

MARU STREAM BIOASSESSMENT: 2023 ANNUAL REPORT



Photo: Stonewall Creek, Sampled in 2023

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1. Introduction

This technical report was produced by the California Water Quality Control Board San Diego Region's (San Diego Water Board) Monitoring, Assessment and Research Unit (MARU). The MARU supports San Diego Water Board regulatory and non-regulatory programs by conducting water quality monitoring and data assessment, as well as by guiding and/or conducting scientific research to better protect and restore beneficial uses of the San Diego Region's waters. MARU regularly conducts stream bioassessment at up to 20 stream sites on an annual basis. In some years, the California Department of Fish and Wildlife (CDFW) conducts bioassessments in Region 9 under the statewide reference condition management program (RCMP).

This report is a summary of 23 bioassessments conducted in 2023 – 17 by MARU and 6 by CDFW. This report also includes a summary of environmental DNA (eDNA) sampling done by MARU in 2023, much of which was done in conjunction with bioassessment.

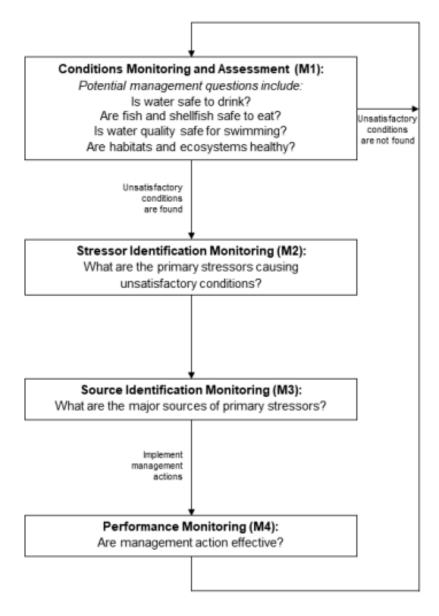
2. Bioassessment Background and Purpose

Biological assessment, or bioassessment, is the science of evaluating the health or integrity of an ecosystem by assessing the organisms that live within it. Bioassessment allows for the San Diego Water Board to better protect and restore waters by facilitating a broader evaluation of the cumulative effects of stressors beyond analyzing for individual chemicals. The use of bioassessment is central to the the Clean Water Act (CWA), as the CWA goal is to protect and restore the biological integrity of waters (33 U.S. Code § 1251). In addition, the Porter-Cologne Act also relies on the use of bioassessment to protect water quality, which is defined to include chemical, physical, biological, bacteriological, and radiological properties (CWC section 13050). Unlike traditional chemistry-based monitoring, which provides only limited information about a relatively narrow portion of the environment at a discrete point in time, bioassessment can account for living organisms exposed to multiple chemicals and other stressors such as altered habitats and changes in water-flow patterns over extended time periods. Bioassessment therefore provides a far more integrated view of the condition of an aquatic ecosystem. Bioassessment is also more closely tied to an environmental manager's end-goal focus on ecosystem protection and serves as an important way to monitor and protect populations of endangered species and fisheries.

Biological assessment is a critical component of the San Diego Water Board's monitoring and assessment framework ("Monitoring Framework", Figure 1). Biological assessment can and should be used to conduct waterbody-oriented monitoring and assessment, specifically to:

- 1) Answer the M1 question: "Are habitats and ecosystems healthy?"
- 2) Guide the M2 and M3 Stressor Identification Process, and
- 3) Evaluate M4 performance monitoring to document success.

Figure 1. Water Body-Oriented Monitoring and Assessment



2.1. Stream Bioassessment Background

While bioassessment can and should be used to assess the condition of all surface waterbody types, this report focuses on bioassessment done on perennial and intermittent wadeable streams. The State of California has been conducting bioassessment on wadeable streams in the San Diego region since the 1990s, with early sampling conducted by the San Diego Water Board, the State Water Board's Surface Water Ambient Monitoring Program (SWAMP), and USEPA.

The San Diego Water Board, in December of 2020, adopted biological objectives for streams using stream bioassessment, specifically the California Stream Condition Index (CSCI, see Mazor et al. 2016), discussed in section 2.2.1 below. Stream bioassessment has been used in multiple San Diego Water Board program areas for over two decades, including municipal stormwater, CWA assessment under sections 305(b) and 303(d), ambient monitoring, CWA Section 401 water quality certifications, and waste discharge requirements.

SWAMP has developed standard operating procedures (SOPs) for bioassessment field sampling, laboratory identification of specimens, quality assurance/control, data management, and reporting. These SOPs are on the SWAMP bioassessment website: https://www.waterboards.ca.gov/water issues/programs/swamp/bioassessment/

Stream bioassessment currently includes measurement of:

- Benthic macroinvertebrates (BMI)
- Benthic algae: diatoms and soft algae, including cyanobacteria
- Physical habitat: instream and riparian
- Water chemistry
- Flow

All San Diego Water Board bioassessment data is available to the public via the California Environmental Data Exchange Network (<u>CEDEN</u>) database.

2.2. Indices of Integrity and Water Quality Objectives

2.2.1. Biological Indices

To assess bioassessment data, biological scoring tools are needed to translate complex species identification information into a stream condition determination. The development of these biological scoring tools, often referred to as indices or metrics of biological integrity, has been on-going since the 1990s, with various regional indices developed throughout the State. In 2015, the State of California released the peer-reviewed statewide CSCI (Mazor et al. 2016) for assessing the biological condition of wadeable streams throughout the State based on benthic macroinvertebrates. The CSCI uses a combined-reference-site approach to determine the site-specific benthic community expected to be present at any sampled site. In 2020, the peer-reviewed statewide Algal Stream Condition Index (ASCI, Theroux et al. 2020) was released. The ASCI uses an approach that mirrors the CSCI, except multiple indices were developed, with a diatom-specific index (d-ASCI) and combined "hybrid" diatom/soft algae index (h-ASCI).

Both the CSCI and ASCI are indices that score on a scale of 0-1. A score of 1 indicates that the condition of a sampled stream site is equivalent to expected conditions at similar reference sites, while a lower score shows deviation from expected condition, or degradation. The CSCI and ASCI publications calculated thresholds for scores, below which a site could be considered impaired (Table 1). The CSCI threshold of 0.79 was adopted by the San Diego Water Board as a water quality objective in December 2020.

2.2.2. Physical Habitat Indices

In addition to biological condition indices, the State of California developed an Index of Physical Habitat Integrity (IPI) in 2018 that relies on in-stream measures of physical habitat. Like the CSCI and ASCI, the IPI uses a reference approach to determine what a site's physical habitat expectations are. The IPI is also scored on a 0-1 scale. The IPI should be used for provisional informational purposes only, as the State has identified some needed improvements for the IPI to better reflect conditions and impacts and prevent Type I and II errors.

Table 1. Impairment Thresholds for Bioassessment Indices

Index	Threshold
CSCI	0.79
d-ASCI	0.86
h-ASCI	0.86
IPI	0.84

2.2.3. Chemical and Physical Water Quality Objectives

While stream bioassessment focuses on the organisms living in the stream, the SWAMP SOP requires a minimum level of sampling for chemical (e.g. nitrogen) and physical (e.g. turbidity) parameters. These parameters, which are collected as grab samples, simply provide a baseline assessment of water quality at the time of sampling. They do not include all potential stressors or matrices (e.g. sediment), though additional parameters and matrices can be added to sampling efforts and are noted in this report where conducted. Data for all sites are available on the (CEDEN) database.

Table 2. Chemical and Physical Water Quality Objectives and Impairment Thresholds

Table 2. Chemical and Physical Water Quali	ty Objectives and impairment Thresholds
Parameter	Threshold
Temperature	Narrative (Deg C)
Dissolved Oxygen	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
рН	6.5-8.5
Total Nitrogen	1.0 mg/L
Total Phosphorus	0.1 mg/L
Turbidity	20 NTU
Chloride	^b waterbody-specific mg/L, ^c 230 & 860 mg/L
Sulfate	^b waterbody-specific mg/L
Conductivity	^a NA uS/cm
Alkalinity	^a NA mg/L
Silica	^a NA mg/L
Dissolved Organic Carbon	^a NA mg/L
Benthic Ash Free Dry Mass (AFDM)	^d 25 g/m ²
Benthic Chlorophyll a (Chl-a)	^d 44 mg/m ²
Benthic Carbon:Nitrogen Molar Ratio (C:N)	^a NA

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively. Assumes an association with sodium.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

2.3. Supplemental Sampling

The San Diego Water Board often will conduct supplemental sampling or special studies at bioassessment sites. This allows for efficiencies in testing new methods, takes advantage of existing sampling of reference sites, or is done to support the work of sister agencies (e.g. California Department of Fish and Wildlife). In 2023, MARU conducted several types of supplemental sampling as described below.

2.3.1. Molecular benthic algal analysis

Molecular benthic algal analysis is a Region 9 funded SWAMP project to evaluate the potential transition from traditional microscope-based benthic algae analysis to a molecular DNA-based approach. For this analysis, there is no change in the SWAMP collection method for benthic algae; only a small subsample of the collected algal composite is needed (Figure 2). Molecular algal analysis is being done by SWAMP and partners statewide, with the San Diego Water Board funding the analysis and development of independent or supplemental indices of biological integrity. MARU has conducted this sampling since 2017. In 2023, samples for molecular algal analysis were collected at all sites.

Figure 2. A filter for benthic algal molecular analysis. Typically, 15-25 ml of the collected algal composite is filtered, and three replicates are prepared.



2.3.2. Environmental DNA (eDNA)

Sampling for eDNA was done at the 17 bioassessment sites plus nine other sites using pre-packaged metabarcoding kits from Jonah Ventures provided by the statewide SWAMP eDNA Metabarcoding Monitoring and Analysis Project (SeMMAP). In brief, eDNA sampling using these kits involved the filtration of 200-400 ml of stream water through a DNA filter, which was then mailed to a lab for analysis. Metabarcoding does not target single species but looks at the overall community.

In addition, targeted species eDNA sampling was done at 16 sites (eight bioassessment and eight others) using a Smith-Root pump device in the field and at 14 sites (eight bioassessment and six others) using vacuum filtration of collected water in the lab. Concurrent use of the Jonah Ventures, Smith-Root, and lab filtration methods allows for comparison of sensitivity and usefulness among the three. Notable eDNA results for higher-trophic level targeted species are presented in the site summaries and section 5.7.

Figure 3. Collection of eDNA samples using a Smith-Root pump (left) and Jonah Ventures kit (right). Water was also collected for vacuum filtration in the lab.



2.3.3. Particulate carbon and nitrogen (PCN)

Analysis of particulate carbon and nitrogen was done for the second year, having first sampled it in 2022. PCN analysis provides insight into the carbon and nitrogen sources typical of a given waterbody. Specifically, the Carbon:Nitrogen ratio (C:N) tells us about a stream's relative proportions of carbon-rich sources (leaves) or nitrogen-rich sources (algae, animal waste). A higher C:N (> 16) indicates a stream is dominated by terrestrial carbon sources, whereas a lower C:N (< 8) indicates a stream is dominated by algal sources or external nitrogen loading. A C:N between 8 and 16 indicates a mix of the two. PCN is being explored by SWAMP as a potential future addition to, or replacement of, the AFDM parameter. PCN analysis requires no change in the SWAMP collection method for benthic algae; only a small subsample of the collected algal composite is needed. In 2023, two filters for each site were analyzed to get a measure of variability, and the molar C:N ratio for both is given in the site summaries below.

2.3.4. Cyanotoxins

Six bioassessment sites were sampled for cyanotoxins using Solid Phase Adsorption Toxin Tracking (SPATT) samplers. A SPATT sampler is a small packet containing resin that absorbs toxins from the water (Figure 4). SPATT samplers were deployed on the day of bioassessment and retrieved seven to nine days later. SPATT samplers were analyzed for a suite of cyanotoxins: microcystin/nodularin, anatoxin-a, cylindrospermopsin, and saxitoxin. In addition, an aliquot of the bioassessment algal composite and a targeted mat sample collected from the bioassessment reach were analyzed for the cyanotoxin suite. This was a Region 9 special study done under the State Water Board's freshwater harmful algal bloom (FHAB) program.

Figure 4. SPATT sampler deployment (left) and retrieval (right). To deploy, a SPATT sampler was sandwiched between two slotted mesh pots and zip tied to a scuba weight.





2.3.5. Bacteria and HF183

E.coli, total coliform, and HF183 were sampled at two sites in 2023 to test for the possible presence of human fecal contamination. While *E. coli* and total coliform can have human or non-human sources, HF183 is a gene sequence unique to human-associated *Bacteroides* bacteria and is specific to human fecal sources. The IDEXX Quantitray method was used to quantify *E.coli* and total coliform, and droplet digital polymerase chain reaction (ddPCR) was used for HF183.

2.3.6. Laboratories Used for Sample Analyses

The laboratories used to process supplemental and standard bioassessment samples were: CSU Chico (BMI identification); CSU San Marcos (Algal identification); Babcock Laboratories (Chemistry); SCCWRP (Molecular algal analyses, eDNA); Jonah Ventures (eDNA); UCSB Marine Science Institute (PCN), Bend Genetics (cyanotoxins); Alpha (bacteria); and WSP (HF183).

