Surface Water Ambient Monitoring Program (SWAMP)

Monitoring Plan for Watershed Dynamics of Cyanotoxin Transport

Region 9

FY 2015/2016

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1 Summary Sheet

Beneficial Uses

The proposed cyanotoxin monitoring plan for the Surface Water Ambient Monitoring Program (SWAMP) addresses a class of contaminants of emerging concern that may potentially impact many of the beneficial uses (BUs) that have been designated for inland and coastal waters in the San Diego Region. These include, but are not limited to:

- Municipal and Domestic Supply (MUN)
- Agricultural Supply (AGR)
- Industrial Service Supply (IND)
- Industrial Process Supply (PROC)
- Contact Water Recreation (REC-1)
- Non-contact Water Recreation (REC-2)
- Warm Freshwater Habitat (WARM)
- Cold Freshwater Habitat (COLD)
- Wildlife Habitat (WILD)
- Commercial and Sport Fishing (COMM)
- Rare, Threatened, or Endangered Species (RARE)
- Preservation of Biological Habitats of Special Significance (BIOL)
- Estuarine Habitat (EST)
- Marine Habitat (MAR)
- Migration of Aquatic Organisms (MIGR)
- Shellfish Harvesting (SHELL)
- Navigation (NAV)

Assessment Questions

The proposed monitoring plan provides details for an initial investigation of the transport of cyanotoxins in various water bodies within the San Diego Region, focusing on the Los Penasquitos Creek, Sweetwater River, and Otay watersheds. These efforts will be used to address the following assessment questions:

a. Do wadeable streams constitute meaningful loading sources for cyanotoxins to receiving waters?
b. To what extent are potential cyanotoxin-producing species, both marine and freshwater, and their associated cyanotoxins present at the land-sea interface?
c. How do month-long deployments of passive, Solid Phase Adsorption Toxin Tracking (SPATT), samplers compare with consecutive 10-day deployments over the same time period?

Link to Framework for Monitoring and Assessment in the San Diego Region

The investigation of cyanotoxin transport and bioaccumulation in the San Diego Region, conducted under this monitoring plan, will support the Framework for Monitoring and Assessment in the San Diego Region (Busse and Posthumus, 2012) that was adopted by the Board on December 12, 2012. The new approach is systematic, logical, question-driven, and is water-body oriented rather than discharge-oriented. The Framework illustrates the need for conditions monitoring (referred to as M1) on an ongoing basis to determine if/how conditions are changing in the water bodies of the San Diego Region. The proposed study will provide valuable input about the conditions of streams, reservoirs, and estuaries in the San Diego Region, which help address the most basic questions that reflect the fundamental concerns about beneficial uses, such as:

- Is the water safe to drink?
- Are the fish and shellfish safe to eat?
- Is water quality safe for swimming and other recreational activities?
- Are habitats and ecosystems healthy?

Clean Water Act Section 305(b)

The data produced by this monitoring plan will be used in water body assessments required under Clean Water Act (CWA) section 305(b).

Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA)

The HABHRCA of 2004 and 2014 implemented through the President's U.S. Ocean Action Plan, recognizes the importance of harmful algal blooms as a high priority national issue and mandates to advance scientific understanding and ability to detect, monitor, assess and predict harmful algal blooms and hypoxia events in coastal waters. The proposed monitoring plan contributes to these efforts and will provide valuable information about water bodies (i.e., inland and freshwater) that have not been studied extensively.

SWAMP Cyanotoxin Projects

The SWAMP Cyanotoxin Program is currently supporting multiple projects that are working toward a long term vision and strategic plan for statewide coordination of cyanobacteria and cyanotoxin monitoring and assessment. These include developing standardized protocols and communication tools, such as field documents for guidance on sample collection, a health and safety plan for sampling, a web portal, and standard operating procedures (SOPs) for the major labs in California that are using enzyme-linked immunosorbent assay (ELISA) and liquid chromatography-mass spectrometry (LC-MS/MS) methods to detect cyanotoxins. In addition, SWAMP is funding the National Oceanic and Atmospheric Administration (NOAA) work to develop satellite imagery that can be used to detect cyanobacteria blooms.

2 Background

2.1 Introduction

Toxic cyanobacteria have been reported in freshwater, brackish, and marine environments all over the world (World Health Organization (WHO), 1999 and Office of Environmental Health Hazard Assessment (OEHHA), 2012). Cyanobacterial blooms, often caused by anthropogenic eutrophication of surface waters, represent a major ecological and human health problem. Cyanobacteria can be found on the water surface, in benthic zones, and within the water column. When cyanobacteria die, a cell breaks, or a benthic mat detaches, cell membranes rupture and can release toxins into the water. Besides releasing toxins when blooms die, cyanobacterial blooms can cause taste and odor problems for drinking water, and the decaying process of cyanobacteria consumes oxygen and may destroy fishery habitats. Harmful cyanobacteria blooms can also impair boating activities by clogging channels and water filters.

Toxins released by various cyanobacteria species include neurotoxins (affect nervous system), hepatotoxins (affect liver), and dermatoxins (affect skin). Table 1 shows some of the specific cyanotoxins responsible for the effects (WHO, 1999), and Table 2 lists the cyanotoxins and taxa known to produce them (WHO, 2003 and Castle and Rogers, 2009).

Table 1. Cyanotoxins listed by toxin type (WHO, 1999)

TOXIN TYPE	CYANOTOXINS
Dermatoxins	Lyngbyatoxins Aplysiatoxins
Neurotoxins	Anatoxins Saxitoxins B-methylamino alanine (BMAA)
Hepatotoxins	Cylindrospermopsins Microcystins Nodularins

Table 2. Taxa known to produce specific cyanotoxin(s) (WHO, 2003 and Castle and Rogers, 2009)

Cyanotoxins	Taxa known to produce toxin(s)
Microcystins in general Microcystin-LR Microcystin-YR Microcystin-RR	*Microcystis* *Planktothrix (Oscillatoria)* *Nostoc* *Dolichospermum (Anabaena)* *Anabaenopsis* *Hapalosiphon* *Nodularia* *Anacystis* *Gloeocapsa* *Eucapsis* *Aphanocapsa*

	Rivularia *Entophysalis* *Schizothrix* *Phormidium* *Synechococcus* *Microcoleus* *Woronichinia naegeliana*
Nodularin	*Nodularia spumigena*
Anatoxin-a (including homoanatoxin-a)	*Dolichospermum (Anabaena)* *Planktothrix (Oscillatoria)* *Plectonema* *Aphanizomenon* *Rhaphidiopsis* *Hyella* *Cylindrospermum*
Saxitoxins	*Dolichospermum (Anabaena)* *Lyngbya* *Cylindrospermopsis raciborskii* *Aphanizomenon* *Planktothrix (Oscillatoria)*
Cylindrospermopsin	*Cylindrospermopsis raciborskii* *Aphanizomenon* *Umezakia* *Raphidiopsis*
Lyngbyatoxin-a	*Lyngbya* *Schizothrix* *Planktothrix (Oscillatoria)*

Humans, pets, livestock, and wildlife may be exposed to cyanotoxins in a variety of ways. Humans can inadvertently ingest contaminated water while participating in recreational water activities such as swimming, boating, and waterskiing. The toxins may also be aerosolized and inhaled or consumed when eating contaminated fish and shellfish. Pets and wildlife may ingest cyanobacterial scum and drink contaminated water. Some animals tend to be attracted to the drying clumps of cyanobacteria (also called crusts or mats) that have washed onto the land (Office of Environmental Health hazard Assessment (OEHHA), 2012). In addition, bioaccumulation has been documented in sensitive species, like sea otters that consumed bivalves contaminated with microcystins in Monterrey Bay, which caused liver poisoning and mortality (Miller et al., 2010).

Presence of high levels of cyanotoxins in recreational or drinking water can cause symptoms in humans that include: fever, headaches, muscle and joint pain, blisters, stomach cramps, diarrhea, vomiting, mouth ulcers, and allergic reactions. There is evidence that some cyanotoxins, especially hepatotoxins, are potent tumor promoters (Carmichael, 2001). In the most severe cases, effects can include seizures, liver failure, respiratory arrest, and (rarely) death. Harmful cyanobacteria and their toxins are contaminants of emerging concern and were placed on the Candidate Contaminant List (CCL) by the United States Environmental Protection Agency in July 2012 (USEPA, 2012). Included on the list are microcystin-LR, anatoxin-a, and cylindrospermopsin.

Cyanobacteria blooms have been documented throughout the State of California in various water body types, including lakes/reservoirs, wadeable streams, depressional wetlands, coastal lagoons, and rivers (e.g., California Department of Public Health, 2012, Fetscher et al., in press, and Howard et al., in progress (Figure 1)).

