not detected on SPATTs deployed on the upper Eel River at Trout Creek in 2016, a canine mortality fitting the pattern of cyanotoxin poisoning occurred that same year (Rich Fadness, personal communication). The NCRWQCB documented a profuse bloom of *Phormidium* (Figure 18) at this location, but was unable to document how dogs might have been exposed. Since *Phormidium* grows in swifter water and riffle habitats, it is unlikely that dogs got exposed there, but mats may have broken down and accumulated in eddies in stream margins. Further study is needed to examine why blooms of *Phormidium* are occurring in the upper Eel River and to explore pathways for canine and human exposure. Bouma-Gregson et al. (2018b) found that *Phormidium* mats in the South Fork Eel contained both anatoxin-a and microcystin.

The highest levels of microcystin were measured on the upper Eel River at Trout Creek in 2016. Although microcystin may be produced by a number of cyanobacterial species, *Microcystis*, a planktonic species which typically occurs in lakes and reservoirs, is the most well-documented source of microcystin in northern California (Kann and Corum 2009, Hill 2010). Benthic taxa capable of producing microcystin are also widespread, however, and microcystin has been detected in many rivers and streams in the region (Fetscher et al. 2015). The State Water Resources Control Board (Carter et al. 2017) has noted occurrences of cyanotoxins in Lake Pillsbury and lists it annually on a precautionary basis (Bernard

2018). The relative contributions of Lake Pillsbury and benthic river algae to the microcystin detected in the Eel River at Trout Creek are unclear.



Figure 18. Thick mats of *Phormidium* at Trout Creek Campground on upper Eel River. Rich Fadness, NCRWQCB. 9/5/2017.

Interannual Variability of Anatoxin-a

One of the biggest challenges in the Eel River watershed is to better understand the annual patterns of cyanotoxin production so we can anticipate, map, and reduce public health risk. The variability in rainfall and flow over the 2013 to 2017 study period was ideal in this regard as we had some of the wettest and driest years on record. The flow on May 1 of each year during the study period at three gauges is contained in Table 2, which is useful for inter-annual comparison.



Figure 19. SF Eel River near Piercy with substrate covered with *Cladophora*. June 16, 2015.



Figure 20. Lush *Cladophora* growth at Angelo Reserve in early June 2013. Photo by Keith Bouma-Gregson.



Figure 21. Cyanobacteria spires emerging from a lush bed of *Cladophora* in the South Fork Eel River downstream of Cedar Creek. 6/28/18.

Power et al. (2015) details the impact of winter and summer flows on algal proliferation for the unusual water year 2012–2013, which was the beginning of a record multi-year drought. On December 2, 2012, the first and last scouring flood of the water year occurred. This flood scoured out predator-resistant over-wintering grazers, including *Dicosmoecus gilvipes*, so they were too sparse during the subsequent low flow season to suppress *Cladophora* blooms. After the single December flood, stable, clear, warming flows gave *Cladophora* a ‘head start’ during a prolonged period of favorable growth conditions, so attached *Cladophora* turfs produced the largest proliferations ever observed in 25 years of field work in the upper South Fork Eel River, attaining lengths greater than 25 feet. On May 1, the South Fork Eel flow was just 178 cfs at Leggett and at 350 cfs at Miranda (Table 2, Figure 8). But on June 24–26, a small spate (that elevated stage just over a foot and discharge by less than $2 \text{ m}^3 \text{ s}^{-1}$ at the Branscomb gage within the Angelo Reserve) detached *Cladophora* streamers, which could not withstand the huge drag forces imposed by the slightly elevated flow. Drifting *Cladophora* accumulated as large floating mats along the slack-water margins and tails of pools a few km downstream. As *Cladophora* mats rotted, they released nutrients that fueled massive *Anabaena* proliferation in pools just downstream. Later in the season, the adjacent Salsler Bar cyanotoxin monitoring station had one high anatoxin-a reading. SPATT results from 2013 indicated that the highest anatoxin accumulations were at Phillippsville on the lower South Fork Eel River (Figure 15) which may have been fed, in part, by algae that floated down with the late June rain spate.

Table 2 Average daily flow on May 1 at three USGS gauges in cubic feet per second.

| Gauge | Y2013 | Y2014 | Y2015 | Y2016 | Y2017 |
|---------------|-------|-------|-------|-------|-------|
| Eel at Scotia | 1530 | 1540 | 931 | 2740 | 8110 |
| SF at Miranda | 350 | 346 | 199 | 557 | 1470 |
| SF at Leggett | 178 | 179 | 99.5 | 251 | 707 |

The May 1 flow levels in 2014 are almost identical to those of 2013 (Table 2), but there was no summer rain storm to detach *Cladophora* or to lower water temperature. Flows declined gradually in the South Fork at Leggett and Miranda during the summer of 2014 (Figure 8), and presumably water temperatures rose (temperature loggers were not deployed until June) as the volume of water shrunk and the transit time increased, setting up ideal conditions for cyanobacteria. This led to the highest anatoxin-a levels occurring further upstream in 2014 (Piercy and Leggett) than in 2013 (Phillippsville) (Figure 15). Because the South Fork Eel River in the reach where these sites (Piercy and Leggett) are located is incised and less depositional than the lower South Fork it tends not to form as many blooms in eddies at the river’s edge. Instead cyanobacteria there grow within *Cladophora* beds that can cover a great deal of the stream bed (Figure 21). Also, *Phormidium* is present in this reach and could contribute to anatoxin-a levels. The steady decline of flow in the lower South Fork Eel River in 2014 could have stranded cyanobacteria to desiccate on the dry river bar. This could have depressed anatoxin-a levels at lower South Fork monitoring stations (Phillippsville and Meyer’s Flat) in 2014.