3. Bioassessment Site Types

Stream bioassessment has been conducted in the San Diego Region for over two decades, with sampling at over 550 sites (**Error! Not a valid bookmark self-reference.**) and with many sites sampled multiple times (1,700+ total samples). Bioassessment samples have been collected by multiple entities and include a mix of site types: reference, non-reference, targeted, probabilistic, trend, and investigation sites. MARU typically conducts stream bioassessment at the following site types:

- 1) Reference Condition Management Program/Reference Sites
- 2) Water Board Program Request Sites
- 3) Causal/Protective Assessment Sites
- 4) Trend Assessment Sites
- 5) Coverage Expansion Sites

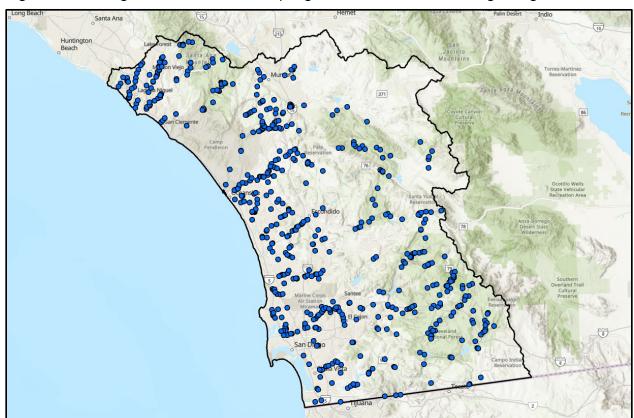


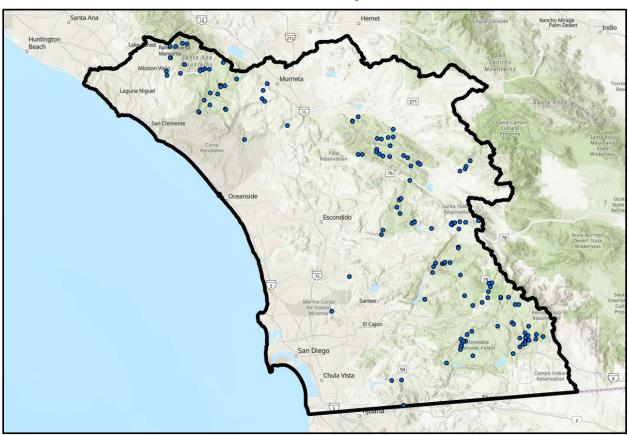
Figure 5. Existing Bioassessment Sampling Sites within the San Diego Region.

3.1. <u>Reference Condition Management Program</u> Sites/Reference Sites

The SWAMP program conducts long-term monitoring at a set of sites that meet GIS reference screening criteria (Ode et al. 2016) as part of the state's <u>reference condition management program</u> (RCMP). This network of sites, and their on-going monitoring, is critical for establishing reference conditions, which are "healthy" or "biologically intact" conditions in streams with little to no upstream anthropogenic stressors. In addition, the RCMP helps assess longer-term impacts to California's streams from large-scale changes, such as those associated with climate change.

The San Diego Water Board supports the RCMP in two ways. First, MARU conducts repeat sampling at long-term RCMP sites. Second, MARU identifies new reference sites to add to the potential State reference site pool (**Error! Not a valid bookmark self-reference.**6, also section 3.5).

Figure 6. Bioassessment Sites in the San Diego Region that meet reference screens. Reference sites are those that meet GIS screening criteria under Ode et al. 2016.



3.2. Program Request Sites

Program request sites are those sites that have been identified by internal San Diego Water Board staff/programs as a priority for monitoring to determine condition (M1 of the Monitoring Framework). Monitoring may be done to investigate discharge impacts, determine the success of mitigation measures, monitor trends for CWA section 305(b)/303(d) assessment, etc. In addition, some program request sites are requests from partner agencies who manage sensitive native species, such as the California Department of Fish and Wildlife (CDFW), United States Geologic Survey (USGS), and United States Fish and Wildlife Service (USFWS), and from partner nonprofit organizations such as the San Diego River Park Foundation.

3.3. Causal/Protective Assessment Sites

For stream bioassessment sites that score poorly, determining the likely stressor(s) causing the poor condition allows for actions to be taken to improve biological condition (M2 of the Monitoring Framework). The process for investigating and determining likely stressors is referred to as "causal assessment." This process can also be used to assess whether sites scoring above an index threshold might be at risk from stressors, especially for sites close to the threshold ("protective assessment"). MARU may conduct stream bioassessment revisits, combined with extra stressor-related sampling, to support causal and protective assessment efforts.

3.4. Trend Sites

Trend sites are sites that have been previously sampled and are targeted for resampling due to the time since prior sampling or a change in upstream development or management, or for regular monitoring associated with other factors (e.g. sensitive species). These sampling events are typically M1 or M4 of the Monitoring Framework.

3.5. Coverage Expansion Sites

While bioassessment has been conducted at more than 550 sites in the San Diego Region (Figure 5), many streams have never been bioassessed. MARU is working to conduct stream bioassessment at previously unsampled streams (M1 of the Monitoring Framework). Doing so helps clarify the condition of streams across the region, identify new reference sites, and determine baseline condition for anti-degradation purposes (e.g. in the event of a spill).

4. Sites Sampled in 2023

4.1. Site Type and Location

In 2023, MARU conducted bioassessment at 17 sites from May to September, all but one completed by mid-July (Table3). CDFW conducted bioassessment at an additional six sites in May (Table 4). MARU collected eDNA samples from all 17 bioassessment sites, plus an additional nine sites (Table 5). Figure 7 shows the locations of MARU's bioassessment sites, CDFW's bioassessment sites, and MARU's eDNA-only sites.

Table 3. Bioassessment sites sampled by MARU in 2023

Site Type	SWAMP Code	Stream	Latitude	Longitude	Date
RCMP	902NP9CWC	Cottonwood Creek Temecula	33.4205	-116.86164	5/25
RCMP	903PCPMPx	Pauma Creek Palomar Mountain Park	33.3484	-116.91457	7/11
RCMP	903FCPSPx	French Creek	33.3517	-116.91387	6/22
RCMP	903NP9UAC	Upper Agua Caliente	33.3204	-116.62265	6/26
RCMP	903ACPCT1	Agua Caliente at PCT	33.2961	-116.63860	6/29
RCMP	909SWCASR	Stonewall Creek	32.9421	-116.55409	6/8
RCMP	911NP9EPC	Espinosa Creek	32.7449	-116.6495	6/14
Program Request	901SJOF1x	San Juan Creek above Ortega Falls	33.6163	-117.42686	6/1
Program Request	902SMAS1x	Santa Margarita River Arroyo Seco	33.4567	-116.97013	5/31
Program Request	902SCSCRx	Sandia Creek	33.4249	-117.24942	7/12
Program Request	554NMCCVM	Ninemile Creek - Casa Vieja Meadows	36.1990	-118.26529	9/29

Causal	910OTJMC4	Jamul Creek	32.6376	-116.88406	5/18
Trend	911TJPVC1	Pine Valley Creek	32.8359	-116.54290	6/27
Coverage Expansion	901NP9LAN	Upper Los Alamos Canyon, North Fork	33.5502	-117.35281	6/19
Coverage Expansion	902R9CCC1	Cole Canyon Creek	33.5575	-117.25437	6/7
Coverage Expansion	903DCBDCP	Doane Creek	33.3419	-116.90434	7/6
Coverage Expansion	909HPCASR	Harper Creek	32.9321	-116.54427	6/20

Table 4. Bioassessment sites in Region 9 sampled by CDFW in 2023

Site Type	SWAMP Code	Stream	Latitude	Longitude	Date
RCMP	909CCCSPx	Cold Stream Cuyamaca State Park	32.94023	-116.565529	5/10
RCMP	909JQCASR	Juaquapin Creek above Sweetwater River	32.91468	-116.56562	5/8
RCMP	909JPCH79	Japacha Creek above Hwy 79	32.91849	-116.57176	5/8
RCMP	911TCCTCx	Troy Canyon Creek	32.80778	-116.44	5/8
RCMP	911TJLCC2	Long Canyon Creek	32.77836	-116.44337	5/9
RCMP	911TJKC1x	Kitchen Creek above Kitchen Creek Road crossing	32.76012	-116.45123	5/10

Table 5. Additional sites sampled for eDNA by MARU in 2023

Site Type	SWAMP Code	Stream	Latitude	Longitude	Date
eDNA	901SMCBTC	San Mateo Creek 200 meters below Tenaja Canyon Trail crossing	33.53053	-117.41112	11/21
eDNA	901SMCUPL	San Mateo Creek Trail crossing at upper pools	33.53646	-117.40238	11/21
eDNA	901SMCABP	San Mateo Creek above upper pools	33.53988	-117.40009	11/21
eDNA	901SMCBTF	San Mateo Creek below Tenaja Falls Creek	33.54844	-117.39608	11/21
eDNA	901LACUTF	Los Alamos Creek above Tenaja Falls Creek	33.55099	-117.39440	11/21
eDNA	909SSWR01	Sweetwater River 1	32.90942	-116.57633	7/5
eDNA	910RJTPND	Rancho Jamul Turtle Pond	32.67707	-116.865111	9/20
eDNA	554UBTNMC	Unnamed Blackrock Tributary to Ninemile Creek	36.19674	-118.269604	9/29
eDNA	554SFKBLC	South Fork of the Kern River below Lost Creek	36.06023	-118.131186	9/30

Rancho Mirage 1 Hemet Indio Santa Ana Huntington Lake Forest Beach Jacinto Santa Ana Mountains Mountales Terres-Martines Murrieta Reservation Laguna Niguel San Clemente Ocotillo Wells State Vehicular Oceanside Recreation Area Escondido Southern Overland Trail Santee Air Station El Cajon San Diego 2023 Site Type Campo India 0 CDFW Reservation Chula Vista

Tecate

and the GIS User Community

(BS). NASAT (SA LUSGS, Sources: Esri, TomTom, Garmin, FAO, NOAA, USGS, © OpenStreetMap contributors,

Figure 7. MARU and CDFW 2023 Sampling Sites. Note map does not include R5 sites.

R9 Bioassessment

☆ eDNA Only

4.2. Overview of Results

The low and high values recorded for indices and other parameters are below. All streams were sampled at base flow condition, and all were narrow (1.1–6.7 m wetted width, on average) and shallow (13–37 cm deep, on average). Slope ranged from a low of 1% (Jamul Creek) to a high of 15% (Cottonwood Creek Temecula) over the 150 m sampled reach.

Range of index scores from the 2023 bioassessed streams

Index	Results	Threshold
CSCI	0.73 to 1.14	0.79
d-ASCI	0.55 to 1.20	0.86
h-ASCI	0.55 to 1.29	0.86
IPI	0.98 to 1.22	0.84

Range of chemical and physical water quality results from the 2023 bioassessed streams

Parameter	Results		S	Threshold
Temperature	7.9	to	18.5	Narrative (Deg C)
Dissolved Oxygen	8.4	to	10.2	5.0 (WARM BU), 6.0 (COLD BU) mg/L
pН	7.2	to	8.5	6.5-8.5
Total Nitrogen	ND ⁺	to	5.8	1.0 mg/L
Total Phosphorus	ND ⁺	to	0.8	0.1 mg/L
Turbidity	0.1	to	3.3	20 NTU
Chloride	10	to	250	^b waterbody-specific mg/L, ^c 230 & 860 mg/L
Sulfate	8	to	370	^b waterbody-specific mg/L
Conductivity	88	to	1783	^a NA uS/cm
Alkalinity	34	to	197	^a NA mg/L
Silica	ND	to	56	^a NA mg/L
Diss. Org. Carbon	3	to	16	^a NA mg/L
Benthic AFDM	8	to	139	^d 25 g/m ²
Benthic Chl-a	8	to	252	^d 44 mg/m ²
Benthic C:N	9.9	to	18.5	^a NA

^{*}Non-detect a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

Out of interest, the number of BMI and algal taxa recorded from each site was compiled by counting taxa in CEDEN's Final ID column. For BMI, Final IDs are at various levels (species, genus, family, group), and "notable taxa" for this report are those in the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT taxa). EPT taxa are relatively sensitive, and their presence is indicative of good stream condition. Each sample from which BMI taxa were recorded was a composite of 11 subsamples (30 x 30 cm), or a total of ~1 m² of stream bed sampled.

For algae, Final IDs are at the level of species or genus, and "sensitive taxa" for this report are those with a California Biological Condition Gradient (BCG) rating of 1 or 2 (Paul et al. 2020). The presence of sensitive algae is indicative of good stream health. Each sample from which algal taxa were recorded was a composite of 11 subsamples (5.3 or 12.6 cm²), or a total of ~100 cm² of stream bed sampled (actual total areas were between 95 cm² and 131 cm²).