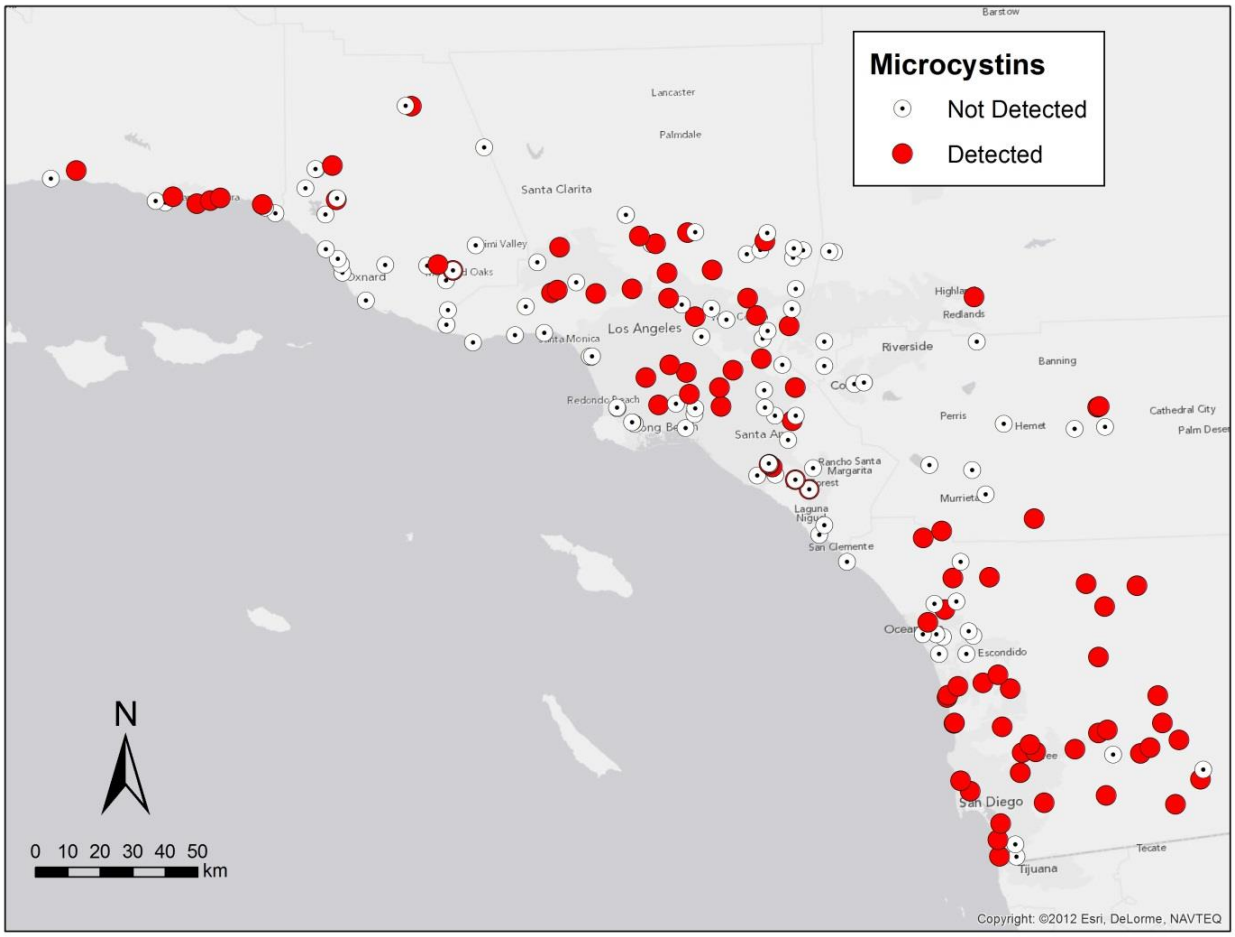

Figure 1. Microcystins detected in depressional wetlands, coastal lagoons, lakes, wadeable streams, rivers, and estuaries of southern California

Many factors affect cyanobacteria bloom formation and persistence. These include light intensity, sunlight duration, nutrient availability, water temperature, pH, an increase in precipitation events that deliver nutrient-rich runoff, altered flow regimes, and water column stability. Many harmful cyanobacteria have the ability to fix nitrogen and can therefore thrive in nitrogen-depleted environments. Others, such as *Microcystis*, do not have nitrogen-fixing capabilities but may be favored by reduced forms of nitrogen (e.g., ammonium and urea rather than nitrate). Rising surface water temperatures, a result of the changing global climate, tend to favor cyanobacteria (O'Neil et al., 2012). At higher temperatures, cyanobacteria are able to outcompete other phytoplankton species. Warmer temperatures result in stronger vertical stratification of lakes and reduce vertical mixing. Cyanobacteria can exploit these conditions using cellular gas vesicles that

create buoyancy, allowing them to rise to the top of warmer waters and cast shade over the non-buoyant phytoplankton (Paerl and Huisman, 2008).

Currently, a cyanobacteria and cyanotoxin monitoring program does not exist in the region, or in the State of California. In 2010, the Blue Green Algae Work Group, comprised of members from the State Water Resources Control Board (SWRCB), the California Department of Public health (CDPH), and Office of Environmental Health and Hazard Assessment (OEHHA), developed voluntary statewide guidance for educating and notifying the recreating public about blue-green algae blooms of non-marine water bodies in the state of California (SWRCB et al., 2010). In May 2012, OEHHA finalized a report that provides calculated health-based water concentration levels (action levels) of microcystins (LA, LR, RR, and YR), anatoxin-a, and cylindrospermopsin for people, pets, and livestock exposed to the cyanotoxins through various scenarios. Health-based concentrations in sport fish and shellfish were also calculated (OEHHA, 2012). In 2015, the United States Environmental Protection Agency (EPA) developed Health Advisories (HA) for the cyanobacterial toxins microcystins and cylindrospermopsin. EPA recommends HA levels at or below 0.3 micrograms per liter for microcystins and 0.7 micrograms per liter for cylindrospermopsin in drinking water for children under the age of six years. For school-age children (6 years and older) through adults, the recommended HA levels for drinking water are at or below 1.6 micrograms per liter for microcystins and 3.0 micrograms per liter for cylindrospermopsin. The state of California has established the following recreational water guidance/action levels, with the recommended action of advisory:

0.8 μg/L Microcystin
90 μg/L Anatoxin-a
4 μg/L Cylindrospermopsin

The state of California has also issued advisory tissue level (ATL) concentrations for microcystins for specific waterbodies in northern California, with an ATL of 26 ng/g in tissue for a consumption rate of no more than one 6-ounce serving per week.

These action levels may be applied as needed on a voluntary basis, by local, regional, state or tribal entities throughout California, to reduce exposures to cyanotoxins. Because cyanobacteria thrive under the conditions created by eutrophication and climate change and can cause detrimental ecological and economic impacts, a great need exists for the monitoring and mitigation of cyanobacteria and cyanotoxins in our region's waters.

Several SWAMP-funded cyanotoxin screening studies were conducted over the past few years in the San Diego region. In 2012, efforts were focused on depressional wetlands and streams. In 2013, the screening took place in lakes/reservoirs and coastal wetlands. Due to high heat and drought conditions, an impromptu study took place in 2014, which was partially SWAMP-funded and collected data from many high-recreational use lake and reservoir systems. Because we have established that cyanotoxins are prevalent throughout the San Diego region, we will expand the screening efforts, under this monitoring plan, to include cyanobacteria and cyanotoxin

watershed dynamics, focusing on potential sources (i.e., transport from wadeable streams) and bioaccumulation (i.e., shellfish in reservoirs and estuaries). Funding for this effort will be provided by SWAMP (FY 2015/2016).

2.2 Past Cyanotoxin Monitoring (SWAMP and non-SWAMP efforts)

2.2.1 Region 9 (San Diego Region)

Cyanotoxin Screenings

Streams and Depressional Wetlands

During FY 2011/2012, a cyanotoxin screening was conducted in streams and depressional wetlands in the San Diego Region. The stream sites were sampled as part of a larger effort by the Stormwater Monitoring Coalition (SMC) and statewide Perennial Stream Assessment (PSA). The depressional wetland sampling sites were selected from a larger study on the extent and conditions of depressional wetlands conducted by the Southern California Coastal Water Research Project (SCCWRP) and the (San Diego, Santa Ana, and Los Angeles) Regional Water Boards (SCCWRP, 2011).

Samples for the screenings were obtained using either discrete (i.e., grab sample) or passive, continuous (i.e., Solid Phase Adsorption Toxin Tracking (SPATT) bag) methods. SPATT bags are sampling devices constructed of resins that adsorb specific toxins, and are deployed in a water body for a fixed amount of time (Kudela, 2011). SPATT bags provide an integrated sample to supplement the grab samples, which are subject to variability due to spatial and temporal heterogeneity in toxin expression in water bodies. SPATT results provide insight into toxin presence, but do not yield toxin concentrations.