The summer of 2015 appears to have set up excellent conditions for cyanotoxin production, with the lowest May 1 flows of the study period, approximately 40–45% lower than 2013 and 2014 (Table 2). Flows were also very low beginning in March (Figure 24 in Appendix A), which allowed for an early start for *Cladophora*. Air temperature and water temperatures from early June through early July were higher in 2015 than other years (Figure 10) which, in combination with the previously mentioned low spring flows, likely allowed cyanobacteria mats to develop earlier than in other years.

The early development and prolonged duration of the cyanobacterial growing season seems to have allowed more complete degradation of the cyanobacterial cells and release of dissolved toxins into the water column where they would be available for uptake onto the SPATTs. In mid-July, a series of weak rain storms caused the main Eel River at Scotia to rise from 78 cfs to peak briefly at 126 cfs. This event may have helped keep warm cyanotoxin producing stream edges connected. The flow at Scotia on September 1 was still only 32 cfs, indicating a long incubation period for cyanobacteria and low dilution factor for cyanotoxins. The Eel River had passed its tipping point in 2015, and toxin-producing cyanobacteria flourished not just in usual South Fork locations, but also in the lower Eel, lower Middle Fork, upper main Eel River, and on the Van Duzen at Carlotta for the only year of the study period.

The anatoxin-a accumulations in 2015 were an order of magnitude greater than other years, raising questions about quality assurance and quality control of the sampling. An alternative explanation for the high 2015 anatoxin-a values is that detached algal mats might have gotten caught on the SPATTs, resulting in SPATT accumulations that reflect toxin concentrations in the algal mats rather than the ambient water. However, the limited records we have from weekly SPATT cleanings do not indicate that the fouling problem was more prevalent in 2015 than other years. Monthly deployment of SPATTs with weekly cleanings since 2015 have greater risk of fouling than in 2013 and 2014 when SPATTs were replaced weekly. Some sites have little problem with fouling, like the main Eel River at Woodman Creek and at Alderpoint, but others, such as Phillippsville, presented challenges in terms of keeping algal mats from interfering with samplers. Various degrees of SPATT fouling are shown in Figure 22. Aside from deployment length, sampling methods were consistent at all sites and in all years, and we could expect that fouling should have led to high values at some sites and low values at others, instead of similarly high values throughout the basin in 2015.

The summers of 2016 and 2017 had much higher flows and fewer problems with cyanotoxins.



Figure 22. Algae fouling on SPATTs. Left: Light fouling – main Eel River at Woodman Creek. Center: Moderate fouling – main Eel River upstream of Dos Rios. Right: Heavy fouling – South Fork eel River at Phillippsville.

Factors Contributing to Eel River Cyanotoxin Blooms

The major factors likely contributing directly to *Anabaena* blooms are depleted summer baseflows, increased water temperature, nutrients, and channel morphology. Problems related to channel morphology are caused by excess sediment supply, which also impacts the other aforementioned drivers. Discussion below includes recommendations for how to remediate impacts and move the Eel River ecosystem back towards its more historic condition and make it less susceptible to cyanobacteria proliferation.

Flow

Low summer discharges are likely a major driver of benthic cyanobacteria blooms because they reduce flow velocity, promote warm water temperatures, and concentrate nutrients. Summer flows measured at long-term flow gauging stations across the Eel River watershed have declined substantially in recent decades, and these declines can only partially be explained by precipitation patterns (Asarian and Walker 2016, Asarian 2016). Large tributaries that were historically perennial but that often lose surface flow in late summer include Outlet and Tomki creeks in the upper main Eel River watershed, Tenmile Creek in Laytonville, an upper South Fork Eel River tributary, and lower South Fork tributaries Salmon Creek and Redwood Creek near Miranda and Redway, respectively (Higgins 2013, 2014; ERRP 2016). Diminished tributary flow may increase the susceptibility to developing cyanotoxins in main river reaches. This change from historic norms is one of the major drivers pushing the river past its tipping point and making it subject to cyanotoxic blooms (Power et al. 2015).

It is commonly assumed that the major driver of the desiccation of the Eel River is direct diversion of water for agricultural use, specifically *Cannabis* (Bauer et al. 2015). However, other factors are likely contributing as well, including climate change (Asarian and Walker 2016, Asarian 2016), accelerated drainage due to roads (Jones and Grant 1996), increased sediment from past logging (Pacific Watershed Associates 1998), and altered structure and composition of vegetation (Asarian 2016). Dense stands of timber regenerating from post WWII harvest have high evapotranspiration rates (Moore et al. 2004, Jassal et al. 2009, Perry and Jones 2017) that can diminish summer baseflows (Stubblefield et al. 2012). In addition, fire suppression has allowed Douglas-fir trees to encroach into grasslands and oak woodlands and caused flow depletion of spring sources (Keter and Busam 1997). Douglas fir encroachment will not occur where the thickness of weathered rock is too shallow to support them, such as on the Central belt mélange geologic unit (Hahm et al. 2017, 2018).