Range of BMI and algal taxa recorded from 2023 bioassessed streams

	# Taxa	# Notable or Sensitive Taxa
BMI	19 to 74	3 to 28
Algae	43 to 80	0 to 12

5. Site Summaries

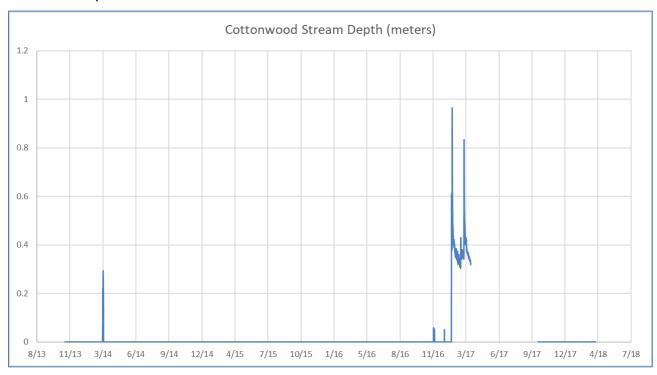
5.1. RCMP/Reference Sites

5.1.1. 902NP9CWC: Cottonwood Creek Temecula



Cottonwood Creek is a reference site that was selected for sampling by R9, beginning in 2013, due to its variable intermittent flow regime. This site, which drains the northern aspect of Palomar Mountain, had a water level logger deployed from 2013 – 2018, though flows were insufficient for conducting bioassessment until 2017 due to drought conditions. It is typically sampled in years with above-average rainfall (2017, 2019, 2023). Drought years typically have insufficient flow to sample.

Figure 8. Cottonwood Creek water level from 2013-2018. Logger results indicated mainly dry conditions during this period. Note the logger experienced a relaunch issue in 2017 that prevented data collection.



CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. Algal scores were mixed, but an h-ASCI above the threshold indicated a likely intact algal community.

Index	Result	Threshold
CSCI	0.88	0.79
d-ASCI	0.83	0.86
h-ASCI	0.95	0.86
IPI	1.09	0.84

Chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan Sulfate was above the drinking water objective of 300 mg/L; there are no aquatic life criteria for sulfate.

incre are no aquatio line criteria for sunate.			
Parameter	Result	Threshold	
Temperature	17.0	Narrative (Deg C)	
Dissolved Oxygen	9.0	5.0 (WARM BU) or 6.0 (COLD BU) mg/L	
pH	8.4	6.5-8.5	
Total Nitrogen	0.3	1.0 mg/L	
Total Phosphorus	0.03	0.1 mg/L	
Turbidity	0.7	20 NTU	
Chloride	51	^b waterbody-specific mg/L; ^c 230 & 860 mg/L	

Sulfate	340	^b waterbody-specific mg/L
Conductivity	1,099	^a NA uS/cm
Alkalinity	169	^a NA mg/L
Silica	42	^a NA mg/L
Dissolved Organic Carbon	5.4	^a NA mg/L
Benthic AFDM	19.6	^d 25 g/m ²
Benthic Chl-a	20.3	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	14.1, 14.1	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	43	6
Algae	59	5

Notable BMI:

- Ephemeroptera: Centroptilum; Baetis; Baetis adonis; Baetis tricaudatus; Callibaetis;
- Plecoptera: none
- Trichoptera: Rhyacophila; Brachycentridae

Sensitive algae:

- Diatoms: Epithemia turgida; Fragilariforma virescens; Meridion circulare
- Cyanobacteria: Calothrix parietina
- Other: Spirogyra varians

eDNA

Method	Detections
Jonah Ventures	none
Smith-Root	NA
Vacuum filtration	NA

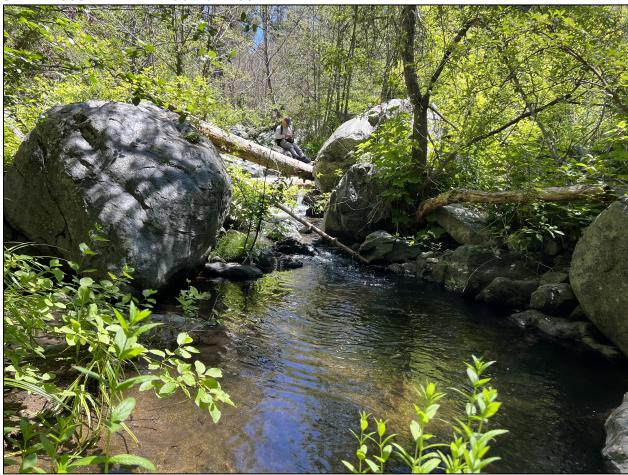
^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

5.1.2. 903PCPMPx: Pauma Creek



Pauma Creek is a RCMP site in Palomar State Park and exhibits perennial flows. Pauma Creek historically contained an isolated population of southern California steelhead, though more recent genetic analysis have documented genetic mixing with planted rainbow trout from upstream Doane Pond, which overtops during high flows.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. Algal scores were mixed, but an h-ASCI above the threshold indicated a likely intact algal community.

Index	Result	Threshold
CSCI	1.10	0.79
d-ASCI	0.77	0.86
h-ASCI	0.95	0.86
IPI	1.26	0.84

Chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan.

Parameter	Result	Threshold
Temperature	13.6	Narrative (Deg C)
Dissolved Oxygen	9.0	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
рН	7.2	6.5-8.5
Total Nitrogen	0.11	1.0 mg/L
Total Phosphorus	0.03	0.1 mg/L
Turbidity	1.0	20 NTU
Chloride	11	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	17	^b waterbody-specific mg/L
Conductivity	195	^a NA uS/cm
Alkalinity	-	^a NA mg/L
Silica	31	^a NA mg/L
Dissolved Organic Carbon	14	^a NA mg/L
Benthic AFDM	32.2	^d 25 g/m ²
Benthic Chl-a	17.4	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	16.3, 17.7	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	49	21
Algae	47	9

Notable BMI:

- Ephemeroptera: Baetis; Baetis tricaudatus; Callibaetis; Centroptilum; Epeorus; Ironodes; Leptophlebiidae; Matriella teresa; Paraleptophlebia
- Plecoptera: Malenka; Nemouridae; Sweltsa; Zapada cinctipes
- Trichoptera: *Hydropsyche;* Hydropsychidae; *Lepidostoma; Neophylax; Parapsyche; Psychoglypha; Rhyacophila betteni* group; *Tinodes*

Sensitive algae:

- Diatoms: Achnanthidium rivulare; Caloneis schumanniana; Gomphonema kobayasii; Planothidium cf haynaldii; Reimeria sinuata
- Cyanobacteria: none
- Other: *Paralemanea californica; Spirogyra varians; Zygnema* aplanosporum; *Zygnema* sp 1

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

eDNA

Method	Detections	
Jonah Ventures	 Green Sunfish (Lepomis cyanellus); Black Bass (Micropterus); Pacific salmon and trout (Oncorhynchus) 	
	Chickadees (<i>Poecile</i>)	
Smith-Root	Oncorhynchus mykiss (KF55)	
Vacuum filtration	Oncorhynchus mykiss (KF55)	

Four small trout were observed while sampling (young-of-year), confirming the eDNA results.

Figure 89. A banana slug (*Ariolimax*) observed during sampling along Pauma Creek. The Palomar population may be an undescribed species.

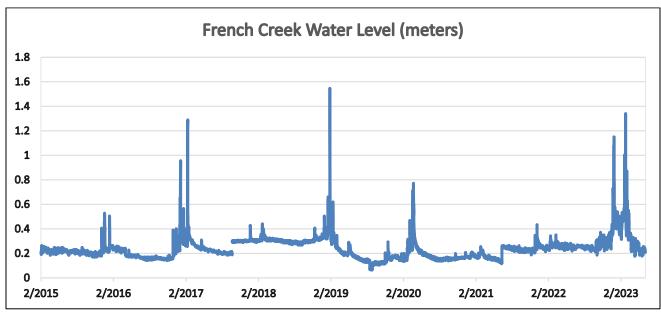


5.1.3. 903FCPSPx: French Creek



French Creek is a long-term RCMP site located within Palomar Mountain State Park. French Creek has had a water level logger deployed since 2015 and has exhibited perennial flow, even during drought years.

Figure 10. Water Level in French Creek from 2015 -2023. Logger results confirmed perennial flow during that period.



All scores were above the thresholds indicating biologically intact benthic macroinvertebrate and algal communities, as well as intact physical habitat.

Index	Result	Threshold
CSCI	1.05	0.79
d-ASCI	1.04	0.86
h-ASCI	1.02	0.86
IPI	1.22	0.84

Chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan.

Parameter	Result	Threshold
Temperature	13.0	Narrative (Deg C)
Dissolved Oxygen	8.5	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
рН	7.8	6.5-8.5
Total Nitrogen	0.18	1.0 mg/L
Total Phosphorus	0.02	0.1 mg/L
Turbidity	1.6	20 NTU
Chloride	10	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	18	^b waterbody-specific mg/L
Conductivity	168	^a NA uS/cm
Alkalinity	34	^a NA mg/L
Silica	32	^a NA mg/L
Dissolved Organic Carbon	13	^a NA mg/L
Benthic AFDM	23.8	^d 25 g/m ²
Benthic Chl-a	32.9	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	16.2, 16.0	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	60	25
Algae	74	11

Notable BMI:

- Ephemeroptera: Baetis; Baetis tricaudatus; Callibaetis; Leptophlebiidae; Paraleptophlebia
- Plecoptera: Chloroperlidae; Isoperla denningi; Malenka; Nemouridae;
- Trichoptera: Agapetus; Glossosomatidae; Hesperophylax; Hydropsyche;
 Hydropsychidae; Hydroptila; Lepidostoma; Neophylax; Parapsyche;
 Psychoglypha; Rhyacophila; Rhyacophila betteni group; Tinodes; Trichoptera;
 Wormaldia

Sensitive algae:

- Diatoms: Epithemia sorex; Epithemia turgida; Meridion circulare; Planothidium cf haynaldii; Reimeria sinuata
- Cyanobacteria: Tolypothrix distorta
- Other: Chaetophora elegans; Paralemanea californica; Zygnema aplanosporum; Zygnema sp 1; Zygnema sp 2

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

eDNA

Method	Detections
Jonah Ventures	 Bluegill (Lepomis macrochirus); Black Bass (Micropterus);
	Pacific salmon and trout (Oncorhynchus)
	 Ducks (Anatidae)
Smith-Root	Oncorhynchus mykiss (KF55)
Vacuum filtration	Oncorhynchus mykiss (KF55)

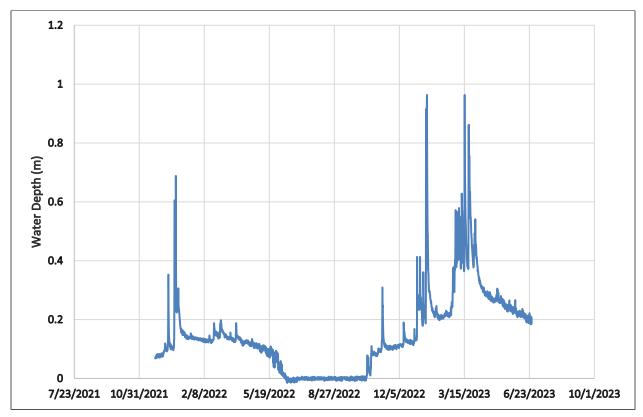
A frog with yellow on its back legs was observed while sampling (identification uncertain), though eDNA did not detect *Rana muscosa* or *Rana draytonii*.

5.1.4. 903NP9UAC: Upper Agua Caliente



Upper Agua Caliente Creek is a long term RCMP site within the Cleveland National Forest. Site visits and water level loggers have documented that Upper Agua Caliente Creek exhibits perennial flow. For 2022 to 2023, the logger was deployed to a new stream location, but it did not accurately depict water level, showing the stream as dry from June to October (Figure 11). It was moved to a new position in 2024 and deployed with a trail camera to test the feasibility of using photographic images to document flow conditions.

Figure 911. Water level at Upper Agua Caliente. This site has been confirmed perennial during drought through site visits, but accurate deployment of water level loggers has proved difficult due to a predominance of bedrock and scouring flows during storm events.



All scores were above the thresholds indicating biologically intact benthic macroinvertebrate and algal communities, as well as intact physical habitat.

Index	Result	Threshold
CSCI	1.03	0.79
d-ASCI	1.20	0.86
h-ASCI	1.29	0.86
IPI	1.22	0.84

Chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan.

Parameter	Result	Threshold
Temperature	14.1	Narrative (Deg C)
Dissolved Oxygen	9.2	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
рН	8.1	6.5-8.5
Total Nitrogen	0.33	1.0 mg/L
Total Phosphorus	ND	0.1 mg/L
Turbidity	0.8	20 NTU
Chloride	27	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	52	^b waterbody-specific mg/L
Conductivity	374	^a NA uS/cm
Alkalinity	80	^a NA mg/L
Silica	49	^a NA mg/L
Dissolved Organic Carbon	16	^a NA mg/L
Benthic AFDM	8.4	^d 25 g/m ²
Benthic Chl-a	10.4	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	13.8, 13.2	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	58	15
Algae	64	12

Notable BMI:

- Ephemeroptera: Baetis; Baetis adonis; Baetis tricaudatus; Centroptilum; Diphetor hageni; Drunella coloradensis; Ironodes
- Plecoptera: Isoperla; Malenka;
- Trichoptera: *Hydropsyche*; Hydropsychidae; *Ochrotrichia*; *Lepidostoma*; *Micrasema*

Sensitive algae:

- Diatoms: Epithemia adnata; Epithemia sorex; Epithemia turgida; Gomphonema pumilum; Gomphonema subclavatum; Planothidium cf haynaldii
- Cyanobacteria: Calothrix marchica; Chamaesiphon polymorphus
- Other: *Paralemanea californica; Spirogyra weberi; Zygnema* aplanosporum; *Zygnema* sp 1

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

eDNA

Method	Detections	
Jonah Ventures	 Coho Salmon (Oncorhynchus kisutch) 	
	 Brush Rabbit (Sylvilagus bachmani) 	
Smith-Root	None detected	
Vacuum filtration	None detected	

California tree frogs were observed while sampling, and a baby red diamond rattlesnake was seen on the trail.