The cyanotoxin screening in streams included 120 samples that were collected in 2011 and 2012 throughout Southern California, using a random design. All samples were analyzed for microcystins using Enzyme-Linked Immunosorbent Assays (ELISA) by SCCWRP. A smaller subset of samples was analyzed at UC Santa Cruz by Raphael Kudela, using LC-MS/MS for microcystin, anatoxin, cylindrospermopsin, saxitoxin, nodularin, and lyngbyatoxin detection. Out of the 120 samples measured by ELISA, 38% contained microcystin. In the smaller subset of samples analyzed by LC-MS/MS, 21% contained lyngbyatoxin, 5% contained saxitoxin, and 3% contained anatoxin. There was no cylindrospermopsin or nodularin found in the samples. These efforts were funded in-kind by SCCWRP and the Southern California Stormwater Monitoring Coalition (SMC).

The depressional wetlands screening was funded by SWAMP and included ten (10) randomly-selected sites in the San Diego Region from the extent and conditions study. The sampling sites are located in seven (7) of the eleven (11) hydrologic units (HUs), found within the San Diego Region (Table 3). Two of the wetland sites experienced dry

conditions upon the beginning of the field work and were unable to be sampled, bringing the total number sample sites down to eight (8).

Table 3. Number of depressional wetland sites sampled per hydrologic unit

HU #	Hydrologic Unit Name	# of Wetlands Sampled
902	Santa Margarita	1
903	San Luis Rey	2
904	Carlsbad	1
905	San Dieguito	1
907	San Diego	1
909	Sweetwater	1
910	Otay	1

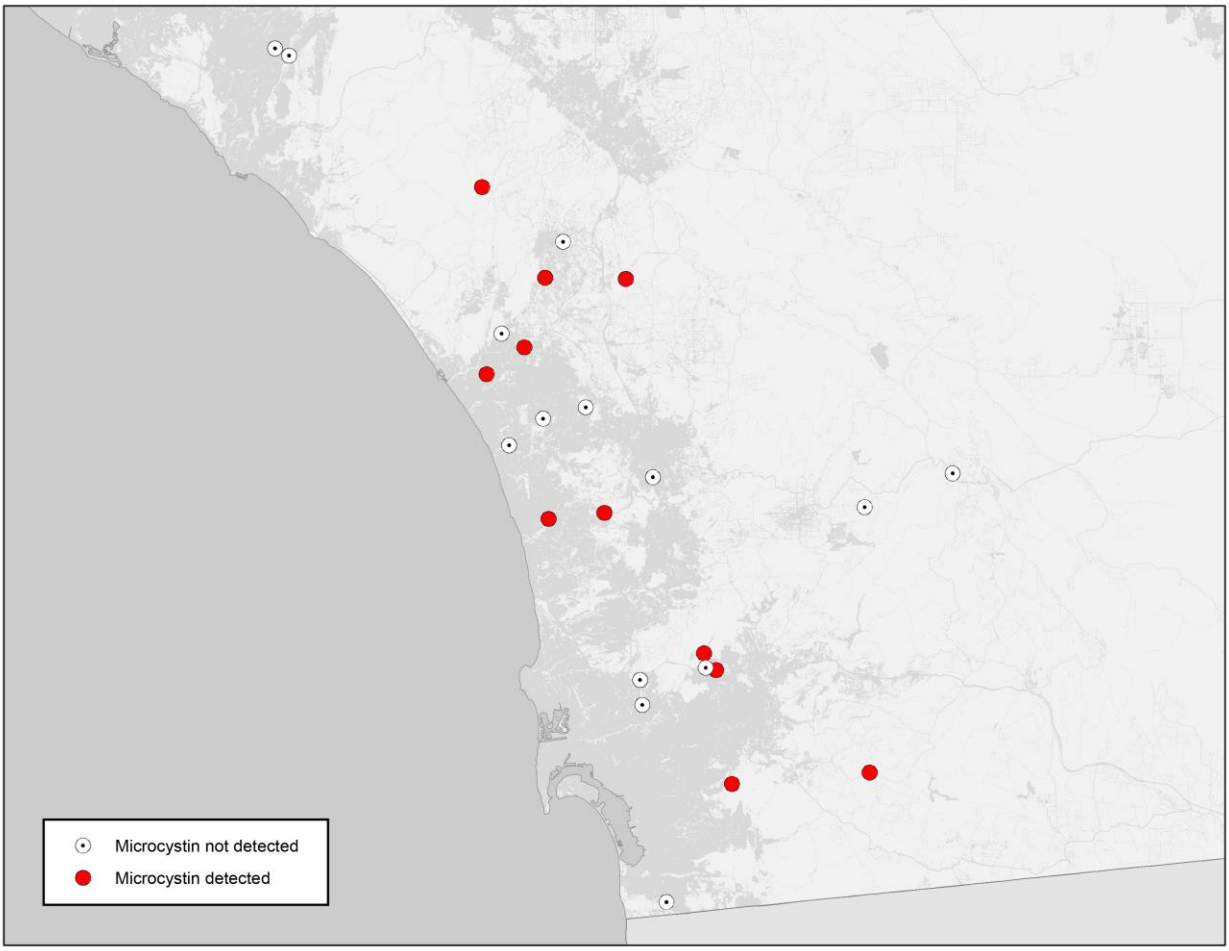

Figure 2. Map of all depressional wetlands sites sampled between 2011-2013 and results of microcystins analysis

Grab samples, taken twice at each site, were analyzed for chlorophyll-a, cyanotoxins, pigments, and nutrients. Of the lab results that are available to date, microcystins were detected at 60% of the sites in the spring and only 30% of the sites in the summer and fall of 2012 (Table 4). Saxitoxin was also detected in 10% of the sites in the spring, 14% in summer and none of the sites in the fall. The SPATT bags retrieved from the final two wetland sites have not yet been analyzed. However, the SPATT samplers detected microcystins at 83% of the sites. These results indicate that grab samples do not capture all toxic events and one-time assessment studies do not provide a comprehensive assessment of cyanotoxin prevalence in water bodies. The SPATT samplers were shown to be an important screening assessment tool to determine if water bodies are routinely toxic for microcystins. The Santee Lake site had high concentrations of microcystins. The cyanotoxins detected to date are listed in Table 5.

Table 4. The percentage of sites where microcystins were detected based on grab samples compared with SPATT samples in San Diego sites, sampled in 2012

Season	% of toxic sites based on grab samples	% of toxic sites based on SPATT samples
Spring	60	Not collected
Summer	29	83
Fall	29	

Table 5. Cyanotoxins detected in depressional wetlands monitored in 2012

SITE ID	City	Cyanotoxin Detected
902CRXFAL	Fallbrook	Microcystin
903CCRVAL	Valley Center	Microcystin-RR
903OLVFAL	Fallbrook	Microcystin-RR
904EMISGC	Oceanside	Microcystin-RR
904MANENC	Encinitas	Microcystin
907SL7SNT	Santee*	Microcystin-LR & Microcystin-LA
909SWASPV	Spring Valley	Microcystin
910LVRJAM	Jamul	Microcystin-LR

*Indicates microcystin concentration exceeded the California recreational health advisory thresholds (0.8 µg/L).

Lakes/Reservoirs and Coastal Wetlands

During the summer and fall of 2013, a screening of lakes/reservoirs and coastal wetlands was conducted throughout Region 9. Targeted sampling included a combination of passive (SPATT) samplers and surface water grabs at the water bodies listed below in Table 6.

Table 6. Targeted sampling sites for 2013 screening of lakes/reservoirs and coastal wetlands

Lake/Reservoirs	Coastal Wetlands
Vail Lake (902TV0111)	San Elijo Lagoon (904SNELLG)
Lake Henshaw (903PLH214)	San Elijo Pond (904SNELPD)
Lake Hodges (905PLH070)	Mission Bay (90606MISS)
Lake Sutherland (905PLS198)	Los Penasquitos Lagoon (906LSPNLG)
Lake Miramar (906PLM142)	San Diego River Estuary (907SDRVES)
Cuyamaca Reservoir (907CUYRES)	San Diego Bay near NTC (908SDBNTC)
Lake Murray (907LKMURR)	San Diego Bay Silver Strand (908SDBYSS)
El Capitan Reservoir (907PEC062)	San Diego Bay Sweetwater (908SDBSW)
Lower Otay Reservoir (910PLO182)	Tijuana River Estuary (911TJRVES)
Morena Reservoir (911PMR110)	

SPATT were deployed for two 1-month intervals, typically from July to August and from August to September. SPATT were analyzed for dissolved microcystins (MC-LR, MC-RR, MC-YR, and MC-LA) at UC Santa Cruz using LC-MS/MS. Grab samples were collected during each SPATT deployment and retrieval, for a total of three (3) times per site. Grab samples were processed and analyzed for nutrients (total nitrogen, total phosphorous, dissolved inorganic nitrogen, particulate nitrogen, and particulate phosphorous), chlorophyll a, pigments, alkalinity, and particulate microcystins. SCCWRP provided most of the analyses, but the particulate microcystins were analyzed at the CA Fish and Wildlife Water Pollution Control Lab (WPCL) using LC-ESI-MS/MS. *In situ* readings were also recorded at each site, which included temperature, pH, DO, conductivity, and salinity.