The timing and amount of winter and spring flows play an important role in shaping river algal dynamics (Power et al. 2008, 2013; Vadeboncoeur and Power 2017). Long term studies of the upper South Fork Eel River at the Angelo Reserve show that scouring winter flows tend to result in increased growth of the filamentous green algae *Cladophora* because large, predator-resistant grazers, such as the caddisfly *Dicosmoecus*, are killed or relocated downstream when high winter flows mobilize much of the stream bed. Power et al. (2008) used estimated bankfull discharge (1.5 year recurrence interval or greater, which is 120 m³/s or 4240 cfs) at the Branscomb gauge as proxy for these bed-mobilizing events, but bed mobilization may occur more frequently at lower gradient sites downstream with finer substrates. Given these uncertainties, identifying bed-mobilizing events at the various gauges in our study area is beyond the scope of our study, but we recognized that the occurrence and timing of bed mobilization could be an important driver of *Anabaena* dynamics since *Anabaena* often grows on *Cladophora*.

Flow has other effects on *Cladophora* besides full bed-mobilization. For example, high flows in late spring can retard or export incipient *Cladophora* growth and, thus delay the start of when dead algal segments accumulate in stream margins and form the medium ideal for *Anabaena* blooms.

Nutrients

Non-point sources of nutrient pollution in the Eel River basin would be hard to discern due to the way that nutrients cycle in a river system. Clear relationships between the productivity of benthic algae and nutrient concentrations are difficult to document because these algae can uptake and store nutrients as well as access nutrients from substrates or local recycling (Wood et al. 2015, Vadeboncoeur and Power 2017). During periods when other factors such as temperature, light, and flow are favorable for *Cladophora* growth, a substantial portion of the phosphorous or nitrogen that enters the Eel River or its tributaries is likely rapidly taken up by *Cladophora*. While *Cladophora* is an essential part of a healthy aquatic food web, it can also be an indication of pollution, if growth is too dense (U.S. EPA 2000).

Excessive nutrients may allow *Cladophora* to overcome suppression by macroinvertebrate grazers. In sunlit reaches where gentle summer flow persists, the South Fork Eel River can be carpeted with *Cladophora* (Figure 19). As attached *Cladophora* segments grow, elongate, and then senesce, they detach, float downstream, and settle out in warm, quiet backwaters and pool margins. Rotting *Cladophora* and epiphytic diatoms release nutrients that then become available to *Anabaena*, which, like many cyanobacteria, can tolerate and grow in much warmer water (Paerl and Huisman 2009). The timing of *Cladophora* blooms and subsequent cyanobacteria blooms each summer may be, in part, driven by the previous winter and spring flow, as described above.

Once cyanobacteria form a mat, the heterotrophic (non-photosynthetic) bacteria that take up residence in the mat also become part of the nutrient cycling, breaking down carbon and releasing phosphorous, thus, further benefitting cyanobacteria. *Phormidium* mats extract nutrients from soil particles that adhere to the mat (Wood et al. 2015). This raises the question as to whether excessive fine sediment loading in the lower South Fork Eel River may also be supplying nutrients that fuel cyanobacteria blooms.

Water Temperature and Channel Morphology

The relationship between water temperature and cyanotoxins is discussed above in the Spatial Variability of Anatoxin-a and Microcystin section. In this section we focus on the factors affecting water temperature. Air temperature and water temperature are generally highly correlated because both respond to the same temporal patterns in solar heating (Johnson 2004). Incoming solar radiation is the most important term in stream energy budgets (Johnson 2003). Poole and Berman (2001) noted that reducing flow decreases the water volume and increases residence time, both of which promote stream warming. Lower flows also reduce hydraulic pressure for down-welling and connection to the sub-surface hyporheic zone (Poole and Berman 2001). Excess sediment may plug interstitial spaces in the stream bed and obstruct connections between surface water and groundwater, further reducing connection with the hyporheic zone and promoting stream warming (Poole and Berman 2001).

A thorough examination of the variables driving water temperatures in the Eel River watershed is outside the scope of this report, but comparison of Figure 8 and Figure 10 indicates that during the summer season, water temperatures appear to respond more strongly to variability in air temperature than variability in flow. For example, although 2017 had the highest summer flows at all sites, daily 7-day average water temperatures still reached levels comparable to other years. In fact, at the South Fork

Eel River at Myers Flat station, the highest temperatures of the study period occurred in early August 2017. Similarly, the Middle Fork Eel River downstream of Williams Creek was warmer in 2017 than in 2016, when there was less flow. Temperatures in the Van Duzen River at Carlotta during the relatively high flow year of 2016 were lower for every day of the summer season than the corresponding date in the very low flow year 2014, even on days where air temperatures were higher in 2016. However, there were periods in which 2017 water temperatures exceeded the corresponding day in 2014 despite 2017 having the flows even higher than 2016 (Figure 10).

Stream channel morphology also appears to have an influence on water temperatures in Eel River streams. Asarian et al. (2016) presented Eel River water temperature data spanning from 1982-2015 and noted that despite very low flows in 2013 to 2015, these years did not have the highest MWMT temperatures during the period of record. Instead, data showed that the highest water temperatures occurred in the years 2006, 2004, 1996, and 1997 (Asarian et al. 2016). The January 1, 1997 storm caused widespread channel scour and loss of riparian vegetation.