Figure 1210. Red diamond rattlesnake along the Pacific Crest Trail.

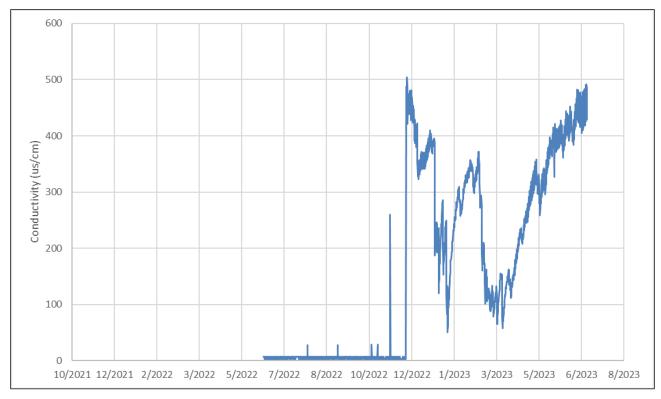


5.1.5. 903ACPCT1: Agua Caliente at PCT



Lower Agua Caliente Creek is a long-term RCMP site located approximately 4 stream kilometers downstream of Upper Agua Caliente. Unlike Upper Agua Caliente, this stream stretch exhibits intermittent flow. This stream has water level and specific conductance loggers deployed. The specific conductance results show the stream began flowing around December 2022 and had sustained flows into early summer. The logger was downloaded on the date of sampling (6/29/2023).

Figure 13. Conductivity logger results for Lower Agua Caliente Creek. Results near zero indicate a dry, no-flow condition.



CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. Algal scores were mixed, but an h-ASCI above the threshold indicated a likely intact algal community.

Index	Result	Threshold
CSCI	1.09	0.79
d-ASCI	0.84	0.86
h-ASCI	1.00	0.86
IPI	1.15	0.84

Parameter	Result	Threshold
Temperature	16.7	Narrative (Deg C)
Dissolved Oxygen	8.7	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
рН	8.2	6.5-8.5
Total Nitrogen	0.10	1.0 mg/L
Total Phosphorus	0.02	0.1 mg/L
Turbidity	0.5	20 NTU
Chloride	36	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	98	^b waterbody-specific mg/L
Conductivity	506	^a NA uS/cm
Alkalinity	83	^a NA mg/L
Silica	51	^a NA mg/L
Dissolved Organic Carbon	5.1	^a NA mg/L
Benthic AFDM	18.3	^d 25 g/m ²
Benthic Chl-a	24.1	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	13.6, 10.2	^a NA

⁺Non-detect

For benthic C:N, the difference between the two replicates may be explained by a note that was written when the filters were prepared. It said, "Rep 2 has a piece of *Nostoc*." Given Rep 2's relatively higher algal contribution on the filter, its lower C:N ratio makes sense.

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	41	9
Algae	62	3

Notable BMI:

• Ephemeroptera: Baetis; Centroptilum; Fallceon

• Plecoptera: Isoperla denningi; Malenka

• Trichoptera: Hydropsyche; Hydroptila; Micrasema; Ochrotrichia

Sensitive algae:

• Diatoms: Caloneis schumanniana; Gomphonema pumilum

• Cyanobacteria: Calothrix marchica

Other: none

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

eDNA

Method	Detections
Jonah Ventures	none
Smith-Root	NA
Vacuum filtration	NA

Tadpoles were observed while sampling (species uncertain).

<u>Cyanotoxins:</u> Agua Caliente Creek is one of six streams tested for the presence of cyanotoxins this year. Algal composite and targeted benthic mat samples were collected June 29, and no cyanotoxins were detected. A SPATT sampler was deployed June 29 and retrieved July 7, and anatoxin-a was detected (1.96 ng/g).

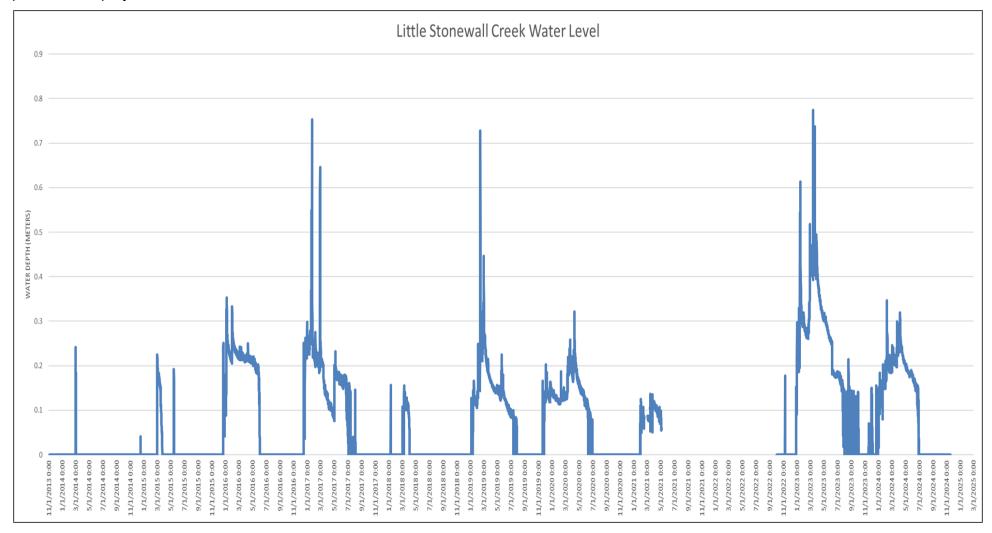
5.1.6. 909SWCASR: Stonewall Creek



Stonewall Creek is a long-term reference site within Cuyamaca Rancho State Park. Stonewall Creek is an intermittent site and has been logged for flow for over 10 years (



Figure 14. Water level for Stonewall Creek from 2013 to 2024. Note a logger relaunch error prevented data readings during a portion of deployment.



All scores above the thresholds indicated biologically intact benthic macroinvertebrate and algal communities, as well as intact physical habitat.

Index	Result	Threshold
CSCI	0.93	0.79
d-ASCI	1.03	0.86
h-ASCI	1.05	0.86
IPI	1.08	0.84

Chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan.

objectives in the basin Flan.		
Parameter	Result	Threshold
Temperature	14.9	Narrative (Deg C)
Dissolved Oxygen	8.6	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.8	6.5-8.5
Total Nitrogen	0.1	1.0 mg/L
Total Phosphorus	0.02	0.1 mg/L
Turbidity	0.4	20 NTU
Chloride	24	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	8.1	^b waterbody-specific mg/L
Conductivity	248	^a NA uS/cm
Alkalinity	51	^a NA mg/L
Silica	46	^a NA mg/L
Dissolved Organic Carbon	2.8	^a NA mg/L
Benthic AFDM	44.6	^d 25 g/m ²
Benthic Chl-a	33.1	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	13.8, 13.8	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	29	7
Algae	75	8

Notable BMI:

• Ephemeroptera: Baetis; Callibaetis; Centroptilum; Paraleptophlebia

• Plecoptera: Isoperla adunca

• Trichoptera: Hesperophylax; Wormaldia

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

Sensitive algae:

- Diatoms: Encyonema silesiacum; Fragilariforma virescens; Meridion circulare
- Cyanobacteria: Calothrix parietina
- Other: Chaetophora incrassata; Paralemanea californica; Zygnema sp 1; Zygnema sp 2

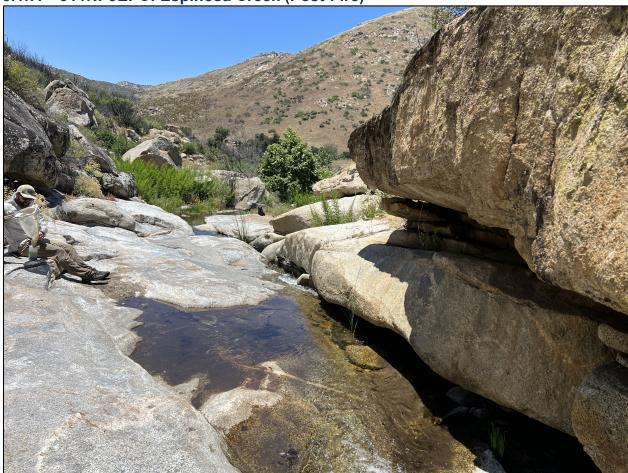
eDNA

Method	Detections
Jonah Ventures	none
Smith-Root	NA
Vacuum filtration	NA

A frog and tadpoles were observed while sampling (species not recorded).

<u>Cyanotoxins:</u> Stonewall Creek is one of six streams tested for the presence of cyanotoxins this year. Algal composite and targeted benthic mat samples were collected June 8, and no cyanotoxins were detected. A SPATT sampler was deployed June 8 and retrieved June 15, and anatoxin-a was detected (1.76 ng/g).

5.1.7. 911NP9EPC: Espinosa Creek (Post-Fire)



Espinosa Creek is a long-term reference site within the Cleveland National Forest. Espinosa Creek exhibits intermittent flows and has had a water level logger deployed since 2013, though the watershed and a logger burned in the 2020 Valley Fire (approx. 2.5 years prior to sampling). The water level logger was not downloaded during sampling.

The CSCI score indicated a potentially impacted benthic macroinvertebrate community, potentially representing a delay in full fire recovery, as prior CSCI scores were 0.94 (2017) and 0.92 (2019). However, some sensitive BMI were observed in portions of the reach (e.g. Blephariceridae), indicating good water quality. Algal scores were mixed, but an h-ASCI above the threshold indicated a likely intact algal community. Prior algal scores were all above thresholds. The IPI score indicated intact physical habitat, suggesting habitat fire recovery. This site will be prioritized for sampling during the next above-average rainfall year.

Index	Result	Threshold
CSCI	0.73	0.79
d-ASCI	0.75	0.86
h-ASCI	0.98	0.86
IPI	1.10	0.84

Parameter	Result	Threshold
Temperature	18.5	Narrative (Deg C)
Dissolved Oxygen	8.6	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
рН	8.2	6.5-8.5
Total Nitrogen	0.21	1.0 mg/L
Total Phosphorus	0.02	0.1 mg/L
Turbidity	1.0	20 NTU
Chloride	74	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	20	^b waterbody-specific mg/L
Conductivity	542	^a NA uS/cm
Alkalinity	103	^a NA mg/L
Silica	55	^a NA mg/L
Dissolved Organic Carbon	4	^a NA mg/L
Benthic AFDM	45.6	^d 25 g/m ²
Benthic Chl-a	35.3	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	13.7, 13.7	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	29	7
Algae	52	5

Notable BMI:

• Ephemeroptera: Baetis; Baetis adonis; Baetis tricaudatus; Diphetor hageni

• Plecoptera: none

• Trichoptera: *Hydroptila;* Hydroptilidae; *Hydropsyche*

Sensitive algae:

• Diatoms: Meridion circulare

• Cyanobacteria: Calothrix parietina

• Other: Paralemanea californica; Zygnema sp 1; Zygnema sterile

eDNA

Method	Detections	
Jonah Ventures	Brush Mouse (<i>Peromyscus boylii</i>)	
Smith-Root	NA	
Vacuum filtration	NA	

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

Two-striped garter snakes (*Thamnophis hammondii*) and California tree frogs (*Pseudacris cadaverina*) were observed while sampling, as well as tadpoles (species uncertain).

<u>Cyanotoxins:</u> Espinosa Creek is one of six streams tested for the presence of cyanotoxins this year. Algal composite and targeted benthic mat samples were collected June 14, and no cyanotoxins were detected. A SPATT sampler was deployed June 14 and retrieved June 23, and anatoxin-a was detected (2.18 ng/g).

5.2. **Program Sites**

5.2.1. 901SJOF1x: San Juan Creek above Ortega Falls (Decker Canyon)



San Juan Creek above Ortega Falls (Decker Canyon) is an intermittent stream in the Cleveland National Forest. This site was identified for sampling due to past discharges of sediment associated with the Caltrans Ortega Highway widening project. While sedimentation conditions had improved when compared to prior inspection visits, substantial excess coarse sediment was still present in sub-habitats (e.g. pools, glides) within the stream.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. ASCI scores indicated a likely impacted algal community.