Microcystins were detected at all of the sites during at least one of the SPATT deployments. Figure 3 shows the location and concentrations of microcystins detected in grab samples and SPATT bags. Table 7 provides sites for which microcystins were detected in both grab and SPATT samples and the range of microcystins detected.

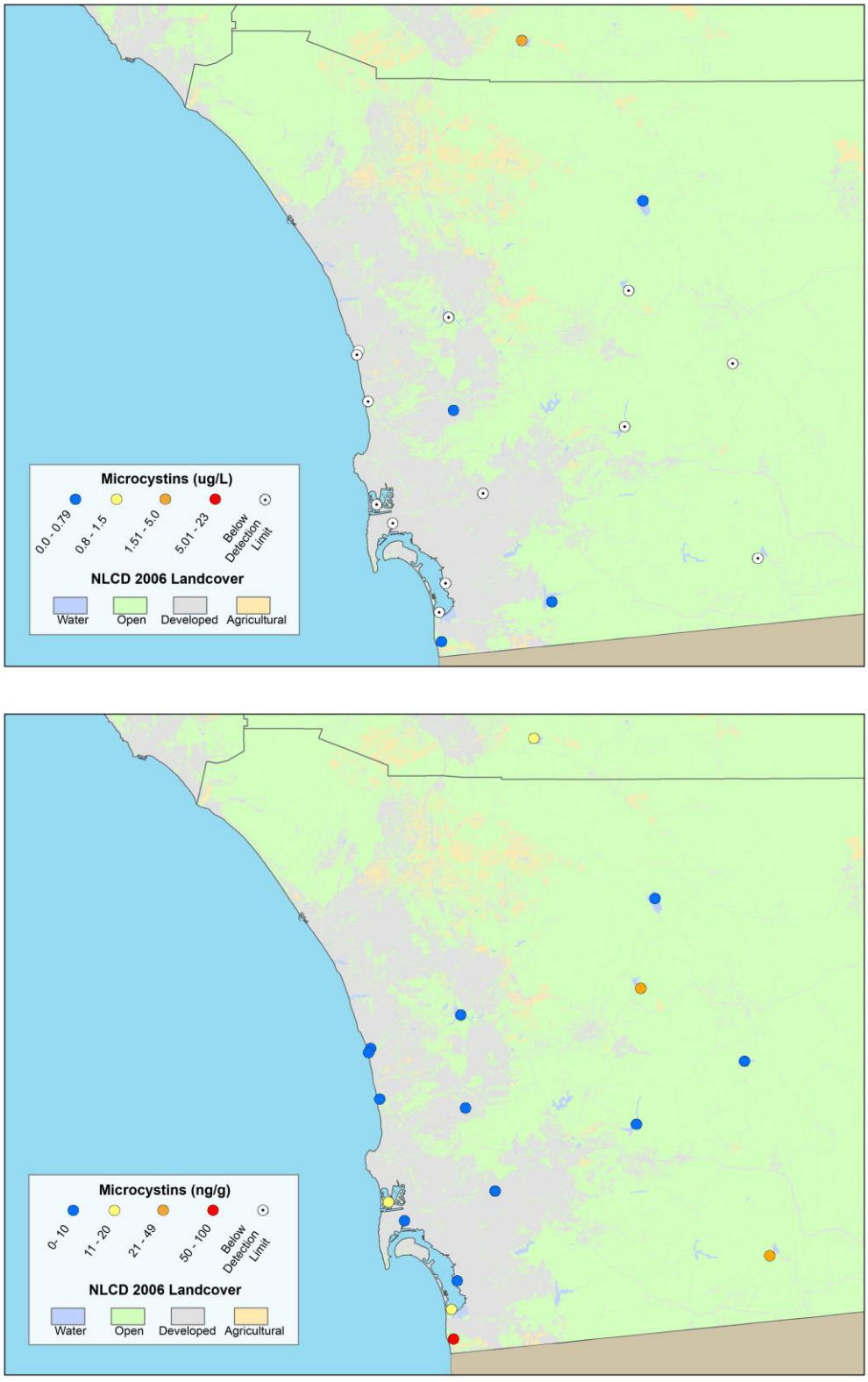

Figure 3. Maps of microcystin concentration results from grab samples (top panel) and SPATT bags (bottom panel) collected in the summer, 2013. The highest concentration is reported (sites were visited multiple times)

Table 7. Sites for which microcystins were detected in 2013 and the range of microcystin concentrations detected by both grab and SPATT samples

Site Name	Range of microcystin concentrations determined from grab samples (µg/L)	Range of microcystin concentrations determined from SPATT samples (ng/g)
Vail Lake	bd – 2.1	bd - 13.3
Lake Henshaw	0.1 - 0.3	1.3 – 2.1
San Elijo Lagoon	bd	1.2 – 1.5
San Elijo Pond	bd	2.3 – 4.5
Lake Hodges	bd	0.5 – 2.7
Lake Miramar	bd – 0.1	5.6 – 7.0
San Diego Bay (near Naval Training Center)	bd	3.2 – 6.0
Morena Reservoir	bd – 23.6	6.1 – 44.7
Tijuana River Estuary	bd – 0.09	2.7 – 100.8

The lakes/estuaries and coastal wetlands screening study showed that microcystins are prevalent in these coastal lakes, estuaries and wetlands. Similar to the depressional wetlands results, grab samples missed toxic events and were not representative of the prevalence of cyanotoxins in these waterbodies. The SPATT bags were again shown to be a very useful screening tool to determine the presence of toxins over time.

The findings of this screening study will be combined with the other studies listed in this section and will be published in a technical report, written with by Meredith Howard (SCCWRP) in 2015.

Additional Lake/Reservoir Screening

Due to the drought conditions and subsequent high volume of cyanobacteria blooms in the San Diego region in 2014, an impromptu cyanotoxin screening took place at lakes with high recreational use in Region 9 (and a few in Region 8) from June through September, 2014. Reservoir managers were asked to notify the San Diego Water Board if/when cyanobacteria blooms occurred in their water bodies, and SCCWRP and Region 9 staff conducted spot checks of smaller lakes during the bloom season. Sampling sites included the following:

Region 8
Lake Elsinore
Canyon Lake
Lake Menifee
Region 9
Skinner Reservoir
Lake Hodges
Lake Henshaw (San Luis Rey River)
Lake Sutherland
Lake Barbara

Lindo Lake
Discovery Lake
Harveston Lake
Pond Park
Guajome Lake
Lake Miramar
Lake Wohlford
Santee Lake #5
Barrett Lake
Lake Poway
Lake Morena
Chollas Reservoir
San Elijo Lagoon
Los Penasquitos Lagoon

Most grab samples were analyzed for particulate (WPCL) and dissolved (SCCWRP) cyanotoxins via ELISA. *In situ* readings were recorded for temperature, pH, dissolved oxygen, conductivity, and salinity. Grab samples were analyzed for nutrients (total nitrogen and total phosphorous) at some sites, and alkalinity. Samples were also collected for species identification at the Caron Lab of University of Southern California. The results of the cyanotoxins and species identification are listed in Table 8.

Microcystins were detected in many of the water bodies sampled, and results included one of the highest concentrations ever recorded in California. In May 2015, the concentration of total microcystins found in San Joaquin Marsh, Pond C (Region 8) was greater than 36,000 μg/L. The results that have been compiled so far also indicate a high abundance of species with the ability to produce cylindrospermopsin in the water bodies sampled, demonstrating the importance of analyzing for other cyanotoxins in addition to the more common microcystins.