While the 2017 water year (10/1/2016 to 9/30/2017) did not have high intensity rainfall events that can trigger landslides, there was substantial bedload movement (Higgins 2018). Tributary stream beds showed some recovery from past flood damage, with depth improving and median particle size becoming more coarse. However, this may also have caused loss of riparian cover in some tributaries and made them more open and subject to warming. Also, large river channels downstream of major sediment sources, like the South Fork Eel River downstream of Salmon Creek near Miranda, may have widened and become shallower as a result of sediment deposition. The lower Eel River channel below Dyerville also likely aggraded as sediment washed down from throughout the watershed. This may have increased the contribution of heat exchange between air and water and resulted in water warming in response to high air temperatures in 2017.

ERRP's Management Recommendations for Increasing Flows and Improving Watershed Health

ERRP recommends continuing work with its partners to address the issues contributing to cyanotoxin blooms in the Eel River watershed:

- Continue to work with farmers and other residents throughout the Eel River basin to prevent nutrient pollution and maximize water conservation in order to restore perennial flow to key tributaries.
- Work in partnership with federal and state agencies and local communities to improve forest health and restore watershed hydrology to improve base-flows in the future.
- Foster development of road associations and local watershed groups to prevent erosion and nutrient transport to streams.

ERRP provided technical assistance to 70 *Cannabis* farmers to help them conserve water and implement measures to avoid water pollution. Data collected from participants is private to protect clients, but a summary of data (ERRP 2016) indicated that few farmers had water meters. This means they lacked capacity to derive water budgets, which is a starting point for making a conservation plan. Many farmers did acquire water meters and implemented conservation measures as a result of outreach efforts, but the study indicated that there is huge potential for improving flows in some watersheds simply by fully

implementing water conservation, including augmenting water storage similar to Sanctuary Forest's community efforts in the upper Mattole River (Camp-Schremmer 2014). ERRP will continue to work with farmers and other residents throughout the Eel River basin to increase water conservation and restore perennial flow to key tributaries.

As noted above in the Factors Contributing to Eel River Cyanotoxin Blooms section, the structure and composition of the Eel River's forests has changed dramatically in the past century and is likely contributing to declining river flows. Computer modeling of water used by a forest in the Mattole River watershed by Stubblefield et al. (2012) predicted that natural mortality of smaller under-story trees due to shading would eventually increase water yield. They noted that this process could be expedited by selective harvest of small and mid-size trees leaving larger trees spaced further apart. This practice, known as thinning from below, may be an important in restoring baseflows over time. ERRP intends to work in partnership with federal and state agencies and local communities to improve forest health and restore watershed hydrology.

High road densities are also recognized as altering the hydrology of watersheds, by essentially extending stream networks and intercepting ground water flows (Jones and Grant 1996). Winter storm flows are intercepted by roads and are routed to streams, increasing damaging storm peaks and also reducing groundwater recharge, resulting in reduced stream base flows in summer. Jones and Grant (1996) observed that sediment yield may decrease within a few years after timber harvest, but that hydrologic perturbations from road networks can persist for decades.

In order to promote improved watershed hydrology that increases base flows: 1) road densities in Eel River watersheds should be reduced through decommissioning, 2) roads should be relocated away from springs and slide prone areas and onto ridge-tops to the extent possible, and 3) as much as possible of the road network in the watershed should be out sloped to help water infiltrate into hillslopes. Communities improve their security by working collaboratively on roads and ERRP will continue to encourage and assist formation of road associations and local watershed groups to work on this problem.

Extreme sediment over-supply can result in a stream bed becoming so aggraded that the stream loses surface flow in late summer. Salmon Creek, a major tributary of the lower South Fork Eel River near Miranda, typically loses surface flow in June or July partially as a result of aggradation (Figure 23). As noted in ERRP (2016), other lower South Fork Eel tributaries with similar problems include Fish Creek near Benbow, and every creek between Dean Creek and Miranda on the east side of the watershed. Sediment over-supply is also likely contributing to loss of surface flow of Redwood Creek near Redway and Tomki Creek in the upper Eel River watershed. Therefore, reducing erosion is necessary to prevent massive aggradation that leads to streams being buried and also prevent widening and warming in downstream reaches.

The widening and shallowing of the Eel River in low gradient reaches as a result of aggradation promotes warming. Consequently, reducing erosion and sediment over-supply will help promote channel recovery and cooler water temperatures. Protection and restoration of riparian zones in tributaries can also promote cooling. ERRP will be working with residents of the Tenmile Creek watershed, a large upper South Fork Eel River tributary, to restore riparian zones throughout that 65 square mile basin.



Figure 23. Mouth of Salmon Creek underground as a result of aggradation on 7/15/2015.

Conclusion

This report presents cyanotoxin data collected using solid phase adsorption toxin tracking (SPATT) samplers in rivers in the Eel River Basin in the years 2013–2017. SPATTs were deployed by the Eel River Recovery Project (ERRP), the Eel River Critical Zone Observatory of the University of California, Berkeley, and the North Coast Regional Water Quality Control Board (NCRWQCB), and were analyzed by the Kudela Lab at University of California at Santa Cruz. To understand the environmental factors which may influence the growth and seasonal progression of cyanobacteria communities, we also present data on flow, water temperature, and air temperature. This project was initiated as part of Keith Bouma-Gregson’s dissertation in 2013–2014 (Bouma-Gregson 2017, Bouma-Gregson et al. 2018b) and then transitioned to ERRP and NCRWQCB in 2015. This transition resulted in adjustments to the number of sites and length of SPATT deployments which make it difficult to make clean quantitative comparisons across the entire study period; however, general conclusions can still be drawn.