Index	Result	Threshold
CSCI	0.83	0.79
d-ASCI	0.68	0.86
h-ASCI	0.68	0.86
IPI	1.14	0.84

Parameter	Result	Threshold
Temperature	14.8	Narrative (Deg C)
Dissolved Oxygen	9.9	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
рН	7.8	6.5-8.5
Total Nitrogen	0.46	1.0 mg/L
Total Phosphorus	0.08	0.1 mg/L
Turbidity	1.4	20 NTU
Chloride	51	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	30	^b waterbody-specific mg/L
Conductivity	440	^a NA uS/cm
Alkalinity	103	^a NA mg/L
Silica	53	^a NA mg/L
Dissolved Organic Carbon	5.2	^a NA mg/L
Benthic AFDM	23.4	^d 25 g/m ²
Benthic Chl-a	176.9	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	14.1, 9.9	^a NA
Total Coliform	>2,419.6	^a NA MPN/100 mL
E. coli	16.1	^a NA MPN/100 mL
HF183	ND	^a copies/ 100mL

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	35	5
Algae	44	1

Notable BMI:

• Ephemeroptera: Baetis; Centroptilum; Baetis adonis; Baetis tricaudatus

• Plecoptera: none

• Trichoptera: Hydropsyche

Sensitive algae:

• Diatoms: none

• Cyanobacteria: none

• Other: Paralemanea californica

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

eDNA

Method	Detections
Jonah Ventures	 Rough-skinned newt (<i>Taricha granulosa</i>) likely CA newt Cloven-hoofed mammals (Bovidae); Golden-mantled Ground Squirrel (<i>Callospermophilus lateralis</i>); Humans Ducks (Anatidae); Crows (<i>Corvus</i>)
Smith-Root	NA
Vacuum filtration	NA

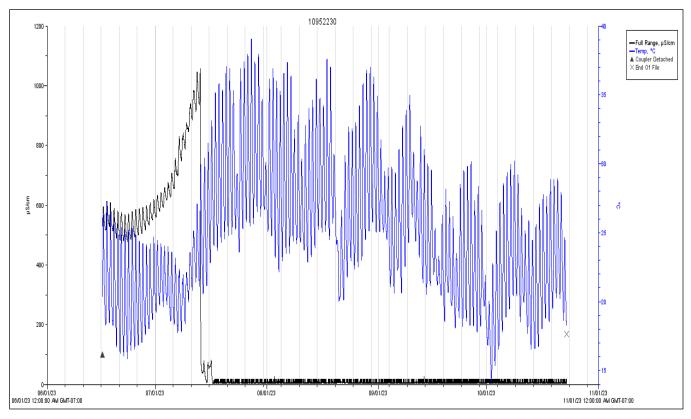
Tadpoles were observed while sampling (species uncertain).

5.2.2. 902SMAS1x: Santa Margarita River Arroyo Seco



Arroyo Seco is an intermittent stream above Dripping Springs Campground in the Cleveland National Forest. This site was requested for sampling by the Water Board cannabis staff due to potential upstream discharge impacts. Historic monitoring of this site found it was subject to periodic dewatering, likely due to upstream diversions. Staff deployed a conductivity logger during sampling to evaluate potential dewatering (after sampling). No evidence of periodic dewatering was observed, as conductivity remained constant until the stream went dry in mid-July (Figure). It is unknown whether flow diversions preceded sampling.

Figure 1511. Conductivity logger results for Arroyo Seco above Dripping Springs Campground from mid-June through mid-October 2023.



CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. ASCI scores indicated a potentially impacted algal community.

Index	Result	Threshold
CSCI	0.80	0.79
d-ASCI	0.76	0.86
h-ASCI	0.83	0.86
IPI	0.98	0.84

Parameter	Result	Threshold
Temperature	17.1	Narrative (Deg C)
Dissolved Oxygen	9.2	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	8.0	6.5-8.5
Total Nitrogen	ND	1.0 mg/L
Total Phosphorus	0.02	0.1 mg/L
Turbidity	0.1	20 NTU
Chloride	42	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	61	^b waterbody-specific mg/L
Conductivity	527	^a NA uS/cm
Alkalinity	127	^a NA mg/L
Silica	ND	^a NA mg/L
Dissolved Organic Carbon	5.7	^a NA mg/L
Benthic AFDM	13.6	^d 25 g/m ²
Benthic Chl-a	9.5	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	13.0, 14.0	^a NA

[⁺]Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	19	3
Algae	55	4

Notable BMI:

• Ephemeroptera: Baetis; Fallceon; Siphlonurus

Plecoptera: noneTrichoptera: none

• Neohermes filicornis (observed, not sampled)

Sensitive algae:

• Diatoms: Meridion circulare; Reimeria sinuata

• Cyanobacteria: Cyanobium diatomicola

• Other: Spirogyra varians

eDNA

Method	Detections	
Jonah Ventures	Arroyo Toad (Anaxyrus californicus)	
	Humans	
Smith-Root	NA	
Vacuum filtration	NA	

Tadpoles were observed while sampling (species uncertain).

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

5.2.3. 902SCSCRx: Sandia Creek



Sandia Creek is a perennial stream that drains a predominantly agricultural area in Riverside and San Diego Counties. The stream was sampled as part of monitoring agricultural impacts in the region. In addition, the stream has documented native species of concern, specifically steelhead and arroyo chub.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. ASCI scores indicated a likely impacted algal community.

Index	Result	Threshold
CSCI	0.98	0.79
d-ASCI	0.60	0.86
h-ASCI	0.55	0.86
IPI	1.07	0.84

Except for total nitrogen, chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan. Sulfate was above the drinking

water objective of 300 mg/L; there are no aquatic life criteria for sulfate.

Parameter	Result	Threshold
Temperature	17.8	Narrative (Deg C)
Dissolved Oxygen	9.2	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.9	6.5-8.5
Total Nitrogen	5.8	1.0 mg/L
Total Phosphorus	0.08	0.1 mg/L
Turbidity	2.1	20 NTU
Chloride	250	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	370	^b waterbody-specific mg/L
Conductivity	1,783	^a NA uS/cm
Alkalinity	-	^a NA mg/L
Silica	46	^a NA mg/L
Dissolved Organic Carbon	16	^a NA mg/L
Benthic AFDM	34.2	^d 25 g/m ²
Benthic Chl-a	114.8	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	11.6, 11.2	^a NA

^{*}Non-detect

Duplicate samples for chemistry were collected at this site. The results were consistent with those listed above, i.e., total nitrogen (5.5 mg/L), total phosphorus (0.03 mg/L), chloride (250 mg/L), sulfate (360 mg/L), dissolved organic carbon (16 mg/L), and silica (49 mg/L).

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	44	11
Algae	62	2

Notable BMI:

• Ephemeroptera: Baetis; Baetis adonis; Fallceon; Tricorythodes

• Plecoptera: none

• Trichoptera: *Cheumatopsyche; Hydropsyche;* Hydropsychidae; Leptoceridae; *Micrasema; Nectopsyche; Neotrichia*

Sensitive algae:

• Diatoms: Reimeria sinuata; Reimeria uniseriata

• Cyanobacteria: none

Other: none

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

A BMI field duplicate was collected at this site. The duplicate had a CSCI of 1.09. It had 34 BMI, with 9 EPT taxa. Notable taxa were *Baetis tricaudatus; Diphetor hageni; Tricorythodes* (Ephemeroptera), *Malenka* (Plecoptera), and *Cheumatopsyche; Nectopsyche;* Hydroptilidae; *Hydropsyche; Micrasema* (Tricoptera). Combined, the two samples contained 55 taxa, including 15 EPT taxa.

eDNA

Method	Detections
Jonah Ventures	 Arroyo Chub (Gila orcuttii); Black Basses (Micropterus);
	Pacific salmon and trout (Oncorhynchus)
Smith-Root	Oncorhynchus mykiss (KF55)
Vacuum filtration	Oncorhynchus mykiss (KF55)

A Pacific tree frog (*Pseudacris regilla*) was observed while sampling, as well as two fish (trout/steelhead) and tadpoles (species uncertain).

5.2.4. 554NMCCVM: Ninemile Creek - Casa Vieja Meadows



Ninemile Creek in Casa Vieja Meadows is a high Sierra meadow site located within the boundary of Regional Board 5 in the federally designated Golden Trout Wilderness on the Kern Plateau. Casa Vieja Meadows has been sampled by the San Diego Water Board as part of an effort to evaluate meadow best management practices to reduce nonpoint source pollution¹. Despite being in designated wilderness, a grandfathered historic grazing lease allows for active grazing in the meadow. Grazing has caused extensive damage to the meadow, which led to a United State Forest Service restoration project that included the installation of locked log and rock grade control structures to increase stream elevation in the meadow. Volunteer groups have also maintained a cattle exclusion fence over a major portion of the meadow to prevent cattle from grazing streamside vegetation and trampling the stream, which increases sedimentation. More recently, the fence was extended to the upper portion of the meadow and beaver dam analogs installed throughout to help capture sediment and further re-water the meadow. The results from this restoration work may be used to guide potential work in San Diego Region meadows with extensive historic grazing.

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¹ This sampling effort also serves as a team-building trip for the Monitoring Assessment and Research Unit.

Figure 12. Photo of the bioassessment sampling reach in 2016 (top, grazed) and 2023 (bottom, ungrazed).



CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. [ASCI scores not available: molecular algae only collected.] The CSCI score increased substantially since 2016 when the CSCI score was 0.82.

Index	Result	Threshold
CSCI	1.14	0.79
d-ASCI	-	0.86
h-ASCI	-	0.86
IPI	1.05	0.84

Chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan.

Parameter	Result	Threshold
Temperature	8.1	Narrative (Deg C)
Dissolved Oxygen	8.4	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pН	8.2	6.5-8.5
Total Nitrogen		1.0 mg/L
Total Phosphorus		0.1 mg/L
Turbidity	1.0	20 NTU
Chloride		^b waterbody-specific mg/L; ^c 230 & 860 mg/L
-Sulfate		^b waterbody-specific mg/L
Conductivity	88	^a NA uS/cm
Alkalinity	45	^a NA mg/L
Silica		^a NA mg/L
Dissolved Organic Carbon		^a NA mg/L
Benthic AFDM	76.1	^d 25 g/m ²
Benthic Chl-a	8.4	^d 44 mg/m ² QA code H (hold time)
Benthic C:N Rep 1, Rep 2	18.5, 18.0	^a NA

^{*}Non-detect

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	74	28
Algae	-	-

Notable BMI:

- Ephemeroptera: Baetis; Baetis tricaudatus; Diphetor hageni; Drunella; Ephemerellidae; Leptophlebiidae; Matriella teresa; Paraleptophlebia
- Plecoptera: Kogotus/Rickera; Malenka; Nemouridae; Sweltsa; Yoraperla; Zapada cinctipes
- Trichoptera: Brachycentridae; *Gumaga; Heteroplectron californicum; Hydroptila; Hydropsyche;* Hydropsychidae; *Lepidostoma;* Limnephilidae; *Micrasema*; Philopotamidae; *Rhyacophila; Rhyacophila brunnea* group; *Wormaldia; Yphria californica*

eDNA

Method	Detections	
Jonah Ventures	 Pacific salmon and trout (Oncorhynchus) 	
Smith-Root	Oncorhynchus mykiss (KF55)	
Vacuum filtration	Oncorhynchus mykiss (KF55)	

An unknown 2-3 inch frog, either a tree frog (*Pseudacris*) or yellow legged frog (*Rana*) was observed in the meadow during sampling.

5.3. Causal Assessment Sites

5.3.1. 910OTJMC4: Jamul Creek



Jamul Creek was selected for sampling as part of participation by the San Diego Water Board in the Stormwater Monitoring Coalition (SMC) causal assessment efforts for the CSCI. Jamul Creek was prioritized for causal assessment due to past poor CSCI scores (e.g. 0.66 in 2008) and concurrent high quality in-stream habitat. The first step in causal assessment is confirmation sampling to determine impairment, concurrent with or followed by supplemental sampling to begin potential identification of the pollutants or pollution causing the impairment. The San Diego Water Board resampled Jamul Creek to confirm condition and also added additional constituents (bacteria and HF183) to explore potential sources. Jamul Creek is largely open space but has an upstream RV park and cattle grazing.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. ASCI scores indicated a likely impacted algal community.

Index	Result	Threshold
CSCI	0.92	0.79
d-ASCI	0.55	0.86
h-ASCI	0.56	0.86
IPI	1.09	0.84

objectives in the basin man.		
Parameter	Result	Threshold
Temperature	16.6	Narrative (Deg C)
Dissolved Oxygen	8.5	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pН	7.8	6.5-8.5
Total Nitrogen	0.43	1.0 mg/L
Total Phosphorus	0.02	0.1 mg/L
Turbidity	-	20 NTU
Chloride	190	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	48	^b waterbody-specific mg/L
Conductivity	1,040	^a NA uS/cm
Alkalinity	-	^a NA mg/L
Silica	30	^a NA mg/L
Dissolved Organic Carbon	5.4	^a NA mg/L
Benthic AFDM	57.1	^d 25 g/m ²
Benthic Chl-a	252.2	^d 44 mg/m ²
Benthic C:N Rep1, Rep 2	11.0, 11.3	^a NA
Total Coliform	>2,419.6	^a NA MPN/100 mL
E. coli	23.3	^a NA MPN/100 mL
HF183	ND	^a copies/ 100mL

^{*}Non-detect

^a Informational

b Basin Plan criteria are specific to non-aquatic use CusePA recommended freshwater criteria for 4-day and 1-hour exposure, respectively. Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	38	9
Algae	43	2

Notable BMI:

• Ephemeroptera: Baetis; Baetis adonis; Callibaetis; Fallceon

• Plecoptera: none

• Trichoptera: Cheumatopsyche; Hydroptila; Hydroptilidae; Hydropsyche; Wormaldia

Sensitive algae:

• Diatoms: Gomphonema pumilum; Reimeria sinuata

• Cyanobacteria: none

• Other: none

eDNA

Method	Detections
Jonah Ventures	 African Clawed Frog (Xenopus laevis)
	California vole (<i>Microtus californicus</i>); Woodrat (<i>Neotoma</i>)
Smith-Root	NA
Vacuum filtration	NA

Crayfish (*Procambarus clarkii*) and tadpoles (species uncertain) were observed when sampling. eDNA detections of African clawed frog likely originate from an infestation on Rancho Jamul that was undergoing removal efforts.