Table 8. Summary of potentially toxic species identified in San Diego Sites sampled in summer of 2014. The sample collection month in 2014 is listed next to the name of the water body. The genera and species identification of HAB organisms in the samples are listed, as well as total microcystins detected in mg/L. All samples analyzed for microcystins were also analyzed for Nodularin and anatoxin-a, and all were below the limit of detection

Month	Name	HAB Genera and Species Identification	Total MCY
August	Barrett Lake	*Cylindrospermopsis raciborskii,* *Cylindrospermopsis spp.,* *Anabaena spp.*	b.d.
August	Chollas Reservoir	Low abundance of non-nitrogen fixing filaments	NA
June	Discovery Lake	*Planktothrix sp., Anabaena variabilis, Anabaena spiroides,* *Cylindrospermopsis sp.* (minor component)	NA
August	Guajome Lake	*Cylindrospermopsis sp., Planktothrix sp.*	NA
June	Harveston Lake	NA	10.0
August	Harveston Lake	Sparse *Microcystis sp.*	NA

June	Lake Barbara	no cyanobacteria observed	b.d.
June	Lake Hodges	*Anabaena sp.*	b.d.
June	Lake Henshaw, downstream	*Microcystis sp.*	b.d.
August	Lake Morena	Mainly eukaryotes, shoreline dominated by *Microcystis spp.*	NA
August	Lake Poway	Sparse *Microcystis sp.* colonies	NA
June	Lake Sutherland	*Microcystis sp.*	b.d.
June	Lindo Lake	*Planktothrix spp., Anabaena variabilis, Anabaena sp.* *Cylindrospermopsis sp.* (minor component)	2.5
August	Lindo Lake	*Planktothrix sp.* and *Cylindrospermopsis spp.* dominant *Microcystis sp.,* and *Anabaena spp.* observed	2.4
July	Santee Lake #5	*Microcystis sp*. floating on surface, *Cylindrospermopsis sp.* dominated water column, *Cylindrospermopsis raciborskii* and *Anabaena spiroides*	11.7

NA = not analyzed; b.d. = below the method detection limit.

Benthic Algae Sampling

Benthic algae were sampled throughout wadeable streams in southern California as part of the SWAMP Bioassessment study in 2008, the Stormwater Monitoring Coalition efforts from 2009-2012, and through funding awarded to SCCWRP from a Proposition 50 grant. The benthic algae sampling efforts included diatoms and soft algae, which supported the development of an Index of Biologic Integrity (IBI) for benthic algae in Southern California. Soft algae sampling included the cyanobacteria community. In 2011 and 2012, 41% of the benthic algal mat and biofilm samples contained some form of cyanotoxin.

Summer and fall 2001 cyanobacterial blooms in Lake Skinner (Region 9) and Silverwood Lake (Region 6) prompted the Metropolitan Water District of Southern California (MWDSC) to develop a cyanotoxin monitoring program in 2003. MWDSC found benthic algal samples from several drinking water reservoirs that tested positive for microcystin, which led to further studies to identify the particular toxin variant(s) involved and the corresponding cyanobacteria. The lakes that were found to contain microcystin include two lakes located in Region 9, Lake Skinner and Diamond Valley Lake (Izaguirre et al., 2007).

2.2.2 Region 1 (North Coast Region)

In 2007, the USEPA provided funding through a Water Quality Cooperative Agreement (CP 96941301-2) to analyze fish tissue and water from the Klamath River, which is an impaired water body on the CWA section 303(d) list for sediment, microcystin toxin, temperature, nutrients, and dissolved oxygen (Kanz, 2008). The study provided a

screening level analysis of microcystin accumulation in a range of aquatic species. Targeted species included yellow perch, yearling Chinook salmon, and freshwater mussels. The study determined that the levels of microcystin found in the fish and shellfish warranted development of advisories for tissue consumption. On December 28, 2010, the USEPA approved Total Maximum Daily Loads (TMDLs) addressing temperature, dissolved oxygen, nutrient and microcystin impairments of the Klamath River.

In partnership with a variety of organizations, the Klamath Basin Monitoring Program (KBMP) developed Klamath Basin Monitoring Maps, which includes The Blue-Green Algae Tracker. The Blue-Green Algae Tracker informs the public and research community of current river conditions and tracks blue-green algae blooms throughout the Klamath Basin. It identifies river segments that pose a threat to public health (i.e., exceed thresholds identified in the statewide voluntary guidance).

2.2.3 Region 2 (San Francisco Bay Region)

A bloom of *Microcystis aeruginosa* has been observed between June-November in the northern reach of the San Francisco Bay Estuary since 1999 (Lehman et al., 2005). A study on its distribution, biomass and toxicity was conducted in 2003, which determined that microcystin is widely distributed, from freshwater to brackish water environments. Samples from all stations throughout the estuary were found to contain hepatotoxic microcystins and indicated the need for long-term monitoring. Part of the monitoring, funded by a special grant from the San Francisco Bay Delta Interagency Ecological Program, has included an investigation of the impacts of the *Microcystis aeruginosa* blooms on the aquatic food webs in the San Francisco Estuary (Lehman et al., 2010). Phytoplankton, cyanobacteria, zooplankton, and fish were collected biweekly throughout the estuary and analyzed for microcystins. Total microcystins were present at all levels of the food web. Greater total concentrations in striped bass, as compared with their prey, indicated that microcystin is accumulating at higher trophic levels.

An ongoing study has also investigated a bloom of *Aphanizomenon flosaquae* in the Sacramento-San Joaquin Delta during the summer of 2011 (Mioni et al., 2012). A lower abundance of *Aphanizomenon flosaquae* was found during summer 2012, but the strain is still present. Other strains of interest in this region include *Anabaena sp*., which has a patchy distribution but can reach significant levels.

2.2.4 Region 3 (Central Coast Region)

The deaths of marine mammals (more than 21 dead and dying sea otters) found along the shores of Monterey Bay, with microcystin intoxication determined to be the cause, prompted a study investigating the land-sea flow and trophic transfer of microcystins through marine invertebrates (Miller et al., 2010). During cyanobacteria bloom events, water samples and surface bloom samples were collected from Pinto Lake, just inland

of Monterey Bay, and its drainage into Corralitos Creek and the Pajaro River. Time-integrative passive samplers (SPATT) were also deployed in fresh and marine systems along the central California coast. Water from Pinto Lake in fall of 2007 confirmed occurrence of an extensive *Microcystis* bloom with high toxin production. Recurrent *Microcystis* blooms, with toxin production, were also confirmed in samples from Pinto Lake and surrounding waters in 2008 and 2009. The most common congener of microcystin found in the lake and surrounding water is microcystin-LA, but others were also detected. Field deployed SPATT, placed in ocean water and the marine interfaces of coastal rivers flowing into Monterey Bay, were analyzed for microcystins and provided results that determined the main source of toxins in Monterey Bay is not of marine origin. Since that time, regular (weekly) monitoring of Pinto Lake using both grab samples and continuous toxin measurements using SPATT showed that grab samples can miss toxic events (Kudela, 2011).

Monitoring of Pinto Lake has continued and includes a study that was conducted from January 2012 through February 2013 that collected bi-monthly, followed by weekly data on cyanobacterial taxa density, intracellular microcystins, presence of microcystin-production gene (mcyB) and environmental factors (e.g., pH, temperature, and nutrients). An increase of cyanobacterial biomass occurred from late summer through early winter, and microcystin levels were more than double that which was detected in previous years. An early peak of *Aphanizomenon* and *Anabaena* occurred in April 2012, with an early peak of microcystins but no *Microcysti*s. *Microcystis* blooms and intracellular *microcystins* peaks were observed later in the year (Blanco, 2013).

2.2.5 Region 5 (Central Valley Region)

Under contract #10-058-150, monitoring was conducted to determine the distribution of harmful cyanobacteria of concern and their associated toxins in the surface waters of Clear Lake and the Sacramento-San Joaquin Delta (Mioni et al., 2012). Samples (discrete and continuous) were collected from June through October of 2011 and analyzed for toxins, nutrients, chlorophyll-a, cyanobacterial deoxyribonucleic acid (DNA), dissolved organic carbon (DOC), and taxonomy. A *Lyngbya* bloom in Clear Lake was the initial focus of the study, but several strains of harmful cyanobacteria are known to bloom in the system. Several successive blooms were found to occur over the year. *Anabaena* and *Aphanizomenon* dominate in spring; *Lyngbya* dominates during summer; and *Microcystis* usually dominates in late summer/early fall. *Woronichinia* can also reach a relatively high abundance in the summer. The data collected during this study were used to determine correlations between individual cyanobacteria taxa and environmental controls. Several environmental drivers in surface waters were found, with the major influencing factors being temperature, and nitrogen and phosphorous concentrations.

2.2.6 Region 8 (Santa Ana Region)

The MWDSC monitoring studies mentioned above in Section 2.2.1, include two lakes that are located in Region 8. Benthic algal samples from Lake Mathews and Lake Perris tested positive for microcystins and were further analyzed to determine which variants were present and which cyanobacteria produced the toxins (Izaguirre et al., 2007).