Anatoxin-a and microcystin were detected in the majority of SPATT samplers analyzed and were detected in every year. The highest detection frequencies for these toxins occurred in the 2015 and 2017, respectively, which is interesting because these two years represent opposite ends of the hydrologic spectrum (2015 was very dry and 2017 was very wet). Despite similar detection frequencies in those two years, the quantity of anatoxin-a that accumulated on the SPATTs was markedly different, with 2015 featuring very high levels.

There appear to be distinct spatial patterns for the two toxins, with anatoxin accumulations and detection frequencies generally being higher at sites in the South Fork Eel River than in other sub-basins (2015 was an exception), while the highest microcystin accumulations were found in the upper Eel River at Trout Creek Campground below Lake Pillsbury. Thermal differences were evident also, with peak anatoxin-a accumulations occurring in sites with water temperatures several degrees warmer than peak microcystin accumulations.

ERRP's collaborative monitoring of cyanotoxins using SPATT samplers has provided valuable information for protecting public health and advancing the scientific understanding of riverine ecology of the Eel River Basin. We recommend continuing this effort because each year is a natural experiment with a unique set of environmental conditions and ecological outcomes. The more years of data we accumulate, the better we can understand the system. Our five-year study period represented a wide range a hydrologic conditions but additional years would provide the replication necessary to draw stronger conclusions. Bouma-Gregson (2017) stated that:

“Comprehensive knowledge of the ecology of benthic cyanobacteria is necessary to understand its impacts to public and ecosystem health and to better predict where and when blooms might occur in rivers.”

Water temperature outside of the summer season remains an important data gap in the Eel River Basin. Only a few sites are consistently monitored year-round and they are all in the southern portion of the Basin. These include Eel River below Scott Dam⁷ monitored by Pacific Gas and Electric Company, Elder Creek⁸ and Cahto Creek⁹ monitored by USGS, and the South Fork of the Eel River near Branscomb monitored by UC Berkeley¹⁰. Water temperature probes at other sites are typically not deployed until June, but temperatures in the spring season are potentially important factors influencing seasonal progression of algal communities. In recent years, protocols have been developed for year-round temperature monitoring using relatively inexpensive equipment (Isaak et al. 2013) which ERRP or others could deploy in the Eel River Basin if resources were made available. Highest priorities would be sites with existing USGS gages such as Eel River at Scotia, Fort Seward, and Van Arsdale; South Fork Eel at Miranda and Leggett; Middle Fork Eel River at Dos Rios; and Van Duzen River at Bridgeville.

The Eel River Recovery Project should continue to organize citizen monitoring of cyanotoxins to explore additional questions and to expand monitoring. The role of nutrient enrichment in *Cladophora* and cyanotoxin blooms also needs to be further explored. Even though we do not have scientific certainty about causal mechanisms of toxic cyanobacteria blooms, work should continue to restore watershed hydrology, to minimize erosion and water pollution, and to get the community to conserve water. In combination, these actions may help push the Eel River reaches back to more functional cold water ecosystems, with the ultimate outcome being a river that resists toxic cyanobacterial blooms.

Recommendations for Further Study

- Continue to work with UCSC and the NCRWQCB to monitor cyanotoxins basin wide and increase timely flow of information to county public health departments.
- Seek resources from the community to make this activity sustainable through focused membership drive specific to this cyanotoxin monitoring.
- Extend cyanotoxin monitoring in upper Eel River to learn more about *Phormidium* and potential pathways for canine and human exposure.
- Capture baseline data in North Fork Eel River in 2019 with local volunteers.
- Develop capacity for year-around water temperature monitoring at key locations near flow gauges and SPATTs.

⁷ http://cdec.water.ca.gov/dynamicapp/staMeta?station_id=ELP

⁸ https://waterdata.usgs.gov/nwis/uv?site_no=11475560

⁹ https://waterdata.usgs.gov/nwis/uv?site_no=11475610

¹⁰ http://sensor.berkeley.edu/data_access.html

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Appendix A: Additional Flow Graph