5.4. Trend Sites

5.4.1. 911TJPVC1: Pine Valley Creek



Pine Valley Creek is a long-term SWAMP site that has been monitored since 1999. The SWAMP site was originally closer to old highway 80 but moved slightly downstream for better access. This stream segment of Pine Valley Creek is perennial and supports a population of stickleback, a native fish.

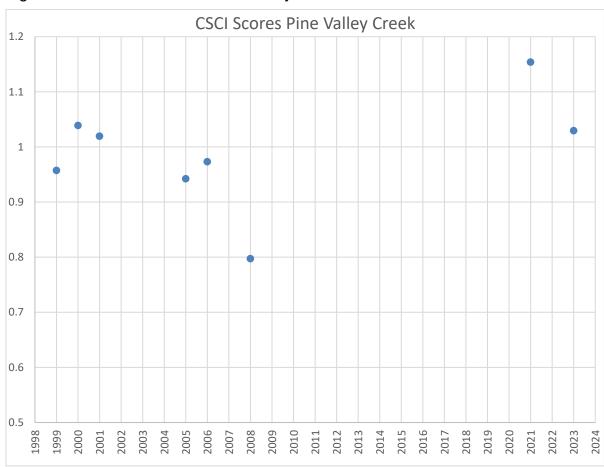


Figure 1317. CSCI scores at Pine Valley Creek 1999-2023.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. ASCI scores indicated a potentially impacted algal community.

Index	Result	Threshold
CSCI	1.03	0.79
d-ASCI	0.76	0.86
h-ASCI	0.70	0.86
IPI	1.05	0.84

Except for total nitrogen, chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan.

Parameter	Result	Threshold
Temperature	12.1	Narrative (Deg C)
Dissolved Oxygen	9.1	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.8	6.5-8.5
Total Nitrogen	1.4	1.0 mg/L
Total Phosphorus	0.02	0.1 mg/L
Turbidity	1.3	20 NTU
Chloride	45	^b waterbody-specific mg/L; ^c 230 & 860 mg/L

Sulfate	70	^b waterbody-specific mg/L
Conductivity	521	^a NA uS/cm
Alkalinity	106	^a NA mg/L
Silica	38	^a NA mg/L
Dissolved Organic Carbon	14	^a NA mg/L
Benthic AFDM	48.7	^d 25 g/m ²
Benthic Chl-a	56.4	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	13.2, 13.9	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	46	12
Algae	56	3

Notable BMI:

- Ephemeroptera: Baetis; Baetis adonis; Baetis tricaudatus; Caenis bajaensis
- Plecoptera: Malenka; Nemouridae
- Trichoptera: Brachycentridae; Gumaga; Hydropsyche; Hydropsychidae; Lepidostoma; Micrasema

Sensitive algae:

• Diatoms: Fragilariforma virescens; Gomphonema kobayasii; Reimeria sinuata

Cyanobacteria: none

• Other: none

eDNA

Method	Detections	
Jonah Ventures	Three-spined stickleback (Gasterosteus aculeatus)	
Smith-Root	None detected	
Vacuum filtration	None detected	

Native fish (sticklebacks) were observed along the entire reach when sampling. Tadpoles were also observed (species uncertain).

<u>Cyanotoxins:</u> Pine Valley Creek is one of six streams tested for the presence of cyanotoxins this year. Algal composite and targeted benthic mat samples were collected June 27, and no cyanotoxins were detected. A SPATT sampler was deployed June 27 and retrieved July 5, and anatoxin-a was detected (2.50 ng/g).

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

5.5. Coverage Expansion Sites

5.5.1. 901NP9LAN: Upper Los Alamos Canyon, North Fork



Upper Los Alamos Canyon Creek North Fork is an upper watershed tributary in the San Mateo Creek Watershed. The monitoring site is located on USFS lands but also drains a large area of rural residential land use in the County of Riverside.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. ASCI scores indicated a likely impacted algal community.

Index	Result	Threshold
CSCI	0.93	0.79
d-ASCI	0.68	0.86
h-ASCI	0.73	0.86
IPI	1.08	0.84

Parameter	Result	Threshold
Temperature	17.4	Narrative (Deg C)
Dissolved Oxygen	10.2	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	8.1	6.5-8.5
Total Nitrogen	0.2	1.0 mg/L
Total Phosphorus	0.04	0.1 mg/L
Turbidity	0.4	20 NTU
Chloride	150	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	75	^b waterbody-specific mg/L
Conductivity	1,010	^a NA uS/cm
Alkalinity	197	^a NA mg/L
Silica	56	^a NA mg/L
Dissolved Organic Carbon	4.6	^a NA mg/L
Benthic AFDM	94.2	^d 25 g/m ²
Benthic Chl-a	252.3	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	10.6, 11.1	^a NA

[⁺]Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	33	6
Algae	49	0

Notable BMI:

• Ephemeroptera: Centroptilum; Baetis; Baetis adonis; Baetis tricaudatus

Plecoptera: none

• Trichoptera: Wormaldia; Hydropsyche

Sensitive algae:

• Diatoms: none

Cyanobacteria: none

• Other: none

eDNA

Method	Detections
Jonah Ventures	Cattle (Bos)
Smith-Root	Oncorhynchus mykiss (KF55)
Vacuum filtration	None detected

A California newt (*Taricha torosa*) was observed when sampling. A tree frog and tadpoles were also observed (species not recorded).

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

5.5.2. 902R9CCC1: Cole Canyon Creek



Cole Canyon Creek is an intermittent tributary of Murrieta Creek that drains the Santa Rosa Plateau, which is a State of California ecological reserve.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. Algal scores were mixed, but an h-ASCI above the threshold indicated a likely intact algal community.

	<u> </u>	
Index	Result	Threshold
CSCI	0.80	0.79
d-ASCI	0.77	0.86
h-ASCI	0.91	0.86
IPI	1.18	0.84

Parameter	Result	Threshold
Temperature	16.5	Narrative (Deg C)
Dissolved Oxygen	8.6	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.8	6.5-8.5
Total Nitrogen	0.2	1.0 mg/L
Total Phosphorus	0.03	0.1 mg/L
Turbidity	0.7	20 NTU
Chloride	110	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	46	^b waterbody-specific mg/L
Conductivity	733	^a NA uS/cm
Alkalinity	141	^a NA mg/L
Silica	39	^a NA mg/L
Dissolved Organic Carbon	3.8	^a NA mg/L
Benthic AFDM	43.7	^d 25 g/m ²
Benthic Chl-a	56.0	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	14.3, 14.7	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	32	6
Algae	50	3

Notable BMI:

• Ephemeroptera: Baetidae; *Baetis; Baetis tricaudatus; Centroptilum; Tricorythodes*

• Plecoptera: none

• Trichoptera: Hydropsyche

Sensitive algae:

• Diatoms: Epithemia adnata

• Cyanobacteria: none

• Other: Paralemanea californica; Spirogyra varians

eDNA

Method	Detections
Jonah Ventures	 Rough-skinned newt (<i>Taricha granulosa</i>), likely truly a California newt (<i>Taricha torosa</i>) Humans
Smith-Root	NA
Vacuum filtration	NA

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

A California newt (*Taricha torosa*) was observed when sampling.

5.5.3. 903DCBDCP: Doane Creek



Doane Creek Below Doane Creek Pond is a perennial stream located within Palomar State Park. Historic bioassessment has occurred upstream of the pond, but sampling had never been conducted on Doane Creek downstream of the pond dam.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. ASCI scores indicated a potentially impacted algal community.

Index	Result	Threshold
CSCI	1.04	0.79
d-ASCI	0.73	0.86
h-ASCI	0.76	0.86
IPI	1.16	0.84

Parameter	Result	Threshold
Temperature	14.6	Narrative (Deg C)
Dissolved Oxygen	8.5	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.9	6.5-8.5
Total Nitrogen	0.19	1.0 mg/L
Total Phosphorus	0.04	0.1 mg/L
Turbidity	0.9	20 NTU
Chloride	11	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	8.6	^b waterbody-specific mg/L
Conductivity	175	^a NA uS/cm
Alkalinity	54	^a NA mg/L
Silica	30	^a NA mg/L
Dissolved Organic Carbon	14	^a NA mg/L
Benthic AFDM	73.3	^d 25 g/m ²
Benthic Chl-a	19.1	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	16.7, 15.2	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	52	19
Algae	52	3

Notable BMI:

- Ephemeroptera: Baetis; Baetis adonis; Baetis tricaudatus; Centroptilum; Ironodes; Leptophlebiidae; Paraleptophlebia
- Plecoptera: Malenka; Nemouridae; Plecoptera; Sweltsa; Zapada cinctipes
- Trichoptera: *Hydropsyche;* Hydropsychidae; *Lepidostoma; Neophylax splendens; Parapsyche almota; Psychoglypha; Rhyacophila betteni* group

Sensitive algae:

- Diatoms: Planothidium cf haynaldii; Reimeria sinuata
- Cyanobacteria: none
- Other: Paralemanea californica

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

An algal field duplicate was collected at this site. The d-ASCI and h-ASCI were 0.85 and 0.90, respectively, somewhat higher than Rep 1. Algal chemistry results were consistent with those above, i.e., benthic chl-a (13.9 mg/m²), and benthic C:N Rep 1 and Rep 2 (16.0, 16.8). [Benthic AFDM results for the duplicate are not available.] There were 54 taxa, with four being sensitive. Sensitive taxa were as above plus *Meridion circulare* (diatom). Combined, the two samples contained 66 algal taxa, including four sensitive taxa.

eDNA

Method	Detections
Jonah Ventures	 Bullheads (Ameiurus); Green Sunfish (Lepomis cyanellus); Black Basses (Micropterus); Pacific salmon and trout (Oncorhynchus) Chickadees (Poecile)
Smith-Root	Oncorhynchus mykiss (KF55)
Vacuum filtration	Oncorhynchus mykiss (KF55)

No fish, frogs, or tadpoles were observed while sampling. eDNA fish results are likely from the upstream pond.

<u>Cyanotoxins:</u> Doane Creek is one of six streams tested for the presence of cyanotoxins this year. Algal composite and targeted benthic mat samples were collected July 6, and no cyanotoxins were detected. A SPATT sampler was deployed July 6 and retrieved July 14; both anatoxin-a (2.81 ng/g) and microcystin/nodularin (3.84 ng/g) were detected.

5.5.4. 909HPCASR: Harper Creek



Harper Creek is located within Cuyamaca Rancho State Park and is an intermittent tributary to the Sweetwater River. Harper Creek passes all GIS reference screens except for active mine in the watershed, though the one active mine upstream may no longer be in operation due to a property transfer to the Anza-Borrego Foundation.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. Algal scores were mixed, but an h-ASCI above the threshold indicated a likely intact algal community.

Index	Result	Threshold
CSCI	0.79	0.79
d-ASCI	0.82	0.86
h-ASCI	0.98	0.86
IPI	1.20	0.84

Parameter	Result	Threshold
Temperature	13.7	Narrative (Deg C)
Dissolved Oxygen	9.5	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	8.2	6.5-8.5
Total Nitrogen	0.09	1.0 mg/L
Total Phosphorus	ND	0.1 mg/L
Turbidity	1.2	20 NTU
Chloride	37	^b waterbody-specific mg/L; ^c 230 & 860 mg/L
Sulfate	49	^b waterbody-specific mg/L
Conductivity	368	^a NA uS/cm
Alkalinity	54	^a NA mg/L
Silica	40	^a NA mg/L
Dissolved Organic Carbon	3.4	^a NA mg/L
Benthic AFDM	139.4	^d 25 g/m ²
Benthic Chl-a	61.4	^d 44 mg/m ²
Benthic C:N Rep 1, Rep 2	12.3, 12.3	^a NA

^{*}Non-detect

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	31	6
Algae	80	8

Notable BMI:

• Ephemeroptera: Baetis; Baetis tricaudatus; Centroptilum; Diphetor hageni

• Plecoptera: none

• Trichoptera: Hydroptila; Hydropsyche

Sensitive algae:

• Diatoms: Caloneis schumanniana; Epithemia sorex; Gomphonema subclavatum; Meridion circulare

• Cyanobacteria: none

• Other: *Paralemanea californica; Zygnema* aplanosporum; *Zygnema* sp 1; *Zygnema* sp 2

Method	Detections
Jonah Ventures	 Bullheads (Ameiurus); Black Basses (Micropterus); Pacific salmons and trout (Oncorhynchus); Sunfishes (Lepomis)
Smith-Root	NA
Vacuum filtration	NA

^a Informational

^b Basin Plan criteria are specific to non-aquatic use

^c USEPA recommended freshwater criteria for 4-day and 1-hour exposure, respectively.