2.3 Proposed SWAMP Cyanotoxin Monitoring in Region 9 for 2016

Data from California clearly show that cyanobacteria and cyanotoxins occur throughout many water bodies of varying types. Toxigenic cyanobacteria have been found to be nearly ubiquitous in California wadeable streams, and cyanotoxins are commonly produced in the benthic environments of these systems, sometimes at high concentrations (Fetscher et al., 2015). Furthermore, the literature indicates that some cyanotoxins (e.g., microcystins and nodularin) can persist for weeks under normal ambient conditions. Taken together, these pieces of information have prompted the question**: *Do wadeable streams constitute meaningful loading sources for cyanotoxins to receiving waters?*** If so, stream cyanotoxin production could potentially affect several aquatic-life-related beneficial uses, as well as drinking water and recreational BUs, both within the streams themselves and in the receiving waters Understanding the potential for non-negligible inputs of cyanotoxins from streams will be important for determining how best to monitor for cyanotoxins in reservoirs, lakes, depressional wetlands, and estuaries, and what (if any) management actions will be most appropriate and effective.

The conventional focus of cyanobacteria and cyanotoxin monitoring has generally been water body-dependent, focused on marine or freshwater toxins, but not both. However, recent studies have shown that cyanotoxins generated in freshwater environments can have far-reaching effects in downstream brackish and marine water (Miller et al., 2010). This study will constitute a first step in answering the question: ***To what extent are potential cyanotoxin-producing species, both marine and freshwater, and their associated cyanotoxins present at the land-sea interface?***

This study will also demonstrate/validate how SPATT samplers can be incorporated into existing monitoring programs and will investigate the question: ***How do month-long deployments of passive, Solid Phase Adsorption Toxin Tracking (SPATT), samplers compare with consecutive 10-day deployments over the same time period?***

Cyanotoxins are known from the literature to be capable of bioaccumulating, thus potentially affecting beneficial uses relating to food webs and human consumption of fish and shellfish. Analyzing tissues in order to inform advisory actions is expensive and time-consuming. SPATT samplers may serve as a cheaper and faster surrogate for assessing tissue consumption risk. Should funding and shellfish bed abundance allow, this study will constitute a first step in answering the optional question: ***Can useful information about the potential for cyanotoxin bioaccumulation in shellfish be acquired using SPATT bags for monitoring?***

The following monitoring plan provides details for an initial investigation of the transport and bioaccumulation of cyanotoxins in various water bodies within the San Diego Region, focusing on Los Penasquitos Creek, Sweetwater River and Otay River watersheds. These efforts will be used to address the following assessment questions:

a. Do wadeable streams constitute meaningful loading sources for cyanotoxins to receiving waters?
b. To what extent are potential cyanotoxin-producing species, both marine and freshwater, and their associated cyanotoxins present at the land-sea interface?
c. How do month-long deployments of passive, Solid Phase Adsorption Toxin Tracking (SPATT), samplers compare with consecutive 10-day deployments over the same time period?
d. ***Optional question:*** Can useful information about the potential for cyanotoxin bioaccumulation in shellfish and/or fish be acquired using passive sampling with Solid Phase Adsorption Toxin Tracking (SPATT) bags?

Results of the assessment will be used to determine future needs for adequately monitoring the water bodies in the San Diego Region for cyanobacteria and cyanotoxins. It will be done in accordance with the Framework for Monitoring and Assessment in the San Diego Region, recently adopted by the Board, considering the following beneficial use questions:

- Is the water safe to drink?
- Are the fish and shellfish safe to eat?
- Is water quality safe for swimming and other recreational activities?
- Are habitats and ecosystems healthy?

3 Study Methods

3.1 Monitoring Design

3.1.1 Site Selection

Los Penasquitos Creek, mouth to lagoon

The Los Penasquitos Creek watershed is located in a highly urbanized region, mostly west of Interstate 15. Los Penasquitos Creek discharges to a lagoon, which receives urban runoff yet supports diverse native fauna and flora. The lagoon system flows to the ocean when the barrier bar is breached or bulldozed open. Dredging will be taking place in May/June 2016 to ensure free flow between the lagoon and ocean. Three sampling sites were chosen to monitor the potential transport of cyanotoxins through the Los Penasquitos Creek watershed: (1) upstream/freshwater, (2) mid-slough/brackish, and (3) coastal receiving water/open - lagoon (Figure 4).

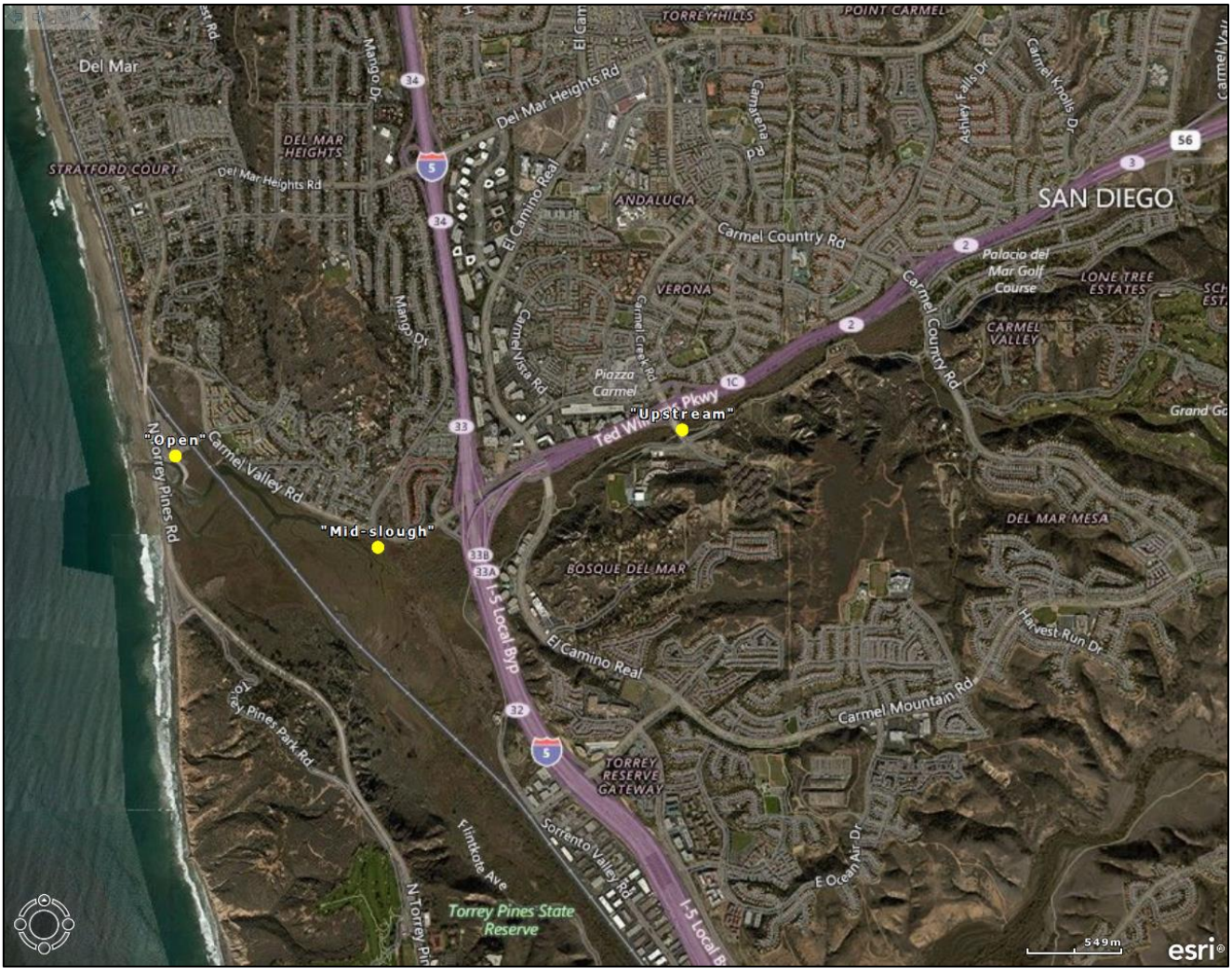

Figure 4. Los Penasquitos Creek cyanotoxin sampling sites.

Sweetwater River, mouth to San Diego Bay

The Sweetwater River watershed encompasses a variety of land use types, including a large portion of undeveloped lands, open space/agricultural, and urban. The Sweetwater River contains two impoundments, which form Loveland Reservoir and Sweetwater Reservoir. At its terminus, the river flows into the San Diego Bay and is the largest of three major watersheds that drain to the Bay. A portion of the river is tidally influenced and known as the Sweetwater River Estuary. Three sampling sites were chosen to monitor the potential transport of cyanotoxins through the Sweetwater River watershed: (1) upstream/freshwater, (2) mid-slough/brackish, and (3) coastal receiving water/open - bay (Figure 5).