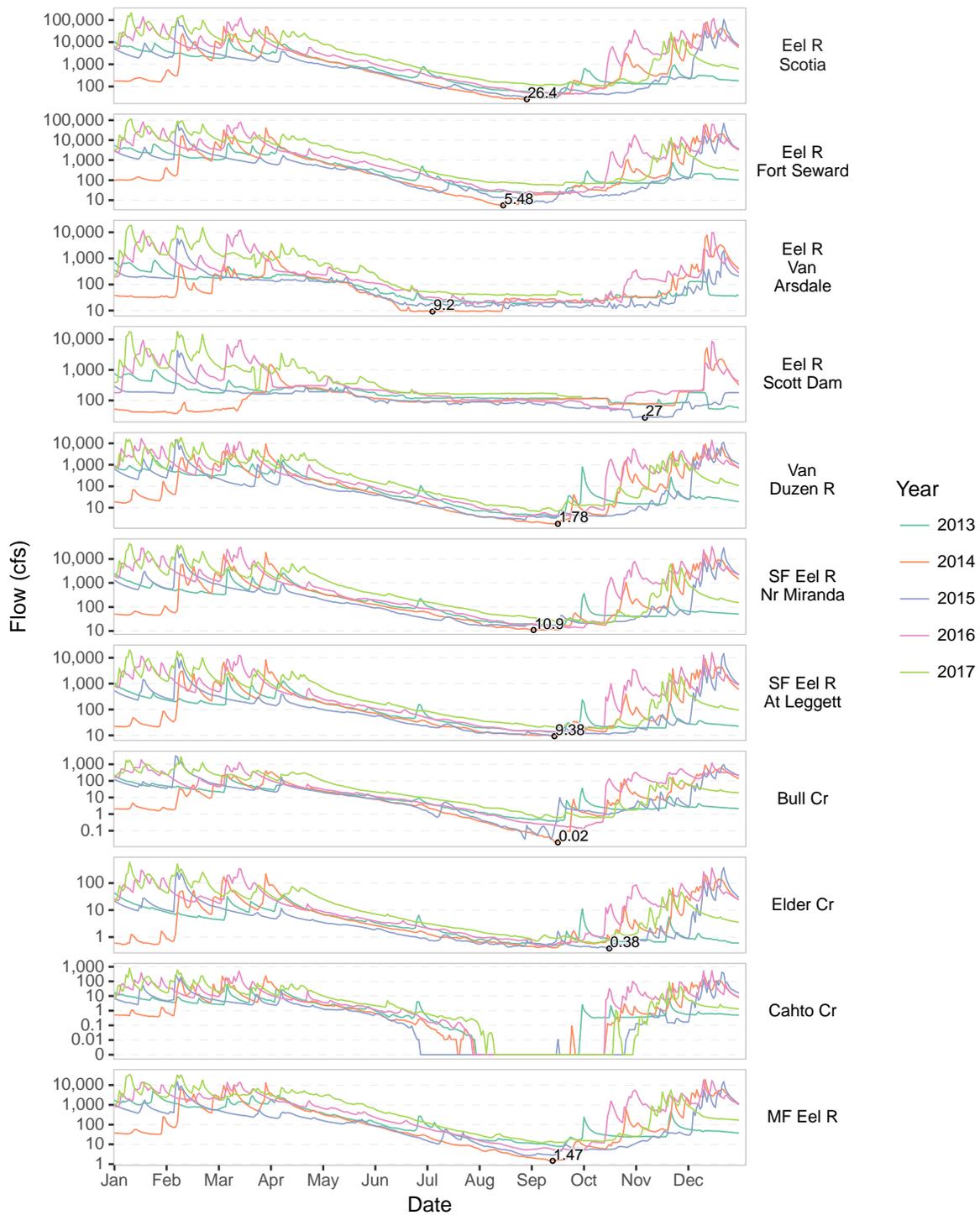


Figure 24. Daily time series of flow in 2013–2017 at all USGS gaging stations in the Eel River Basin. The black circles and associated labels indicate the lowest flow observed at each station during the study period.

Appendix B: Tables Summarizing Detection Frequency by Site and Year

Table 3. Frequency of anatoxin-a and total microcystin detection on SPATT samplers, summarized by site and year.