^d Mazor et al. 2022 threshold for obtaining 10th percentile ASCI score

A two-stripe garter snake (*Thamnophis hammondii*) was observed when sampling. Tadpoles were also observed (species uncertain).

<u>Cyanotoxins</u>: Harper Creek is one of six streams tested for the presence of cyanotoxins this year. Algal composite and targeted benthic mat samples were collected June 20, and no cyanotoxins were detected. A SPATT sampler was deployed June 20 and retrieved June 28, and anatoxin-a was detected (2.24 ng/g).

5.6. CDFW-sampled RCMP sites

The San Diego Water Board provides financial support to the State's RCMP program through supplemental funding of aspects of monitoring. This allows more sites to be sampled by the program state-wide. In 2023 CDFW sampled four sites in the San Diego Region, targeting older RCMP sites for resampling.



Cold Stream is a perennial stream within Cuyamaca Rancho State Park. This location is approximately 2 km upstream of a site sampled by the San Diego Water Board in 2021.

CSCI and IPI scores indicated a biologically intact benthic macroinvertebrate community and intact physical habitat. ASCI scores indicated a potentially impacted algal

community.

Index	Result	Threshold
CSCI	1.09	0.79
d-ASCI	0.79	0.86
h-ASCI	0.74	0.86
IPI	1.04	0.84

Chemical and physical water quality results met relevant aquatic life water quality objectives in the Basin Plan.

objectives in the Basin i lan.		
Parameter	Result	Threshold
Temperature	8.3	Narrative (Deg C)
Dissolved Oxygen	9.2	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pН	7.9	6.5-8.5
Turbidity	2.9	20 NTU
Conductivity	233	^a NA uS/cm
Alkalinity	100	^a NA mg/L

[†]Non-detect ^a Informational

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	46	19
Algae	60	5

Notable BMI:

- Ephemeroptera: Ameletus; Baetis; Leptophlebiidae; Matriella teresa
- Plecoptera: Chloroperlidae; Isoperla; Nemouridae; Suwallia; Taenionema
- Trichoptera: Hydropsyche; Rhyacophila; Gumaga Agapetus; Hesperophylax; Dolophilodes; Lepidostoma; Tinodes; Neophylax

Sensitive algae:

- Diatoms: Epithemia sorex; Gomphonema kobayasii; Gomphonema subclavatum;
 Meridion circulare; Reimeria sinuata
- Cyanobacteria: none
- Other: none





Juaquapin Creek is an intermittent tributary to the Sweetwater River in Rancho Cuyamaca State Park. Juaquapin Creek was last sampled in 2011 with a CSCI of 1.08, d-ASCI of 0.87, and h-ASCI of 0.90.

Juaquapin Creek scores indicated biologically intact benthic macroinvertebrate and algae communities, as well as intact physical habitat.

Index	Result	Threshold
CSCI	0.93	0.79
d-ASCI	1.11	0.86
h-ASCI	1.11	0.86
IPI	0.93	0.84

Parameter	Result	Threshold
Temperature	7.9	Narrative (Deg C)
Dissolved Oxygen	9.6	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pН	8	6.5-8.5
Turbidity	3.3	20 NTU
Conductivity	226	^a NA uS/cm
Alkalinity	105	^a NA mg/L

^{*}Non-detect a Informational

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	55	14
Algae	52	4

Notable BMI:

- Ephemeroptera: Ameletus; Baetis tricaudatus; Centroptilum; Leptophlebiidae
- Plecoptera: Isoperla; Nemouridae; Taenionema; Perlodidae;
- Trichoptera: Lepidostoma; Limnephilidae; Rhyacophila; Rhyacophila sibirica

Sensitive algae:

- Diatoms: Achnanthidium deflexum; Eunotia soleirolii; Gomphonema kobayasii; Gomphonema subclavatum
- Cyanobacteria: none
- Other: none





Japacha Creek is an intermittent tributary to the Sweetwater River in Rancho Cuyamaca State Park. Juaquapin Creek was last sampled in 2011 with a CSCI of 1.05, d-ASCI of 0.74, and h-ASCI of 0.87.

Japacha Creek scores indicated biologically intact benthic macroinvertebrate and algae communities, as well as intact physical habitat.

Index	Result	Threshold
CSCI	0.94	0.79
d-ASCI	1.09	0.86
h-ASCI	1.11	0.86
IPI	1.06	0.84

Parameter	Result	Threshold
Temperature	12.4	Narrative (Deg C)
Dissolved Oxygen	8.7	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pН	7.5	6.5-8.5
Turbidity	1.0	20 NTU
Conductivity	245	^a NA uS/cm
Alkalinity	60	^a NA mg/L

^{*}Non-detect a Informational

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	44	12
Algae	58	6

Notable BMI:

• Ephemeroptera: Ameletus; Baetis; Baetis tricaudatus; Centroptilum; Leptophlebiidae; Matriella teresa; Paraleptophlebia

• Plecoptera: Capniidae; Chloroperlidae; Isoperla; Plecoptera

• Trichoptera: Wormaldia

Sensitive algae:

• Diatoms: Achnanthidium deflexum; Achnanthidium rivulare; Gomphonema kobayasii; Gomphonema subclavatum; Meridion circulare; Reimeria sinuata

• Cyanobacteria: none

Other: none

5.6.4. 911TCCTCx: Troy Canyon Creek



Troy Canyon Creek is a long-term reference site on the Cleveland National Forest that was extensively sampled by SWAMP from 2000-2011. This site has been identified by sister agency staff as perennial. Juaquapin Creek was last sampled in 2011 with a CSCI of 1.06, d-ASCI of 1.19, and h-ASCI of 1.33.

Troy Canyon Creek scores indicated biologically intact benthic macroinvertebrate and algae communities, as well as intact physical habitat.

Index	Result	Threshold
CSCI	1.02	0.79
d-ASCI	1.05	0.86
h-ASCI	1.12	0.86
IPI	0.86	0.84

Parameter	Result	Threshold
Temperature	9.2	Narrative (Deg C)
Dissolved Oxygen	9.4	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	no data	6.5-8.5
Turbidity	2.4	20 NTU
Conductivity	345	^a NA uS/cm
Alkalinity	156	^a NA mg/L

^{*}Non-detect a Informational

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	53	10
Algae	70	5

Notable BMI:

• Ephemeroptera: *Baetis*; Leptophlebiidae

• Plecoptera: Isoperla; Malenka; Nemouridae

• Trichoptera: Hesperophylax, Psychoglypha, Lepidistoma, Gumaga

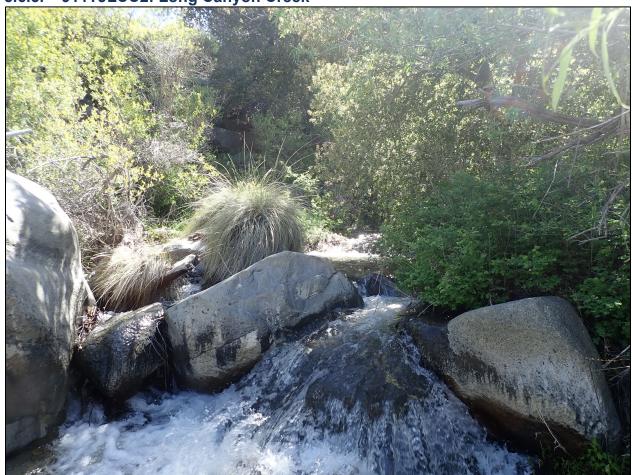
Sensitive algae:

• Diatoms: Eunotia soleirolii; Gomphonema kobayasii; Gomphonema subclavatum; Meridion circulare

• Cyanobacteria: Chamaesiphon

• Other: none

5.6.5. 911TJLCC2: Long Canyon Creek



Long Canyon Creek is an intermittent stream in the Cleveland National Forest. The sampling site is just upstream of Cibbets Flat Campground. Long Canyon Creek is a long-term SWAMP site and has been sampled 14 times from 2001-2023. Long Canyon Creek was last sampled in 2021 with a CSCI of 1.13, d-ASCI of 1.20, and h-ASCI of 1.29.

Long Canyon Creek scores indicated biologically intact benthic macroinvertebrate and algae communities, as well as intact physical habitat.

Index	Result	Threshold
CSCI	0.85	0.79
d-ASCI	1.10	0.86
h-ASCI	1.13	0.86
IPI	0.92	0.84

Parameter	Result	Threshold
Temperature	8.7	Narrative (Deg C)
Dissolved Oxygen	9.9	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pН	8.5	6.5-8.5
Turbidity	1.0	20 NTU
Conductivity	387	^a NA uS/cm
Alkalinity	210	^a NA mg/L

^{*}Non-detect a Informational

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	33	12
Algae	42	5

Notable BMI:

• Ephemeroptera: *Baetis; Centroptilum;* Ephemerellidae; *Ironodes;* Leptophlebiidae; *Matriella teresa*

• Plecoptera: Isoperla

• Trichoptera: Dolophilodes; Gumaga; Hydroptilidae; Micrasema; Rhyacophila

Sensitive algae:

• Diatoms: Cymbella affinis; Epithemia turgida; Gomphonema kobayasii; Gomphonema subclavatum; Meridion circulare

• Cyanobacteria: none

Other: none

5.6.6. 911TJKC1x: Kitchen Creek



Kitchen Creek is an intermittent stream within the Cleveland National Forest. Kitchen Creek has been sampled on an almost annual basis since 2012.

Kitchen Creek scores indicated biologically intact benthic macroinvertebrate and algae communities. Physical habitat scored less than the threshold, though the index is currently insufficient for assessing bedrock dominated streams like this site.

Index	Result	Threshold
CSCI	0.82	0.79
d-ASCI	1.05	0.86
h-ASCI	1.17	0.86
IPI	0.83	0.84

Parameter	Result	Threshold
Temperature	9.7	Narrative (Deg C)
Dissolved Oxygen	9.6	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	8.4	6.5-8.5
Turbidity	0.7	20 NTU
Conductivity	435	^a NA uS/cm
Alkalinity	212	^a NA mg/L

^{*}Non-detect a Informational

Taxa

	# Taxa	# EPT or Sensitive Taxa
BMI	19	6
Algae	73	5

Notable BMI:

• Ephemeroptera: Baetis; Centroptilum

• Plecoptera: Isoperla

• Trichoptera: Ochrotrichia; Rhyacophila

Sensitive algae:

Diatoms: Epithemia sorex; Epithemia turgida; Gomphonema subclavatum;
 Meridion circulare

Cyanobacteria: noneOther: *Chaetophora*

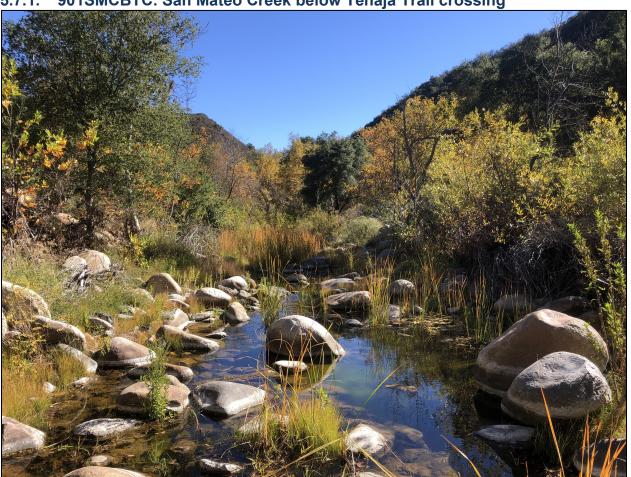
A BMI field duplicate was collected at this site. The CSCI was 0.97. The duplicate had 41 taxa, including 9 EPT taxa. Notable taxa were *Baetis tricaudatus; Centroptilum; Leptophlebiidae; Matriella teresa* (Ephemeroptera), *Isoperla; Plecoptera* (Plecoptera), and *Gumaga; Hydroptilidae; Ochrotrichia* (Tricoptera). Together, the two samples contained a combined total of 48 taxa, including 11 EPT taxa. The difference between the two samples appears to be driven by the predominance of Ostracoda in the first replicate. This can occur at Kitchen Creek due to the prevalence of bedrock pools containing the alga, *Chara*.

An algal field duplicate was collected at this site. The d-ASCI and h-ASCI were 1.02 and 1.09, respectively, similar to the results above. The duplicate had 74 taxa, with five being sensitive. Sensitive taxa were the diatoms *Achnanthidium rivulare; Epithemia sorex; and Meridion circulare;* cyanobacterium *Calothrix marchica;* and green alga *Chaetophora*. Combined, the two samples contained 99 algal taxa, seven considered sensitive.

5.7. eDNA-only sites

Targeted eDNA-only sampling was conducted at sites to assist in long-term incorporation of eDNA monitoring in bioassessment to better capture biological integrity for higher-trophic level aquatic species.