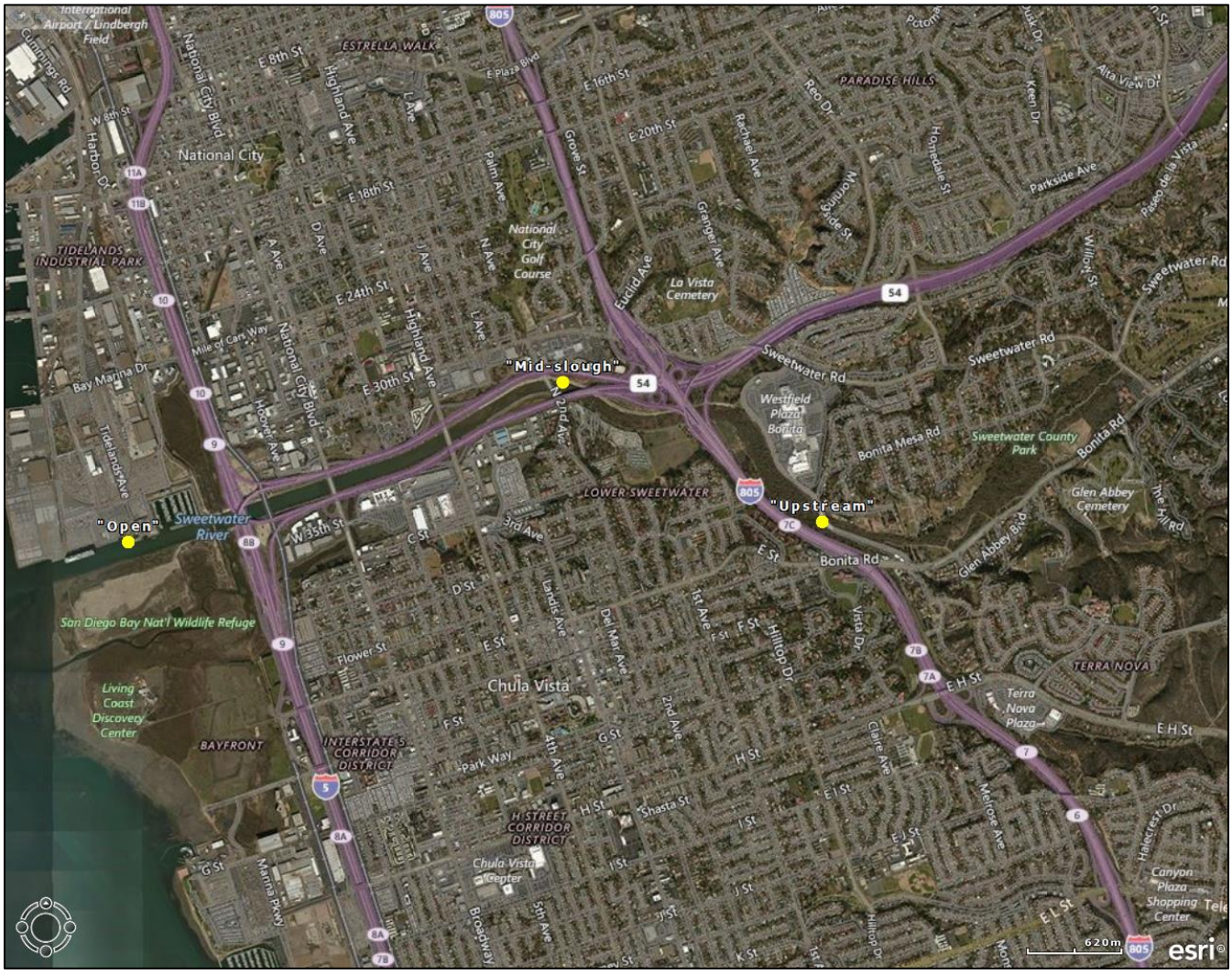

Figure 5. Sweetwater River cyanotoxin sampling sites.

Otay River, mouth to San Diego Bay

The Otay River watershed consists largely of open space and urban/residential land. The Otay River begins at San Miguel Mountain and flows through Upper Otay Reservoir and Lower Otay Reservoir, which also receives imported water. Further downstream, the river flows through South San Diego Bay National Wildlife Refuge, a riparian habitat that supports threatened and endangered wildlife, and then empties to San Diego Bay. Three sampling sites were chosen to monitor the potential transport of cyanotoxins through the Sweetwater River watershed: (1) upstream freshwater, (2) mid-slough brackish, and (3) coastal receiving water/open - bay (Figure 6).

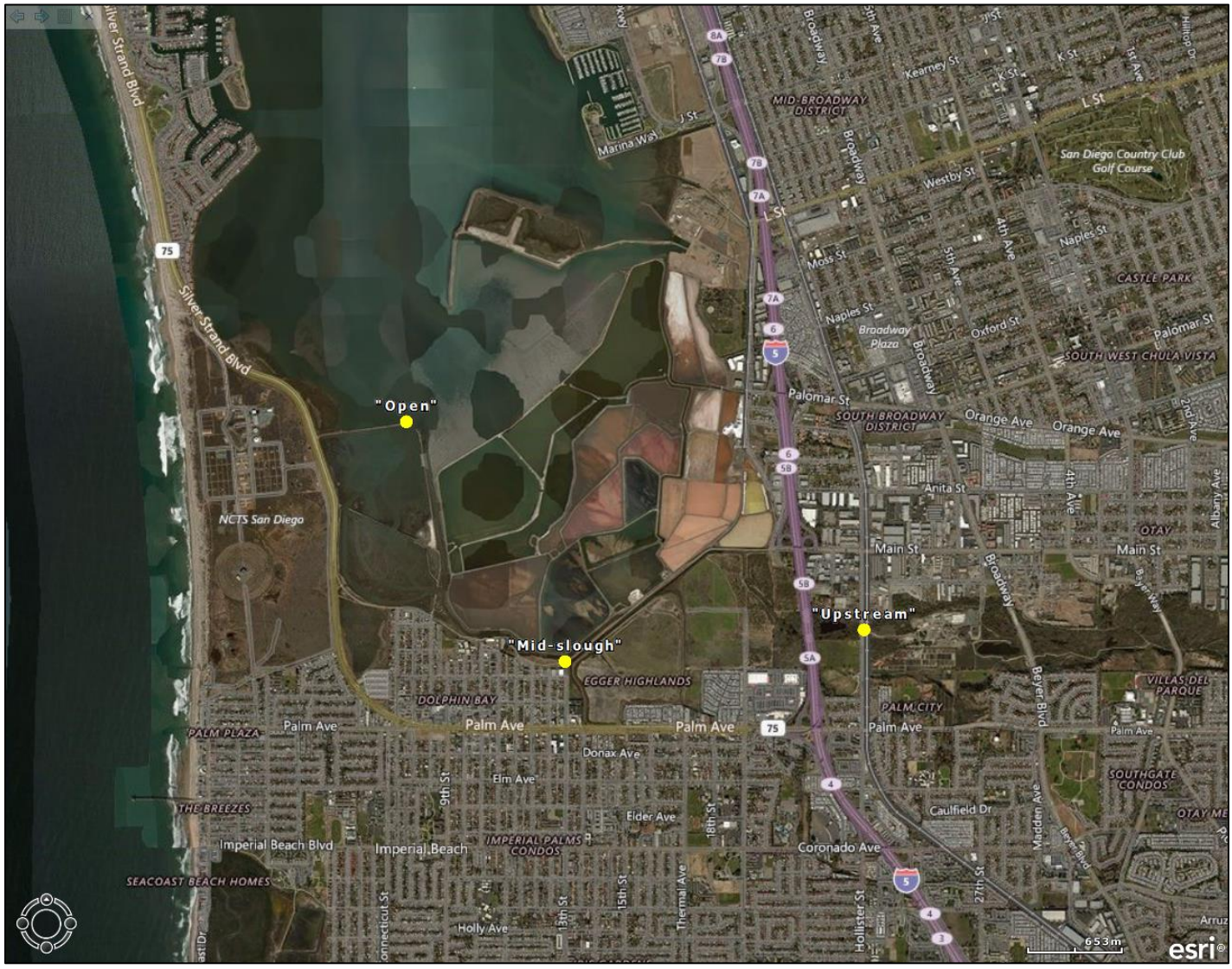

Figure 6. Otay River cyanotoxin sampling sites.

3.2 Sample Design

3.2.1. SPATT Bags and Water-Column Grab Sampling

Sampling efforts will be centered on the deployment, retrieval, and analyses of SPATT bags. Water-column grab samples will also be collected, but due to funding constraints, decisions on grab sample analyses may be made based on SPATT results.

Three (3) SPATT bags will be deployed at each site: one in the tributary, just upstream from the coastal discharge point (freshwater); one mid-slough (brackish); and one in the coastal receiving water (bay/lagoon). SPATT bags will be deployed for ten-day intervals during the bloom season. An additional set of SPATT bags will be co-deployed for a single, one-month interval to compare with the consecutive 10-day deployments during that same time period.