| Sub-basin | Site Standardized | Anatoxin-a | | | | | | Total Microcystins | | | | | |
|-----------------------------|-----------------------------|-------------|--------------|-------------|-------------|-------------|-----------------------|--------------------|--------------|-------------|-------------|--------------|-----------------------|
| | | 2013 | 2014 | 2015 | 2016 | 2017 | All Years (2013-2017) | 2013 | 2014 | 2015 | 2016 | 2017 | All Years (2013-2017) |
| Lower Eel | Eel at Fernbridge | 43% (6/14) | 19% (3/16) | | | | 30% (9/30) | 71% (10/14) | 19% (3/16) | | | | 43% (13/30) |
| | Eel DS Twin | | | 100% (2/2) | 100% (3/3) | 100% (3/3) | 100% (8/8) | | | 100% (2/2) | 33% (1/3) | 100% (3/3) | 75% (6/8) |
| | Eel at Holmes | | 50% (5/10) | | | 67% (2/3) | 50% (7/14) | | 40% (4/10) | | | 100% (3/3) | 50% (7/14) |
| Main Eel | Eel at Alderpoint | | | 67% (2/3) | | | 67% (2/3) | | | 67% (2/3) | | | 67% (2/3) |
| | Eel US Woodman | | | 100% (3/3) | | | 100% (3/3) | | | 100% (3/3) | | | 100% (3/3) |
| | Eel US MF Eel | | | 100% (4/4) | | | 100% (4/4) | | | 75% (3/4) | | | 75% (3/4) |
| | Eel DS Outlet | 0% (0/9) | 45% (5/11) | | | | 25% (5/20) | 89% (8/9) | 20% (2/10) | | | | 53% (10/19) |
| | Eel US Outlet | | | | 0% (0/7) | | 0% (0/7) | | | | | 57% (4/7) | 57% (4/7) |
| | Eel Trout Campground | | | | 0% (0/7) | | 0% (0/7) | | | | | 86% (6/7) | 86% (6/7) |
| Van Duzen | VD Carlotta | 45% (5/11) | 47% (8/17) | 100% (4/4) | 0% (0/6) | 67% (2/3) | 46% (19/41) | 73% (8/11) | 53% (9/17) | 100% (4/4) | 67% (4/6) | 100% (3/3) | 68% (28/41) |
| | VD Carlotta (RWQCB) | | | | 0% (0/5) | | 0% (0/5) | | | | 80% (4/5) | | 80% (4/5) |
| | VD side-channel at Runeburg | | | 100% (4/4) | | | 100% (4/4) | | | 100% (4/4) | | | 100% (4/4) |
| SF Eel | SF Eel at Myers Flat | | 36% (5/14) | 100% (4/4) | 33% (1/3) | 67% (2/3) | 50% (12/24) | | 20% (3/15) | 50% (2/4) | 0% (0/3) | 100% (3/3) | 32% (8/25) |
| | SF Eel at Phillipsville | 100% (9/9) | 67% (8/12) | 67% (2/3) | | | 79% (19/24) | 78% (7/9) | 33% (4/12) | 100% (3/3) | | | 58% (14/24) |
| | SF Eel DS Dean (Redway) | | | | 20% (1/5) | | 20% (1/5) | | | | 60% (3/5) | | 60% (3/5) |
| | SF Eel DS Sproul | | | 67% (2/3) | 50% (2/4) | 100% (3/3) | 70% (7/10) | | | 100% (3/3) | 75% (3/4) | 100% (3/3) | 90% (9/10) |
| | SF Eel at Piercy | 89% (8/9) | 77% (10/13) | | | | 82% (18/22) | 44% (4/9) | 38% (5/13) | | | | 41% (9/22) |
| | SF Eel at Leggett | 88% (7/8) | 92% (11/12) | | | | 90% (18/20) | 75% (6/8) | 33% (4/12) | | | | 50% (10/20) |
| | SF Eel Big Bend | | 43% (3/7) | 33% (1/3) | 17% (1/6) | | 31% (5/16) | | 29% (2/7) | 100% (3/3) | 83% (5/6) | | 62% (10/16) |
| | SF Eel Angelo Salsar Bar | 33% (3/9) | 62% (8/13) | 57% (4/7) | | | 52% (15/29) | 100% (9/9) | 100% (13/13) | 100% (7/7) | | | 100% (29/29) |
| MF Eel | MF Eel at Dos Rios | | | 67% (2/3) | 0% (0/5) | 0% (0/3) | 18% (2/11) | | | 100% (3/3) | 40% (2/5) | 100% (3/3) | 73% (8/11) |
| | MF Eel DS Tramp | | | | 33% (2/6) | | 33% (2/6) | | | | 67% (4/6) | | 67% (4/6) |
| | MF Eel DS Williams | | | 67% (2/3) | 14% (1/7) | 100% (2/2) | 42% (5/12) | | | 67% (2/3) | 71% (5/7) | 100% (2/2) | 75% (9/12) |
| | Williams Cr | | | | 17% (1/6) | | 17% (1/6) | | | | 0% (0/6) | | 0% (0/6) |
| Annual Totals for all Sites | | 55% (38/69) | 53% (66/125) | 77% (36/47) | 17% (12/71) | 70% (14/20) | 50% (166/332) | 75% (52/69) | 39% (49/125) | 87% (41/47) | 58% (41/71) | 100% (20/20) | 61% (203/332) |

Table 4. Frequency of cylindrospermopsin and nodularin detection on SPATT samplers, summarized by site and year.

| Sub-basin | Site Standardized | Cylindro-spermopsin | | | Nodularin |
|-----------------------------|-----------------------------|---------------------|------------|--------------------------|-------------|
| | | 2016 | 2017 | All Years (2016-2017) | 2017 |
| Lower Eel | Eel at Fernbridge | | | | |
| | Eel DS Twin | 0% (0/3) | 33% (1/3) | 17% (1/6) | 100% (3/3) |
| | Eel at Holmes | | 67% (2/3) | 67% (2/3) | |
| Main Eel | Eel at Alderpoint | | | | |
| | Eel US Woodman | | | | |
| | Eel US MF Eel | | | | |
| | Eel DS Outlet | | | | |
| | Eel US Outlet | 0% (0/7) | | 0% (0/7) | 86% (6/7) |
| | Eel Trout Campground | 0% (0/7) | | 0% (0/7) | 86% (6/7) |
| Van Duzen | VD Carlotta | 0% (0/6) | 33% (1/3) | 11% (1/9) | 33% (2/6) |
| | VD Carlotta (RWQCB) | 0% (0/5) | | 0% (0/5) | 20% (1/5) |
| | VD side-channel at Runeburg | | | | |
| SF Eel | SF Eel at Myers Flat | 33% (1/3) | 33% (1/3) | 33% (2/6) | 100% (3/3) |
| | SF Eel at Phillipsville | | | | |
| | SF Eel DS Dean (Redway) | 0% (0/5) | | 0% (0/5) | 60% (3/5) |
| | SF Eel DS Sproul | 0% (0/4) | 100% (3/3) | 43% (3/7) | 100% (4/4) |
| | SF Eel at Piercy | | | | |
| | SF Eel at Leggett | | | | |
| | SF Eel Big Bend | 0% (0/6) | | 0% (0/6) | 100% (6/6) |
| | SF Eel Angelo Salser Bar | | | | |
| MF Eel | MF Eel at Dos Rios | 0% (0/5) | 0% (0/3) | 0% (0/8) | 100% (5/5) |
| | MF Eel DS Tramp | 0% (0/6) | | 0% (0/6) | 83% (5/6) |
| | MF Eel DS Williams | 0% (0/7) | 0% (0/2) | 0% (0/9) | 71% (5/7) |
| | Williams Cr | 0% (0/6) | | 0% (0/6) | 50% (3/6) |
| Annual Totals for all Sites | | 1% (1/71) | 40% (8/20) | 75% (53/71) | 75% (53/71) |