San Mateo Creek was selected for targeted eDNA sampling using ddPCR for southwestern pond turtle, steelhead, California newt, and red-legged frog. Jonah Ventures qPCR metabarcoding detections are also presented for comparative purposes.

Parameter	Result	Threshold
Temperature	14.2	Narrative (Deg C)
Dissolved Oxygen	9.9	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.6	6.5-8.5
Conductivity	689	^a NA uS/cm

^a Informational

Method	Detections
Jonah Ventures	Sunfishes (<i>Lepomis</i>); Golden shiner (<i>Notemigonus crysoleucas</i>)
Smith-Root	Steelhead/rainbow trout (O. mykiss)
Vacuum filtration	No detections

5.7.2. 901SMCUPL: San Mateo Creek Trail crossing at upper pools



San Mateo Creek was selected for targeted eDNA sampling using ddPCR for southwestern pond turtle, steelhead, California newt, and red-legged frog. Jonah Ventures qPCR metabarcoding detections are also presented for comparative purposes.

Parameter	Result	Threshold
Temperature	12.0	Narrative (Deg C)
Dissolved Oxygen	10.3	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	8.0	6.5-8.5
Conductivity	658	^a NA uS/cm

^a Informational

Method	Detections
Jonah Ventures	None
Smith-Root	Steelhead/rainbow trout (O. mykiss)
Vacuum filtration	Sample not yet analyzed





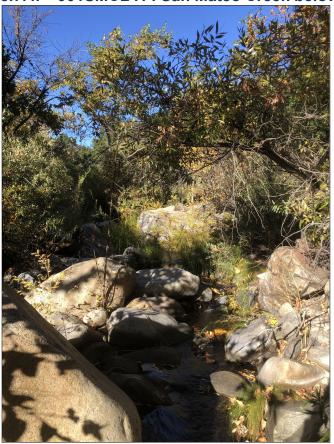
San Mateo Creek was selected for targeted eDNA sampling using ddPCR for southwestern pond turtle, steelhead, California newt, and red-legged frog. Jonah Ventures qPCR metabarcoding detections are also presented for comparative purposes.

Parameter	Result	Threshold
Temperature	12.9	Narrative (Deg C)
Dissolved Oxygen	8.1	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.8	6.5-8.5
Conductivity	648	^a NA uS/cm

^a Informational

Method	Detections
Jonah Ventures	Sunfishes (<i>Lepomis</i>)
Smith-Root	Steelhead/rainbow trout (O. mykiss)
Vacuum filtration	No detections





San Mateo Creek was selected for targeted eDNA sampling using ddPCR for southwestern pond turtle, steelhead, California newt, and red-legged frog. Jonah Ventures qPCR metabarcoding detections are also presented for comparative purposes.

Parameter	Result	Threshold
Temperature	15.4	Narrative (Deg C)
Dissolved Oxygen	8.6	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.5	6.5-8.5
Conductivity	636	^a NA uS/cm

^a Informational

001111	
Method	Detections
Jonah Ventures	Sunfishes (Lepomis)
Smith-Root	Steelhead/rainbow trout (O. mykiss), CA Newt (T. torosa)
Vacuum filtration	No detections

5.7.5. 901LACUTF: Los Alamos Creek above Tenaja Falls Creek



San Mateo Creek was selected for targeted eDNA sampling using ddPCR for southwestern pond turtle, steelhead, California newt, and red-legged frog. Jonah Ventures qPCR metabarcoding detections are also presented for comparative purposes.

Parameter	Result	Threshold
Temperature	15.7	Narrative (Deg C)
Dissolved Oxygen	7.0	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.3	6.5-8.5
Conductivity	613	^a NA uS/cm

^a Informational

Method	Detections
Jonah Ventures	Sunfishes (<i>Lepomis</i>)
Smith-Root	Steelhead/rainbow trout (O. mykiss)
Vacuum filtration	None detected

5.7.6. 909SSWR01: Sweetwater River 1



The Sweetwater River in Cuyamaca Rancho State Park was selected for targeted eDNA sampling using ddPCR for southwestern pond turtle, steelhead, California newt, and red-legged frog. Jonah Ventures qPCR metabarcoding detections are also presented for comparative purposes.

Parameter	Result	Threshold
Temperature	11.6	Narrative (Deg C)
Dissolved Oxygen	9.6	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.1	6.5-8.5
Conductivity	323	^a NA uS/cm

^a Informational

Method	Detections
Jonah Ventures	Three-spined stickleback (<i>Gasterosteus aculeatus</i>); Riffle daces (<i>Rhinichthys</i>)
Smith-Root #1	Steelhead/rainbow trout (<i>O. mykiss</i>); Southwestern pond turtle (<i>A. pallida</i>)
Smith-Root #2	Steelhead/rainbow trout (O. mykiss)
Vacuum filtration	Steelhead/rainbow trout (O. mykiss)

5.7.7. 910RJTPND: Rancho Jamul Turtle Pond



Rancho Jamul turtle pond is a small groundwater-fed pond on CDFW preserve land that has been restored for southwestern pond turtles through the removal of bullfrogs. Jonah Venture qMRA metabarcoding was conducted on the pond.

Parameter	Result	Threshold
Temperature	21.4	Narrative (Deg C)
Dissolved Oxygen	1.9	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pН	7.5	6.5-8.5
Conductivity	1,659	^a NA uS/cm

^a Informational

Method	Detections
Jonah Ventures	Bullheads (Ameiurus); Mosquitofish (Gambusia)



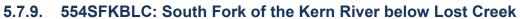


The unnamed tributary to Blackrock Creek flows into a meadow restoration area on the Golden Trout Wilderness. The small tributary is used by golden trout for spawning and has an intermittent connection with Ninemile Creek. It was selected as a comparator site to evaluate eDNA results from an established trout population in a small stream.

Parameter	Result	Threshold
Temperature	9.1	Narrative (Deg C)
Dissolved Oxygen	8.5	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	8.0	6.5-8.5
Conductivity	96	^a NA uS/cm

^a Informational

Method	Detections
Jonah Ventures	Pacific salmons and trouts (Oncorhynchus)
Smith-Root	Steelhead/rainbow trout (O. mykiss)





The South Fork Kern River is a perennial river/stream in the South Sierra Wilderness. The river was selected as a comparator site to evaluate eDNA results from an established trout population in a larger river/stream.

Parameter	Result	Threshold
Temperature	10.6	Narrative (Deg C)
Dissolved Oxygen	9.1	5.0 (WARM BU) or 6.0 (COLD BU) mg/L
pH	7.6	6.5-8.5
Conductivity	162	^a NA uS/cm

^a Informational

Method	Detections
Jonah Ventures	Steelhead trout (<i>Oncorhynchus mykiss</i>); Brown trout (<i>Salmo trutta</i>); Common suckers (Catostomus)
Smith-Root	Steelhead/rainbow trout (O. mykiss)

6. Summary

- 2023 marked the 28th year of State of California bioassessment monitoring in Region 9. To date, over 500 sites in the region have been bioassessed at least once, and more than 1,700 bioassessments have been done.
- In 2023, 23 streams were bioassessed by MARU (17) and CDFW (6).
- CSCI scores indicated intact macroinvertebrate communities at all but one site.
 - Espinosa Creek, a reference site, scored lower than expected (0.73), likely reflecting residual post-fire impacts.
- ASCI scores indicated intact algal communities at most but not all sites.
 - Both d-ASCI and h-ASCI scores were above the threshold at eight sites, all reference: Stonewall, French, Upper Agua Caliente, Juaquapin, Japacha, Troy, Long, and Kitchen.
 - The h-ASCI was above the threshold and d-ASCI below at six sites: four reference (Cottonwood, Espinosa, Agua Caliente at PCT, Pauma) and two expansion (Cole, Harper).
 - Both d-ASCI and h-ASCI scores were below the threshold at eight sites: slightly below at some reference (Cold), trend (Pine), expansion (Doane), and program (Arroyo Seco) sites, and well below at some causal (Jamul), expansion (Los Alamos), and program (Sandia, San Juan) sites.
 - o ASCI scores were not available for one site (Ninemile Creek).
- IPI scores indicated intact physical habitat at all but one site.
 - Kitchen Creek scored below the threshold, likely due to its bedrock dominated substratum.
- Chemistry results met Basin Plan objectives for aquatic life at all but two sites.
 - Total nitrogen levels at Sandia Creek (5.8 mg/L) and Pine Valley Creek
 (1.4 mg/L) were above the Basin Plan objective of 1.0 mg/L.
 - While there are no aquatic life criteria for sulfate, sulfate levels at Sandia Creek (370 mg/L) and Cottonwood Creek (340 mg/L) were above the drinking water objective of 300 mg/L.
 - Benthic AFDM at 11 of the 17 sites tested was above a published threshold for California, as was benthic chl-a at 7 of 17 sites, but these parameters are documented to have a high degree of variability.

- The maximum number of BMI taxa in a sample was 74 (Ninemile Creek). Not counting this Sierra site, the maximum number of BMI taxa was 60 (French Creek). Overall, pooling taxonomic data from the 22 streams sampled within the region, 211 BMI taxa were recorded in Region 9 in 2023.
- The maximum number of algal taxa in a sample was 80 (Harper Creek).

 Overall, pooling taxonomic data from the 22 streams sampled within the region,

 276 algal taxa were recorded in Region 9 in 2023.
- eDNA metabarcoding kits detected notable aquatic wildlife at 12 of the 25 sites where they were used. The Jonah Ventures eDNA kits detected three sensitive native fish species, five non-native fish species, two native amphibians and one non-native amphibian.
 - The native species were steelhead/rainbow trout (Oncorhynchus), stickleback (Gasterosteus), arroyo chub (Gila orcuttii), newt (Taricha granulosa, likely truly a California newt Taricha torosa), and arroyo toad (Anaxyrus californicus).
 - The non-native species were bass (*Micropterus*), green sunfish (*Lepomis cyanellus*), bluegill (*Lepomis macrochirus*), bullheads (*Ameiurus*), mosquitofish (*Gambusia*), and African clawed frogs (*Xenopus laevis*).
 - eDNA metabarcoding kits also detected useful information regarding potential stressors, including cattle (Upper Los Alamos Canyon Creek North Fork) and horses (San Juan Creek above Ortega Falls, horse facility upstream).
 - o MARU plans to use eDNA metabarcoding kits again in 2024.
- Targeted eDNA analyses using ddPCR successfully detected rare, sensitive species of interest at 14 of the 16 sites where it was used. The Smith-Root sampling device and ddPCR analysis targeted for certain species proved to be more effective than metabarcoding at detecting sensitive species.
 - In San Mateo Creek, steelhead/rainbow trout were detected at all five sites sampled. Notably, co-collected metabarcoding samples did not detect steelhead/rainbow trout at any San Mateo sites.
 - California newt (*Taricha torosa*) was detected at one San Mateo site known to have newts just upstream. Notably, the co-collected metabarcoding sample did not detect newt at the site.
 - In the Sweetwater River, steelhead/rainbow trout and southwestern pond turtle were detected. Again, notably, co-collected metabarcoding samples did not detect these species.
 - The fact that ddPCR detected steelhead/rainbow trout, California newt, and southwestern pond turtle when metabarcoding did not highlights the need to do targeted eDNA analysis using ddPCR for the detection of rare taxa.
 - MARU plans to use the Smith-Root device and ddPCR again in 2024.

- Carbon:Nitrogen ratios were between 8 and 16 at all but four sites, indicating that most of the streams had a mix of carbon-rich (leaves) and nitrogen-rich (algae, animal waste) sources.
 - Marginally higher ratios at French Creek (16.0, 16.2), Doane Creek Rep 1 (15.2, 16.7), Doane Creek Rep 2 (16.0, 16.8), Pauma Creek (16.3, 17.7), and Ninemile Creek (18.0, 18.5) suggest these streams have a slight predominance of terrestrial carbon sources.
 - Continued sampling of particulate carbon and nitrogen is planned for 2024.
- Cyanotoxins were not detected in algal composite or targeted benthic mat samples, but they were detected in low concentration in SPATT samplers.
 - Among the six sites where SPATT samplers were deployed, anatoxin-a
 was detected at all (highest was 2.81 ng/g, Doane Creek) and microcystin
 was detected at one (3.84 ng/g, Doane Creek), suggesting that
 cyanotoxins may be present at low background levels even in high-quality
 streams.
- E. coli and HF183 results were acceptable at the two sites tested.
 - E. coli did not exceed the Basin Plan objective at San Juan Creek above Ortega Falls (Program) or Jamul Creek (Causal).
 - HF183 was "non-detect" at both sites.
- According to the CEDEN database, a total of 52 bioassessments were done in Region 9 in 2023. MARU and CDFW did 22, and 30 were done by other entities. Bioassessment data from 2023 can be found in CEDEN under these Projects:

#	Project
16	RWB9 Bioassessment 2023
6	Statewide Ref Condition Management Plan 2023
3	Alvarado Creek Bioassessment Monitoring – 2023
4	OCPW San Diego Region Bioassessment
3	SDSU Mission Valley Campus Master Plan Restoration
10	SMC Regional Watershed Monitoring SCCWRP
10	SMC Regional Watershed Monitoring 2023

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