At each deployment or retrieval of SPATT, whole-water grab samples will be collected for analyses from the same general area. A portion of the whole-water sample will be filtered, and resulting fractional samples frozen, and archived. In the case of samples for microcystins analysis, the filter bearing particulate matter will be kept. In the case of cylindrospermopsin, the filtrate will be kept. If funds are sufficient and other data suggest value in analyzing them (e.g., if the corresponding SPATT bag was found to have absorbed toxin), they will be shipped for analyses. Analysis of the fractional sample material will provide information about the possibility that any detected toxin came from a planktonic HAB within the stream/lagoon/estuary. The whole-water grab samples will be analyzed from each sampling event, and the concentration of toxin will be compared with OEHHA action levels. This will facilitate an assessment of the severity/level-of-threat of the toxin concentration from the standpoint of human and animal health.

3.2.2 Optional Tissue Sampling

Tissue sampling may be conducted in the Sweetwater River watershed (open site), during a maximum of two sampling events, as funding and shellfish abundance allow. Targeted shellfish will be the California mussel *Mytilus californianus* and/or Pacific oyster (*Crassostrea gigas*). Shellfish are prolific non-selective filter feeders capable of accumulating high levels of toxins, such as microcystin, with little organismal impacts (Sipiä et al. 2001). Monitoring and research for mussels has shown them capable of high rates of toxin accumulation (over 300 ng/g, Sipiä et al. 2001, Kann et al. 2010). While rapid depuration of microcystin in clean water conditions has been well documented in the literature, studies to date have shown lower levels of microcystin may persist for weeks following lab dosing or large bloom events (Amorium and Vasconcelos 1999, Kanz 2008). We will target locations where shellfish are utilized for consumptive purposes. While *Mytilus* will be the focus of collections, the non-native

Pacific oyster may also be sampled due to its recent documented invasion of local estuaries (Crooks et al. 2015).

A SPATT bag will be deployed as close as feasible to each bed where shellfish tissue is sampled to make the comparison between observed toxin loads in the shellfish and toxin levels absorbed on the SPATT bag.

3.2.3 *Supporting Water Quality Data*

During each site visit for deployment and retrieval of the SPATT bags, *in situ* multi-meter (YSI 556 MPS) readings will be recorded. These parameters include:

1. Dissolved oxygen
2. Temperature
3. Specific conductance
4. pH
5. Salinity

A portion of the grab sample water will be used to measure alkalinity and turbidity in the field using a HACH Alkalinity Test Kit (Model AL-DT) and turbidity meter (La Motte 2020e Nephelometer), respectively.

In addition, grab sample water will be used to measure planktonic chlorophyll a. Field processing for chlorophyll *a* samples will include filtering 250 mL of grab sample water through a 0.7-μm Whatman glass microfiber (GF/F) filter. The filter will be folded in half, placed inside of a petri dish with cover, wrapped in foil, and frozen immediately. Chlorophyll *a* samples will be sent to SCCWRP for analysis using the method EPA 445.0. These analyses are not SWAMP-funded, and the results will not be submitted to the SWAMP database.

3.3 Cyanotoxin Sampling and Lab Analyses

SPATT

Retrieved SPATT bags will be visually examined for damage and placed in Ziploc bags, frozen immediately, and shipped to University of California, Santa Cruz (UCSC) for LC-MS/MS analyses. The analyses will include microcystins, anatoxin-*a*, cylindrospermopsin (presence/absence only), okadaic acid, domoic acid, and possibly nodularins.

Grab Samples

The whole water samples will be frozen immediately and shipped to WPCL for LC-ESI-MS/MS and ELISA analyses. The LC-ESI-MS/MS analyses will include microcystins, domoic acid, okadaic acid, and nodularin. ELISA analyses will include anatoxin-a and cylindrospermopsin. The archived filters (particulate fraction) that are collected at each sampling event will be analyzed for microcystins at WPCL using LC-ESI-MS/MS, and the filtrate for cylindrospermopsin, if deemed beneficial information for the assessment. Fractions will not be analyzed for anatoxin-a, due to the likelihood that any detected anatoxins-a in the whole-water samples will be in the dissolved phase.

TISSUE

Funding permitting, mussels will be collected whole (in shell) by hand utilizing pre-cleaned tools to provide at least 10 grams of tissue for analyses. Mussels will be handled in accordance with the SWAMP QAPP for (CITE QAPP) and shipped frozen to the California Department of Fish and Wildlife's Water Pollution Control Lab for dissection and analysis. Individual whole mussels will be lab dissected and composited by site for toxin analyses (microcystins, and potentially anatoxin-a and/or cylindrospermopsin, depending on the SPATT and/or grab sample results).

3.4 Data

3.4.1 Data Quality Evaluation and Data Reporting

Data quality evaluation and data reporting will follow the specifications in the SWAMP Quality Assurance Project Plan (QAPP). Quality control will include a 5% field duplicate level for all parameters. We do not anticipate needing additional special data quality evaluation or data reporting procedures.

3.4.2 Data Management

Data generated from the proposed monitoring plan will be stored in the SWAMP database. SCCWRP will be responsible for entering all field-generated data and UCSC (i.e., SPATT) data into the database, with the help of the SWAMP database management team, as needed. Results from the WPCL laboratory analyses will be uploaded into the SWAMP database by WPCL staff. It is expected that the data will also eventually be uploaded to the California Environmental Data Exchange Network (CEDEN).

4 Collaborations

The work described in this monitoring plan is part of a larger project funded by National Oceanic and Atmospheric Administration (NOAA), Monitoring and Event Response for Harmful Algal Blooms (MERHAB), titled “Improving tools for monitoring multiple HAB toxins at the land-sea interface in coastal California.” Principal investigators include:

- Meredith Howard, Senior Scientist, SCCWRP
- Raphael Kudela, Kudela Laboratory, University of California Santa Cruz
- David A. Caron, University of Southern California
- Keith Loftin, U.S. Geological Survey

The San Diego Regional Water Quality Control Board will be responsible for coordinating with local managers at the chosen study sites, collecting all of the samples and field measurements described in this monitoring plan, and shipping samples to the corresponding laboratories conducting the analyses.

The Kudela Laboratory of Biological Oceanography at the University of California, Santa Cruz, will be responsible for all laboratory activities involved in the cyanotoxin analyses of the SPATT bags used in this screening study.

The data analyses, assessment, and technical report writing will be managed by Meredith Howard (SCCWRP).

5 Deliverable Products

A technical report will be produced to present the findings of the screening effort outlined in this monitoring report in conjunction with the data collected for the larger MERHAB project (2015-2019). The MERHAB technical report will be finalized in 2019. A summary of our findings and those of the larger MERHAB project will be presented to the Board when available.

6 Project Schedule

Task 1 – Conduct reconnaissance site visits to determine appropriate salinities and SPATT deployment locations. Coordinate permissions for sample collection with site managers. Date: April 2016.

Task 2 – First SPATT deployment at each study site. Collect whole water grab samples. Process and ship samples to labs. Date: July 27, 2016.

Task 3 – Collect first SPATT and deploy second SPATT at each study site. Collect whole water grab samples. Process and ship samples to labs. Date: August 5, 2016.

Task 4 – Collect second SPATT and deploy third SPATT at each study site. Collect whole water grab samples. Process and ship samples to labs. Date: August 15, 2016.

Task 5 – Collect third SPATT and deploy forth SPATT at each study site. Collect whole water grab samples. Process and ship samples to labs. Date: August 24, 2016.

Task 6 – Collect forth SPATT and deploy fifth SPATT at each study site. Collect whole water grab samples. Process and ship samples to labs. Date: September 2, 2016.

Task 7 – Collect fifth SPATT at each study site. Collect whole water grab samples. Process and ship samples to labs. Date: September 12, 2016.

Task 8 – Laboratory analyses of samples (WPCL and UC Santa Cruz) and report to Region 9. Deliverable date: December 2016.

Task 9 – Enter data into SWAMP database (WPCL and Region 9). Deliverable date: February 2017.

Task 10 – Analyze data and write technical report. A report of the Region 9 data collected according to this monitoring plan will be presented in a technical report. Deliverable date: June 2017.

*The larger MERHAB technical report, written with the study collaborators and including data collected outside of Region 9, will be completed at the end of the “Improving tools

for monitoring multiple HAB toxins at the land-sea interface in coastal California” study. Deliverable date: mid-2019.

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8 Appendix

Notes on Tissue Concentration Levels:

Agency/Source	Organism	Toxin	Advice	Concentration ug/kg
State of California	Any	MC	1 six once serving week	Less than 26
State of Ohio	Fish Fillet	MC	Do Not Eat	28
Van Buynder et al. 2001	Fish	MC/Nodularan	Australia Health Alert Level	250
Van Buynder et al. 2001	Shrimp	MC/Nodularin	Australia Health Alert Level	1100
Van Buynder et al. 2001	Bivalves	MC/Nodularan	Australia Health Alert Level	1500
Germany	All	MC	Adults Do Not Eat	28
Germany	All	MC	Children Do Not Eat	7