Appendix C: Additional Water Temperature Figure and Table

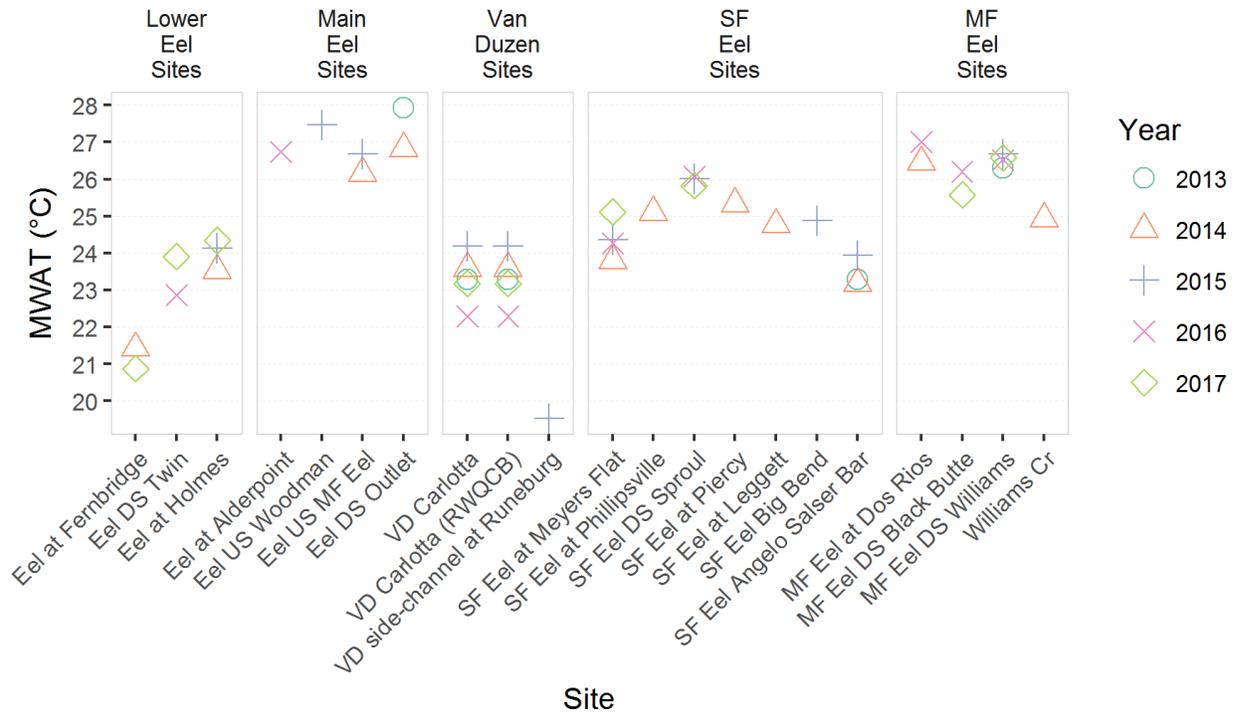


Figure 25. Maximum weekly average water temperature (MWAT) at SPATT sites in the Eel River basin, for each year 2013-2017. Sites are grouped by sub-basin. Within each sub-basin, sites are sorted from downstream (left side) to upstream (right side). MWAT values were only calculated for site-years in which the temperature logger was deployed for all days in both July and August, so there are some site-years in which there were SPATTs but not MWAT values.

Table 5. Maximum weekly maximum water temperature (MWMT) at SPATT sites in the Eel River basin, for each year 2013-2017.

| Sub-basin | Site Standardized | MWMT (°C) | | | | | |
|-----------|-----------------------------|-----------|-------|-------|-------|-------|-------|
| | | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 |
| Lower Eel | Eel at Fernbridge | | | 23.46 | | | 22.88 |
| | Eel at Holmes | | | 26.69 | 27.12 | | 26.24 |
| | Eel DS Twin | | | | | 24.55 | 25.93 |
| Main Eel | Eel at Alderpoint | | | | | 30.75 | |
| | Eel DS Outlet | | 30.74 | 29.03 | | | |
| | Eel US MF Eel | | | 29.26 | 28.79 | | |
| | Eel US Woodman | | | | 29.82 | | |
| MF Eel | MF Eel at Dos Rios | | | 28.78 | | 29.55 | |
| | MF Eel DS Black Butte | | | | | 29.54 | 28.66 |
| | MF Eel DS Williams | | 28.82 | | 29.95 | 30.24 | 29.64 |
| | Williams Cr | | | 28.93 | | | |
| SF Eel | SF Eel Angelo Salser Bar | 21.75 | 25.24 | 25.23 | 25.99 | | |
| | SF Eel at Leggett | | | 26.78 | | | |
| | SF Eel at Myers Flat | | | 26.90 | 27.20 | 26.71 | 27.37 |
| | SF Eel at Phillipsville | 27.36 | | 28.36 | | | |
| | SF Eel at Piercy | | | 28.12 | | | |
| | SF Eel Big Bend | | | | 26.66 | | |
| | SF Eel DS Sproul | | | | 29.34 | 28.99 | 28.57 |
| Van Duzen | VD Carlotta | | 24.38 | 25.50 | 25.93 | 23.92 | 26.38 |
| | VD Carlotta (RWQCB) | | 24.38 | 25.50 | 25.93 | 23.92 | 26.38 |
| | VD side-channel at Runeburg | | | | 20.56 | | |